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Photoionization at Elevated or Atmospheric Pressure: Applications of APPI and LPPI

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8.1 Introduction

Electrospray ionization (ESI) and atmospheric pressure chemical ionization (APCI) have dominated liquid chromatography mass spectrometry (LC-MS) instrumentation for many years. Compared to ESI and APCI, atmospheric pressure photoionization (APPI) is a relatively new technique, which has expanded the range of compounds that can be ionized in LC-MS toward lower polarity, and it has become the first choice for many applications. If not for the legacy and history of ESI and APCI, APPI might arguably be more than just the third option of choice (Robb 2000; Syage 2000). Some of the attributes of APPI that are compelling for many applications are as follows:

- Ionization of a broad range of compound classes including nonpolar compounds that are difficult to detect by ESI or APCI
- Low susceptibility to ion suppression and matrix effects, which makes APPI very effective in detecting compounds quantitatively in complex matrices
- A wide linear dynamic range, which is generally limited by the mass spectrometer
- Very low noise because most solvent molecules are not ionized in APPI, which reduces background signal from solvent ions and solvent ion complexation with analyte and other background compounds

This chapter focuses on the practical application of APPI and low-pressure photoionization (LPPI) and illustrates a number of applications for which these ionization methods are well suited.

8.2 Atmospheric Pressure Photoionization

8.2.1 Atmospheric Pressure Photoionization Ion Source

The APPI source has been described in several research papers and review articles (Raffaelli 2003; Bos 2006; Robb 2008a; Syage 2008; Marchi 2009a;

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First Edition. Edited by Ralf Zimmermann and Luke Hanley.

Kauppila 2017), and therefore, only a limited description is given here. A vacuum ultraviolet (VUV) lamp provides high-energy photons sufficient to ionize analyte and/or dopant molecules. Besides direct photoionization, a multitude of reactions can take place in APPI, such as proton and charge transfer in positive polarity, and electron capture (and many ancillary reactions) in negative polarity. These reactions are described in more detail below. An example configuration of an open source APPI relative to the vaporizer and the mass spectrometer inlet is illustrated in Figure 8.1. In addition, APPI configuration using a narrow drift tube to enhance dopant chemistry has been introduced (Robb et al. 2000). Current commercial APPI sources utilize VUV lamps, which are based on discharge of a gas mixture primarily consisting of Kr and operate on the atomic emission lines at 10.0 and 10.6 eV. The discharge can be driven by a variety of means, but the most common are radio frequency (RF) electrodeless excitation and high-voltage direct current (DC) electrode excitation. APPI is similar to APCI where the analyte molecules must be vaporized for effective ionization, and therefore, APPI in essentially all commercial mass spectrometry (MS) systems uses the same nebulizer/vaporizer assembly used for APCI. Direct APPI (without dopant) leads to formation of ions that are not efficiently formed by APCI, such as molecular ions of nonpolar compounds. APPI with dopant ionization, on the other hand, leads to similar ion chemistry as APCI, such as proton transfer in positive ion mode, and a variety of negative ion mechanisms in negative ionization. A significant difference, however, is that APCI occurs in a region of high electric field because this is what sustains the discharge, whereas APPI can occur in a field-free region. This can have important ramifications on the ensuing ion chemistry after ionization and is an area of research that has not been adequately investigated but could offer some interesting properties.

8.2.2 General Principles of Atmospheric Pressure Photoionization

8.2.2.1 Ionization Mechanism in Positive Ion APPI

The ionization in APPI is initiated by the 10.0 (and 10.6) photons emitted by the krypton discharge VUV lamp. The photons can ionize any compounds that

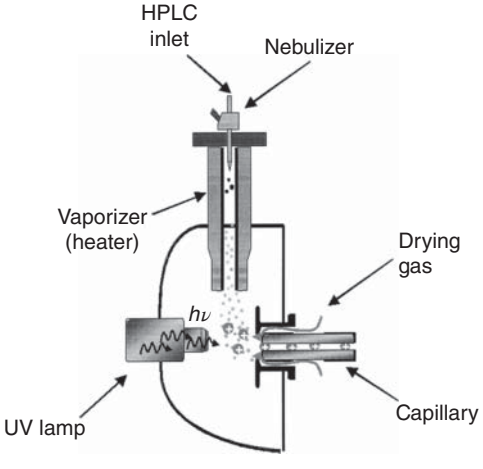

Figure 8.1 Standard configuration of an APPI orthogonal. Source: Hanold et al. (2004). Copyright 2004, Reprinted with permission of American Chemical Society.

have ionization energies (IEs) below their energy, which include most organic compounds, but leaves out atmospheric gases and the most typical liquid chromatography (LC) solvents that are present in the ion source. Therefore, APPI is more selective than APCI, where the gases and LC solvents are also ionized by the corona discharge-initiated ionization reactions. The initial ionization reaction in direct APPI is the photoionization of the analyte and formation of a molecular ion of the analyte ($M^{+\cdot}$, Scheme 8.1, Reaction 1). If a solvent that has energy below 10 eV is present (e.g. several normal-phase LC solvents), the solvent can also be ionized by the 10 eV photons (Scheme 8.1, Reaction 2). After the initial photoionization reaction, compounds that possess high proton affinities (PAs) tend to form protonated molecules ($[M+H]^+$) by self-protonation, whereas compounds with low PA rather remain in the form of a molecular ion.

In atmospheric pressure, the photons can go through numerous collisions with neutral molecules, where they lose their energy. The lifetime of the photons is therefore rather short and the ionization efficiency in direct APPI is often low. The ionization efficiency can be increased by introducing a dopant, a solvent that has ionization energy below 10 eV, to the ion source. This dopant is ionized by the photons (Scheme 8.1, Reaction 3), after which it can react with the analytes through charge exchange, if the IE of the analyte is lower than the IE of the dopant (Scheme 8.1, Reaction 4). The dopant molecular ions ($D^{+\cdot}$) can be solvated by water or solvent clusters (Scheme 8.1, Reactions 5 and 6) (Klee et al. 2013). If the dopant contains a proton, it can be deprotonated by the cluster, or it can transfer its charge to the cluster (Scheme 8.1, Reactions 7 and 8, respectively). The charged solvent clusters can react with the analytes by ligand-switching or association reactions (Scheme 8.1, Reactions 9 and 10, respectively) (Klee et al. 2013). At the MS entrance, the clusters will break because of the electrical field-driven

$M + h\nu$	$\rightarrow$	$M^{+\cdot} + e^-$,	if IE (M) < 10 eV	(1)
$S + h\nu$	$\rightarrow$	$S^{+\cdot} + e^-$,	if IE (S) < 10 eV	(2)
D (dopant) + $h\nu$	$\rightarrow$	$D^{+\cdot} + e^-$		(3)
$D^{+\cdot} + M$	$\rightarrow$	$M^{+\cdot} + D$,	if IE (M) < IE (D)	(4)
$D^{+\cdot} + nH_2O$	$\rightarrow$	$[D^{+\cdot}(H_2O)_n]$		(5)
$D^{+\cdot} + nS$	$\rightarrow$	$[D^{+\cdot}(S)_n]$		(6)
$[D^{+\cdot}(H_2O)_n]$	$\rightarrow$	$[(H_2O)_n+H]^+ + [D–H]^{\cdot}$		(7)
$[D^{+\cdot}(H_2O)_n]$	$\rightarrow$	$(H_2O)_n^+ + D^{\cdot}$		(8)
$[(H_2O)_n+H]^+ + M$	$\rightarrow$	$[M+(H_2O)_n+H]^+ + H_2O$		(9)
$[(H_2O)_n+H]^+ + M$	$\rightarrow$	$[M+(H_2O)_n+H]^+$		(10)
$[M+(H_2O)_n+H^+]$	$\rightarrow$	$[M+H]^+ + nH_2O$		(11)

Scheme 8.1 Ionization reactions in APPI (Kauppila et al. 2002; Syage 2004; Klee et al. 2013).

collisional decomposition reactions and the proton will end up to the species with highest proton affinity, resulting in formation of $[M+H]^+$ (analyte), $[S+H]^+$, or $[D+H]^+$ (Scheme 8.1, Reaction 11).

8.2.2.2 Effect of the Dopant

In most cases, introduction of a dopant in APPI leads to a significant increase in the signal of the analytes, typically in the order of 1–2 magnitudes (Rauha et al. 2001; Kauppila et al. 2002; Rodil et al. 2009; Rivera et al. 2011). The most typical dopants that have been explored in APPI are toluene, acetone, anisole, and chlorobenzene. Besides these, there are numerous solvents or combinations of solvents that have been tested as dopants (Robb et al. 2008b; Cai et al. 2009b; Smith et al. 2009; Dousty et al. 2013). A suitable APPI dopant should have an IE below 10.6 eV, so that it can be directly ionized by the APPI lamp photons (Table 8.1). However, other properties of the dopant determine what kind of reactions take place after the initial photoionization reaction and what kind of analyte ions are likely to form. Selection of the dopant has a direct effect on the sensitivity and selectivity of the ionization and is therefore one of the parameters that most affects the method performance in APPI. Here, the most common dopants and how they affect the ionization of the analytes are briefly discussed.

Toluene has thus far been the most popular dopant. In photoionization, toluene (similar to the other dopants) forms a molecular ion ($M^{+\cdot}$), which can react with the analytes (and solvents) by both charge exchange and protonation reactions (Scheme 8.1, Reactions 3–11). If solvent clusters that possess higher PAs than benzyl radical (deprotonated toluene $M^{+\cdot}$) are present (e.g. in case of methanol and acetonitrile solvents), the toluene $M^{+\cdot}$ can protonate the solvent molecules (or clusters) via proton transfer and is depleted from the ion source atmosphere. The ionization route is then directed toward protonating reactions that include the protonated solvent clusters (Scheme 8.1, Reactions 6–11). Only when low PA solvents, such as water, hexane, and chloroform (Kauppila et al. 2002), or a gas chromatographic system, which is free of other solvents (Kauppila et al. 2014), is used, the toluene molecular ion can survive in the gas phase and enable ionization of analytes via charge exchange.

Anisole has higher PA than toluene, and therefore, the $M^{+\cdot}$ of anisole does not react with the solvent molecules by proton transfer as readily as the $M^{+\cdot}$ of toluene (Kauppila et al. 2004a). As a consequence, the $M^{+\cdot}$ of anisole is available for charge

Table 8.1 Properties of the most typical dopants.

Dopant	IE (eV)	PA (kJ/mol)
Toluene	8.83	784.0
Anisole	8.20	839.6
Chlorobenzene	9.07	753.1
Acetone	9.70	812.0

IE = ionization energy, PA = proton affinity
Source: From Linstrom and Mallard (2015).

exchange with the analytes even when high PA solvents are present, and a significantly higher signal for low PA analytes can be achieved with anisole than with toluene. The formation of $[M+H]^+$ of the analytes with anisole, however, can be compromised because of the higher PA of anisole. Another limitation of anisole is that the IE of anisole is rather low, and therefore, a large amount of compounds cannot be ionized through charge exchange with anisole. Chlorobenzene was suggested as an APPI dopant in order to overcome this problem: it has higher IE than anisole, and it can therefore ionize a larger group of compounds through charge exchange (Robb et al. 2008b). Acetone, on the other hand, goes through self-protonation after the initial photoionization (Tzeng et al. 1989), and therefore, acetone $M^{+\cdot}$ ions are not at all available for charge exchange. However, the protonated acetone molecules and clusters can efficiently protonate analytes of high PA. The ionization with acetone dopant is often more selective than with, e.g. toluene, which often results in lower background.

8.2.2.3 Ionization Mechanism in Negative Ion APPI

In negative ion APPI, the ionization reactions are initiated by thermal electrons that are either released in the photoionization of the dopant (Scheme 8.2, Reaction 1) or from the metal surfaces of the ion source (Basso et al. 2003; Kauppila et al. 2004b). These electrons can be captured by gases, solvents, or analytes that possess positive electron affinities (EAs; Scheme 8.2, Reactions 2–4). In electron capture, a negative molecular ion ($M^{-\cdot}$) of the compound is formed. Oxygen has been shown to play an important role in negative ion APPI. It has positive EA, and it can therefore efficiently capture electrons, thus forming superoxide ions, $O_2^{-\cdot}$ (Scheme 8.2, Reaction 4). $O_2^{-\cdot}$ can react with the analytes through charge exchange, if the analyte has higher EA than $O_2^{-\cdot}$; in that case, a negative molecular ion of the analyte is formed (Scheme 8.2, Reaction 5). $O_2^{-\cdot}$ has also basic properties, and it can therefore also accept protons from analytes or solvents that

D (dopant) + $h\nu$	$\rightarrow$	$D^{+\cdot} + e^-$		(12)
$M + e^-$	$\rightarrow$	$M^{-\cdot}$,	if EA (M) > 0 eV	(13)
$S + e^-$	$\rightarrow$	$S^{-\cdot}$,	if EA (S) > 0 eV	(14)
$O_2 + e^-$	$\rightarrow$	$O_2^{-\cdot}$		(15)
$M + O_2^{-\cdot}$	$\rightarrow$	$M^{-\cdot} + O_2$,	if EA (M) > EA (O_2)	(16)
$M + O_2^{-\cdot}$	$\rightarrow$	$[M-H]^- + HO_2^{\cdot}$,	if $\Delta_{acid}G$ (M) < $\Delta_{acid}G$ ($HO_2^{\cdot}$)	(17)
$S + O_2^{-\cdot}$	$\rightarrow$	$[S-H]^- + HO_2^{\cdot}$,	if $\Delta_{acid}G$ (S) < $\Delta_{acid}G$ ($HO_2^{\cdot}$)	(18)
$M + [S-H]^-$	$\rightarrow$	$[M-H]^- + S$,	if $\Delta_{acid}G$ (M) < $\Delta_{acid}G$ (S)	(19)
$M + O_2^{-\cdot}$	$\rightarrow$	$[M-X+O]^- + OX^{\cdot}$	X = H, Cl, NO_2	(20)
$M^- + O_2$	$\rightarrow$	$[M-X+O]^- + OX^{\cdot}$		(21)

Scheme 8.2 Ionization reactions in negative ion APPI (Kauppila et al. 2004b).

have higher gas-phase acidities (Scheme 8.2, Reactions 6 and 7). $O_2^{-\cdot}$ is therefore an important reagent ion in proton transfer in negative ion mode, which is the main route of ionization for acidic compounds. Also, solvent-derived ions (e.g. acetate ions from the LC buffer) can act as reagent ions in negative ion APPI and react with the analytes through proton transfer (Scheme 8.2, Reaction 8). Aryl compounds also tend to form oxidation products that can somewhat complicate the negative ionization-atmospheric pressure photoionization (NI-APPI) mass spectra (Scheme 8.2, Reaction 10).

Rather few compounds possess positive EAs – typically compounds with halogenated or nitro groups – or a high degree of conjugation (e.g. quinones). Besides these, acidic compounds are efficiently ionized in negative ion APPI. Ionization of nitroaromatic compounds has been successfully achieved in negative ion APPI via anion attachment, by introducing halogen salts to the ionization area (Song et al. 2007). Because negative ion APPI requires certain functionalities in the analyte structure in order to be efficient, it is more selective than positive ion APPI and can be used to gain excellent signal-to-noise ratios.

8.2.3 APPI in Liquid Chromatography Mass Spectrometry

APPI was originally developed as an interface for LC-MS, and it is still the most important area, where APPI is used. In comparison to the earlier invented LC-MS ionization methods, ESI and APCI, APPI has expanded the range of LC-MS analyzable analytes toward completely nonpolar compounds. APPI has also been reported to have other advantages, such as a broader linear range than in ESI (Hakala et al. 2003). The ionization in APPI is more selective than in APCI, and therefore, the background in APPI is typically lower, as illustrated in Figure 8.2,

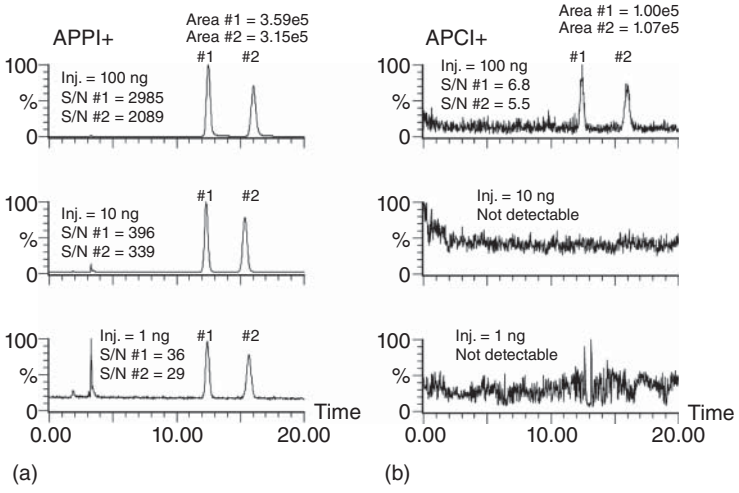

Figure 8.2 LC separation of benzoin enantiomers using (a) APPI and (b) APCI. The APPI and APCI peak areas differ by no more than 4×, but S/N ratios differ by over two decades. Poor APCI sensitivity was mainly due to elevated baseline noise generated from ionization of column bleeding components. Source: Cai et al. (2007a). Copyright 2007, Reprinted with permission of American Chemical Society.

where column bleeding components disturb the signals of analytes in APCI, but not in APPI (Cai et al. 2007a). In comparative studies between the three ionization methods, APPI has typically been the most sensitive technique for neutral and nonpolar compounds (see Section 8.4, for examples of APPI applications), but for polar and ionic compounds, ESI usually gives the best sensitivity.

Unlike in ESI, where the solvent has to be conductive, in APPI, there are no such restrictions. Therefore, APPI allows a wider range of LC solvents than ESI, and even completely nonpolar normal-phase liquid chromatography (NP-LC) solvents can be easily combined with atmospheric pressure photoionization mass spectrometry (APPI-MS) (Hakala et al. 2003; Wang et al. 2005; Cai et al. 2007a). However, the LC solvent can have a significant effect on the APPI mechanism, and it is important to be aware of the possible consequences to the ionization route and sensitivity (Kostiainen and Kauppila 2009). In reversed-phase liquid chromatography (RP-LC), the solvent is typically a mixture of water and methanol or acetonitrile, where ammonium acetate or other volatile buffer is often added to ensure the optimal chromatographic behavior of the analytes. In dopant-assisted APPI, gas-phase reactions between dopant $M^{+\cdot}$ ions and neutral solvent molecules (e.g. methanol, acetonitrile, and ammonia) or their clusters can take place, as already mentioned above. This depletes especially toluene $M^{+\cdot}$ ions from the system, making charge exchange reactions impossible and turning the ionization to the direction of proton transfer (Kauppila et al. 2002). In this case, compounds that have high PAs can still be ionized by the protonation route, but ionization of low-polarity compounds with low PAs becomes highly inefficient. Because these are the compounds that are poorly ionized by other LC-MS techniques, it is the charge exchange route that is often pursued instead of proton transfer. In RP-LC conditions, efficient charge exchange can be achieved using dopants such as anisole and chlorobenzene, as already discussed above (Kauppila et al. 2004a; Smith et al. 2009). Also, with toluene, charge exchange can be achieved if low PA solvents, such as water, hexane, and chloroform, are used (Kauppila et al. 2002). Many NP-LC solvents (e.g. hexane, heptane, octane, isooctane, isopropanol, and tetrahydrofuran) possess sufficiently low IEs for direct photoionization and therefore do not require additional dopant (Wang et al. 2005; Cai et al. 2007b; Black et al. 2013). NP-LC utilizing APPI as the ionization method has been shown to be well suited to, e.g. analysis of lipids (Cai and Syage 2006a), pharmaceuticals (Wang et al. 2005), environmental contaminants (Straube et al. 2004), and food toxins (Cavaliere et al. 2006).

In negative ion APPI, solvents that possess positive EAs (e.g. chloroform and other halogenated solvents) have been reported to suppress the ionization (Kauppila et al. 2004b). Solvent additives, such as acetate and formate buffers, can cause signal suppression if the analyte is a weaker acid than the additive. However, for cases where the analyte is a stronger acid, the presence of such additives may increase the signal and decrease the noise because of improved selectivity. Also, in positive ion mode, the introduction of buffers has been reported to reduce the ionization efficiency in APPI, and therefore, the concentration of LC buffer should be kept in minimum (Rauha et al. 2001; Kauppila et al. 2002).

In liquid chromatography atmospheric pressure photoionization mass spectrometry (LC-APPI-MS), an important issue that has to be considered is the

flow rate of the LC solvent. The signal of analytes in APPI has been observed to saturate and even decay at higher solvent flow rates (above 200 µl/min), and therefore, much lower flow rates are recommended for APPI than for APCI (Yang and Henion 2002). This has been suggested to be due to absorption of photons by the increasing amount of solvent molecules, formation of larger solvent clusters with higher PAs, which can prevent the ionization of the analytes, and increased number of recombination processes between positive and negative ions in the ion source (Hanold et al. 2004; Kauppila et al. 2005; Robb and Blades 2005). In dopant-assisted APPI, the analytes that are ionized through charge exchange reaction are more severely affected by this phenomenon than compounds that form protonated molecules, which may be due to depletion of dopant $M^{+\cdot}$ ions by the increased amount of solvent and solvent impurities (Kauppila et al. 2005). More efficient charge exchange can therefore be achieved using a lower solvent flow rate (50 µl/min instead of 200 µl/min), since then, the dopant $M^{+\cdot}$ ions are not depleted, but are available for the charge exchange reaction, even in the presence of high PA solvents (e.g. methanol and acetonitrile) (Robb and Blades 2006).

Even lower solvent flow rate (0.5–10 µl/min) is used in microchip atmospheric pressure photoionization (µAPPI, Figure 8.3), which allows the interfacing of capillary-LC with APPI-MS (Kauppila et al. 2004c; Haapala et al. 2007a). µAPPI uses a microfabricated heated nebulizer made of two glass layers; the top layer has channels and connections for the sample solution and nebulizer gas, while the bottom layer has platinum heaters that can heat the microchip to over 350 °C (Saarela et al. 2009). CapLC-µAPPI-MS applications using the microchips include the analysis of anabolic steroids and analysis of pesticides from tomato (Hintikka et al. 2010; Kruve et al. 2011; Hintikka et al. 2013). The same microchip can also be utilized for gas chromatography atmospheric pressure photoionization mass spectrometry (GC-APPI-MS) (Haapala et al. 2007a) and desorption atmospheric pressure photoionization (DAPPI) (Haapala et al. 2007b) (see below).

8.2.4 APPI in Gas Chromatography Mass Spectrometry

There are several advantages for coupling gas chromatography (GC) with an atmospheric pressure ionization mass spectrometer (API-MS). GC can achieve a far higher number of theoretical plates than LC and is thus capable of separating a much higher number of compounds. Although traditionally gas chromatography mass spectrometry (GC-MS) utilizes electron ionization (EI) or chemical ionization (CI), the coupling of GC with an LC-MS instrument makes the measurement of unfragmented molecular ions or protonated molecules possible, which reveals the molecular mass of a compound. Also, multiple MS/MS modes, such as product and precursor ion scans, neutral loss, and single or multiple reaction monitoring, or MS^n that can give structural information about the analyte and increase the sensitivity and specificity, are available with the API-MS instruments (depending on the mass analyzer).

APPI is a promising candidate for coupling GC with API-MS because it requires that the analytes are in neutral and gaseous form before the ionization and because it is capable of selectively ionizing the analytes with very low

background. Further, the odd electron $M^{+\cdot}$ ions formed by charge exchange in APPI follow the same fragmentation patterns as the $M^{+\cdot}$ ions formed in EI, and therefore, the product ion spectra of these ions can be compared with EI library spectra, and even unknown compounds can be identified. In GC-APPI-MS, there are no LC solvents that could deplete the dopant $M^{+\cdot}$ ions from the APPI source, and ionization of analytes through charge exchange is more likely than in LC-MS conditions.

Several self-made solutions for coupling APPI with GC-MS have been introduced. Groups by McEwen, Lee, and Kostiainen all used simple setups, where a heated transfer line from a GC was used to deliver the gaseous sample to a commercial API-MS instrument (McEwen 2007; Lee et al. 2012; Suominen et al. 2013). The setups were used for perfume, environmental contaminants, and trimethylsilyl (TMS)-derivated neurosteroids from human urine (Suominen et al. 2013). Another GC-APPI-MS setup by Kostiainen's group utilizes the same heated nebulizer microchip that was described above for CapLC-APPI-MS (Figure 8.3). In the GC-μAPPI-MS setup, a heated transfer line was used to connect the GC column to the microchip, which was heated to approximately 300 °C. The microchip setup has been used to analyze TMS-derivatized and underivatized anabolic steroids from urine (Hintikka et al. 2010, 2013; Luosujärvi et al. 2010a) and polychlorinated biphenyls (PCBs) from soil extracts (Luosujärvi et al. 2008). Kersten et al. introduced another setup for GC-MS coupling, called capillary atmospheric pressure photoionization (cAPPI), where a windowless miniaturized argon spark discharge lamp was embedded into an ion transfer capillary, which separates the atmospheric and differentially pumped low-pressure regions of the mass spectrometer (Kersten et al. 2011). Because of the close proximity to the MS inlet, the source was reported to have a

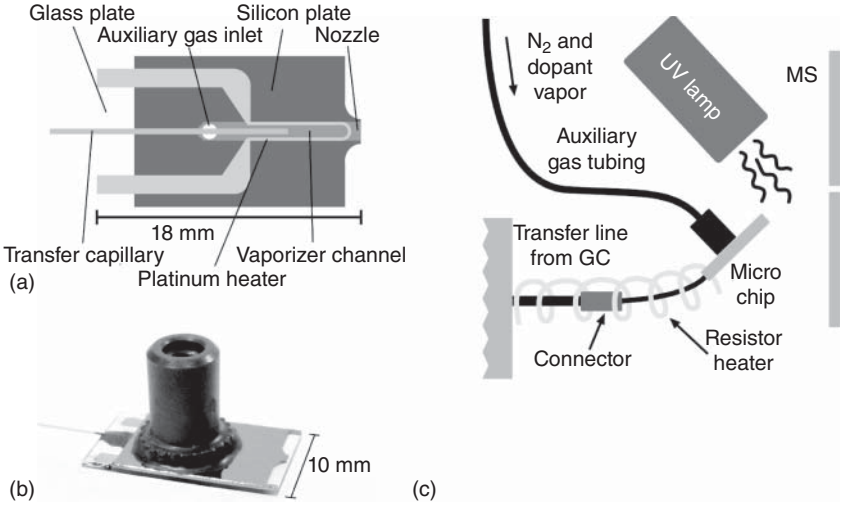

Figure 8.3 (a) Schematic view of the microfabricated heated nebulizer from the heater side. (b) A photograph of a microchip with a Nanoport connector and transfer capillary. (c) A schematic of the GC-μAPPI-MS setup. Source: Haapala et al. (2007a). Copyright 2007, Reprinted (adapted) with permission of American Chemical Society.

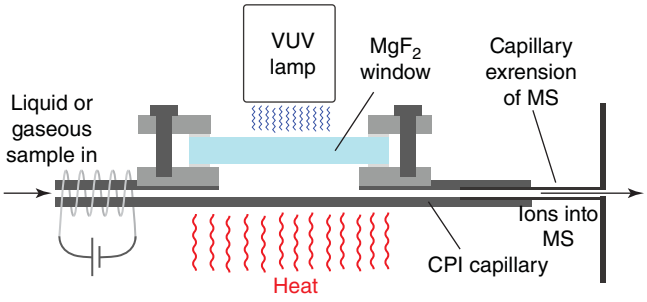

Figure 8.4 Schematic of the capillary photoionization device connected to a mass spectrometer. Source: Haapala et al. (2013). Copyright 2013, Reprinted (adapted) with permission of American Chemical Society.

temporal resolution of milliseconds, which could be utilized in real-time studies of rapidly changing chemical systems. Similar to cAPPI, in the setup introduced by Haapala et al., the photoionization and the subsequent ionization reactions took place inside a transfer capillary (Figure 8.4) (Haapala et al. 2013). This setup called capillary photoionization (CPI) utilized a heated transfer capillary with a VUV transparent MgF_2 window, through which 10 eV photons from a krypton discharge lamp entered. The sample could be either liquid or gaseous, and it was introduced together with a dopant directly into the heated transfer capillary, where it was vaporized (in case of a liquid sample) and photoionized. The setup was used as an interface between GC and MS for the highly sensitive analysis of TMS-derivatized steroids from biological samples with limit of detections (LODs) in the range of 2–6 pg/ml. Yet another setup by Kersten et al. was developed for the coupling of GC with Orbitrap MS using an APPI interface (Kersten et al. 2016). In this setup, the ionization took place inside a small, airtight, cone-shaped unit, where a vortex was maintained by a highly purified (99.999999%) nitrogen flow, to the eye of which the minor GC stream was placed (Figure 8.5). The photoionization lamp was placed directly on a

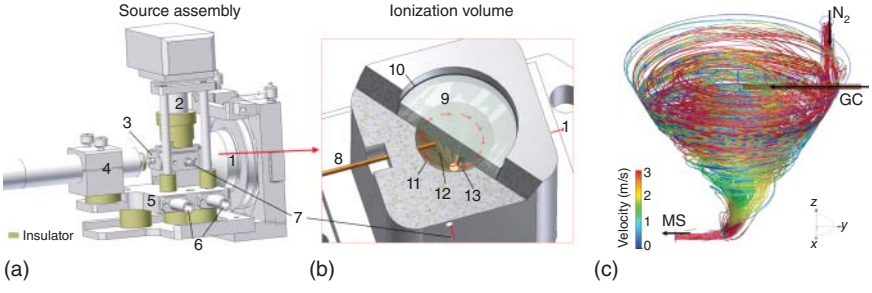

Figure 8.5 Schematic of the GC-APPI-Orbitrap interface by Kersten et al. (a) 1. MS, 2. VUV lamp, 3. GC column adapter, 4. GC transfer line assembly, 5. heater table, 6. heater cartridges, 7. makeup gas adapter, 8. GC column, 9. MgF_2 window; (b) 10. sealing with cement and inorganic coating, 11. makeup gas inlet, 12. conical ionization volume, 13. exit to MS, (c) massless particle trace simulation of the makeup gas inside the conical ionization volume. Source: Kersten et al. (2016). Copyright 2016, Reprinted with permission of Springer Nature.

MgF_2 window. The setup has been shown to ionize a variety of GC compounds by direct photoionization, i.e. without a dopant (Kauppila et al. 2014, 2015). Femtogram-on-column sensitivity has been reported for different environmental protection agency (EPA) standard mixtures (Kersten et al. 2016).

8.2.5 APPI As an Interface for Capillary Electrophoresis Mass Spectrometry

Several groups have used APPI as the ionization method for capillary electrophoresis mass spectrometry (CE-MS). ESI is traditionally the method of choice for capillary electrophoresis (CE) because the CE separation demands that the compounds are in ionic form, but nevertheless, several advantages that APPI has over ESI in CE-MS have been reported. Nonvolatile phosphate and borate buffers that are common in CE can cause severe signal suppression in ESI, but APPI is not severely affected by the buffers, as shown in Figure 8.6 for a group of pharmaceuticals (Nilsson et al. 2003; Hanold et al. 2004). Also, the background has been reported to be lower in APPI than in ESI. APPI-MS has also been used as the detection method for micellar electrokinetic chromatography (MEKC), which is suitable for both polar and nonpolar compounds, and unlike in ESI, the signal in APPI was not affected by sodium dodecyl sulfate (SDS) that is introduced to the electrolyte for micelle formation (Mol et al. 2005a,b; Axén et al. 2010). Thus, APPI allows a wider variety of CE buffers than ESI. Capillary electrophoresis atmospheric pressure photoionization mass spectrometry (CE-APPI-MS) has been used for the analysis of pharmaceuticals, doping substances, and drug impurity profiling (Nilsson et al. 2003; Schappler et al. 2007, 2008; Hommerson et al. 2009). Besides LC-, GC-, and CE-MS, APPI has also been used as the interface for coupling supercritical fluid chromatography (Bolanos et al. 2004) and as ion source for ion mobility spectrometry (Borsdorf et al. 2002).

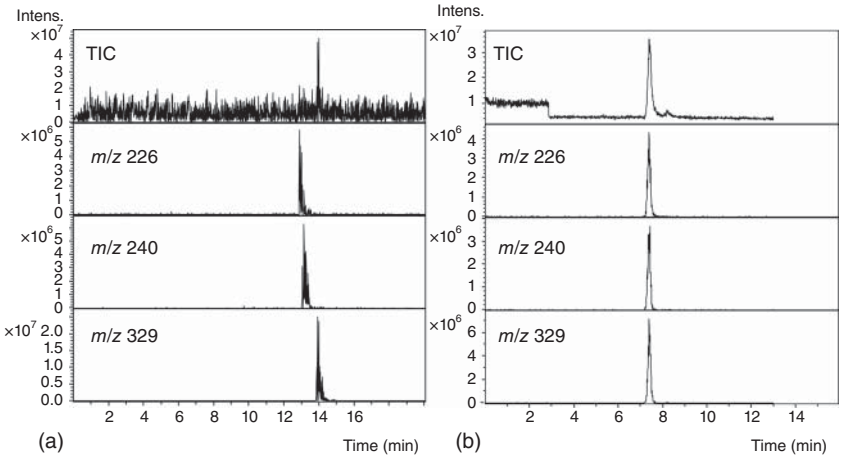

Figure 8.6 CE chromatograms using phosphate buffer for a three-drug mixture comparing signal response for (a) ESI and (b) APPI. Source: Hanold et al. (2004). Copyright 2004, Reprinted with permission of American Chemical Society.

8.2.6 APPI in Multimode Ion Sources

APPI has been combined with other ionization techniques as multimode ion sources for GC- and LC-MS in order to widen the range of compounds that can be analyzed in one chromatographic run. Syage et al. (2004) introduced a dual source for LC-MS that combined APPI with either ESI or APCI (Syage 2004). The source could use either ionizer alone, both simultaneously, or rapidly switch between the ionizers during a chromatographic run. The APPI/APCI combination seemed to work well, but in ESI/APPI, the ESI ionization of proteins that normally form multiply charged ions was suppressed when the APPI source was on. This was explained by ion/molecule reactions between the ions formed by the two sources. Later, another multimode source for LC-MS that combined APPI and ESI was developed by Syage's group, this time combining APPI and ESI (Short et al. 2007). At high flow rate (>200 µl/min) and low temperature (>400 °C), the APPI ionization was reported to take place by solvent-mediated reactions, whereas at low flow rate, $M^{+\cdot}$ ions were formed, probably by direct photoionization. The dual source was reported to perform favorably in comparison to a dual ESI/APCI source, especially for unstable compounds, because of softer ionization by APPI than APCI.

8.2.7 Ambient Mass Spectrometry Utilizing Photoionization

Photoionization has also been utilized for direct surface analysis in ambient mass spectrometry. In DAPPI, the analytes are thermally desorbed from the surface under study, after which they are ionized via photon-initiated reactions in the gas phase, similarly to conventional APPI (Haapala et al. 2007b). The DAPPI setup utilizes a similar microchip heater for the thermal evaporation process and delivery of the dopant and nebulizer gas, as was described above for capLC-µAPPI-MS and GC-µAPPI-MS (Figure 8.7). Thus far, this DAPPI setup has been applied to the analysis of pharmaceuticals in tablets (Haapala et al. 2007b), drugs of abuse in powder, tablet, resin, plant, and paper form (Kauppila et al. 2008, 2011, 2013; Luosujärvi et al. 2009), drug metabolites and endogenic steroids from urine (Suni et al. 2011; Vaikkinen et al. 2015b), pesticides in plant

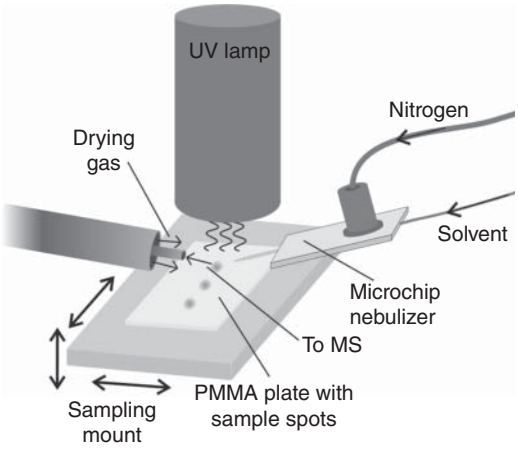

Figure 8.7 Schematic of the desorption atmospheric pressure ionization (DAPPI) source. Source: Haapala et al. (2007b). Copyright 2007, Reprinted with permission of American Chemical Society.

material (Luosujärvi et al. 2010b; Vaikkinen et al. 2015c), polyaromatic hydrocarbons (PAHs) in soil (Luosujärvi et al. 2010b), lipids from capsules, butter and milk (Suni et al. 2012; Vaikkinen et al. 2015a), explosives (Kauppila et al. 2016), insect defense compounds from insect skin (Rejšek et al. 2015), and mouse tissue (Pól et al. 2009). DAPPI has also been used for the characterization of pyrogenic black carbon in a setup, where the sample was held in front of the heated nebulizer of a commercial APPI source (Podgorski et al. 2012). Photoionization has also been combined with direct analysis in real time (DART) (Jorabchi et al. 2013), by adding a VUV lamp to a conventional DART source. Compared to conventional DART operation, the sensitivity of positive ion direct analysis in real-time atmospheric pressure photoionization (DART-APPI) was reported to be three to five times better. Both nitrogen and helium could be used as desorption gases, with similar performance. Besides DART, photoionization has also been added to atmospheric solid analysis probe (ASAP) (Ballesteros-Gomez et al. 2014). In ASAP, a melting probe capillary is used as a sample probe and placed in a conventional ESI or APCI source, where thermal desorption and ionization take place (McEwen et al. 2005). In direct probe APPI, an APPI source is used instead of ESI or APCI. Another ambient MS technique utilizing photoionization is extractive atmospheric pressure photoionization (EAPPI), which used ultrasonic nebulization for sample extraction, nebulization, and vaporization (Liu et al. 2016). The solid sample is placed in a nebulization cell, where the analytes are extracted from the matrix and transformed into aerosols by ultrasonic waves. The formed aerosols are driven to a heated transform tube, mixed with gaseous dopant, and ionized by photon-initiated reactions. EAPPI has been used for the fast screening of compounds in capsules, soul, natural products, and viscous compounds. Photoionization is also used as a postionization method in combination with laser-induced acoustic desorption (LIAD) (Chen et al. 2013; Benham et al. 2016). In LIAD, the sample is placed on a metal foil, and the backside of the foil is irradiated with a nanosecond laser pulse (Pérez et al. 2000). The pulses cause the desorption of neutral, largely intact molecules from the sample side of the foil. In laser-induced acoustic desorption atmospheric pressure photoionization (LIAD-APPI), the photons are generated by a Lyman-a (10.2 eV) microhollow cathode discharge microplasma photon source and toluene dopant is used to enhance the ionization. Laser ablation atmospheric pressure photoionization (LAAPPI) uses infrared (IR) laser for ablation of analytes from water-rich samples and VUV lamp for the photoionization, similarly to DAPPI (Vaikkinen et al. 2012). Even 50 μm sampling spot size can be reached with proper focusing of the IR laser (Hieta et al. 2017), and therefore, LAAPPI can be used for MS imaging of tissue samples. LAAPPI has been used for imaging of natural compounds in sage leaves and mouse brain tissue (Vaikkinen et al. 2014; Hieta et al. 2017).

8.3 Low-Pressure Photoionization (LPPI)

An efficient means for achieving sensitive detection of air or vapor samples by photoionization is through the use of a capillary inlet sampling tube, such as

a gas chromatography (GC) column, followed by photoionization in a reduced pressure environment, then followed by transit of the ions through another pressure drop stage (e.g. an aperture or skimmer) into the vacuum regime of the mass analyzer. Figure 8.8 shows a LPPI source that has been used for fast GC separations, as well as sampling of liquid and air samples. The entire assembly is heated and has been configured like a GC injector for syringe injections of liquid samples and solid-phase microextraction (SPME) samples as well as insertion of a probe containing solid sample on the tip (Wu et al. 2004; Syage et al. 2006). The ambient sampling probe in Figure 8.8 has been operated as both APPI and LPPI sources. This source offers simplicity because no carrier

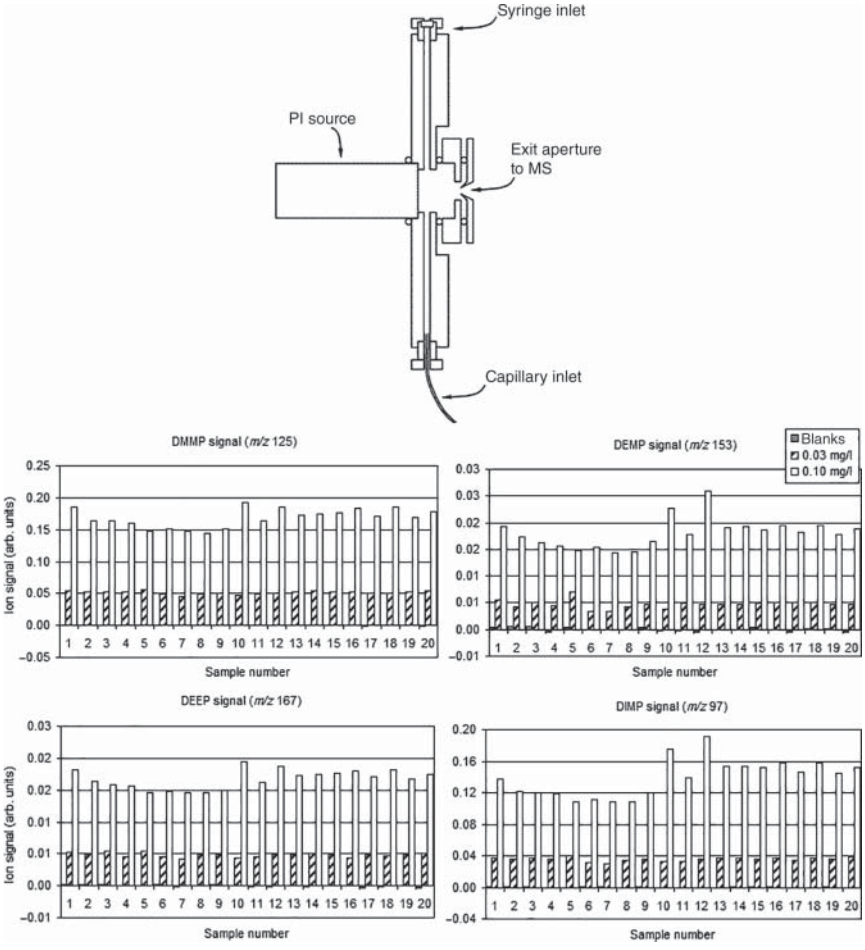

Figure 8.8 Drawing of an ambient insertion photoionization source that can be operated at atmospheric pressure (APPI) or low pressure (LPPI) (above). Plot of repeatability to detection of a class of chemical weapon stimulants at differing low concentrations. Dimethylmethyl phosphonate (DMMP), diethylmethyl phosphonate (DEMP), diethyl ethyl phosphonate (DEEP), and di-isopropyl methyl phosphonate (DIMP) (below). Source: Syage et al. (2006). Copyright 2006, Reprinted (adapted) with permission of American Chemical Society.

gas or nebulizer/vaporizer is needed, but because of the resilience of APPI as an ionizer, room air suffices for screening operation. The source also allows for a continuous capillary flow that can serve as a GC column, calibrant, a dopant, or another sample introduction port. All flows are controlled by the pressure differential created by the vacuum behind the MS inlet.

An example of the use of the ambient source as LPPI source is shown in Figure 8.8. Plotted are the repeated injections of a class of chemical weapons stimulants at different low concentrations. The injections contained all four compounds simultaneously, as can be seen by the consistencies of the relative intensities for all four compounds for variations in the injection.

8.4 Applications of APPI and LPPI

Examples of typical application areas, where APPI has been used, are discussed below and listed in Table 8.2. Literature search of the topic "atmospheric pressure photoionization" in 2000–2017 resulted in over 800 publications, but because of space restrictions, only a fraction of the cases are discussed here.

8.4.1 Drugs, Pharmaceuticals, and Metabolites

LC-MS is extensively used by pharmaceutical industry and academic research, and APPI as the ionization method has been found useful in applications studying pharmacokinetics (Theron et al. 2007; Mascher et al. 2008), drug permeation (Hakala et al. 2003), bioavailability of drugs (do Carmo Borges et al. 2011), and toxicology (Marchi et al. 2009b; Wang et al. 2010), as discussed below. Frequently, the analyses have to be performed from challenging matrices, such as urine, plasma, or serum.

In a comparison of ESI, APCI, and APPI for a large group of drug standards and candidates with diverse structures and polarities, APPI was shown to be an excellent general ionization method (Cai et al. 2005). Of over 201 test compounds, APPI was able to detect 98%, whereas both APCI and ESI detected only 91%. In addition, although neutral compounds in ESI and APCI could only be ionized by modifying the mobile phase with buffers to produce ammonium or acetate adducts, APPI produced $M^{+\cdot}$ ions of the neutrals without modification of the mobile phase. Also, the general ionization efficiency in APPI was higher. APPI was especially superior in the analysis of less polar compounds, most of which could not be ionized by ESI or APCI. The authors concluded that the wider coverage of compound detection by APPI makes it attractive for high-throughput compound identification, pharmaceutical property profiling analysis, and bioanalytical and metabolite ID work.

Mascher et al. used LC-APPI-MS for the quantitative analysis of ciclesonide, ciclesonide-M1-metabolite, and fluticasone propionate, which are fairly apolar corticosteroids and as such well suited to APPI (Mascher et al. 2008). The analyses were performed from human serum. The analytes were detected as acetate adducts in negative ion mode, and the sensitivity was reported to be four times

Table 8.2 Examples of APPI and LPPI applications.

Ionization techniques	Sample introduction	Analytes/purpose of the study	Sample matrix	Dopant in APPI	References
Drugs, pharmaceuticals, and metabolites					
ESI, APCI, APPI (+/−)	LC	Pharmaceuticals and drug discovery compounds		Toluene	Cai et al. (2005)
APPI (+)	Chiral NP-LC	Omeprazole and its main metabolites	Human serum	None	Martens-Lobenhoffer et al. (2007)
APPI (−)	LC	Ciclesonide, ciclesonide-1-metabolite, and fluticasone propionate	Human serum	Acetone	Mascher et al. (2008)
ESI, APCI, APPI (+)	LC	Apomorphine, dobutamine, and entacapone phase II metabolites	Rat urine and hepatocytes and human microsomes	Toluene	Keski-Hynnilä et al. (2002)
ESI, **APPI** (+)	LC	Venlafaxine and its major metabolite *O*-desmethylvenlafaxine	Human plasma	Toluene	Theron et al. (2007)
ESI, APPI (+/−)	LC	Drug cocktail (drug permeation studies)	Caco-2 cells	Toluene	Hakala et al. (2003)
APPI	LC	Budesonide (bioavailability)	Human plasma	Toluene	do Carmo Borges et al. (2011)
APPI (+)	LC	Clozapine and lonafarnib (pharmacokinetics) and drug discovery products	Plasma	Toluene	Hsieh et al. (2003)
APCI, APPI (+)	LC	Lanosterol and its major metabolite FF-MAS (inhibition assay of CYP51 by azoles)		Toluene	Trösken et al. (2004)
Toxicology/Forensic					
ESI, APCI, APPI (+)	Isotope dilution UHPLC	Illicit drugs and their metabolites	Oral fluid	None, toluene	Wang et al. (2010)
APPI (+)	LC	Alprazolam, flunitrazepam, and their main metabolites	Hemolyzed blood	Acetone	Marchi et al. (2009b)
DAPPI	Solid samples	Illicit drugs and steroids	Plant, resin, tablet, paper, oil	Toluene, acetone	Kauppila et al. (2008, 2011)

(*continued*)

Table 8.2 (Continued)

Ionization techniques	Sample introduction	Analytes/purpose of the study	Sample matrix	Dopant in APPI	References
Steroids					
ESI, APCI, APPI (+)	LC	Anabolic steroids	Urine	**Toluene,** acetone	Leinonen et al. (2002)
μAPPI (+)	GC	Anabolic steroids (trimethylsilyl derivatives)	Urine	Chloro-benzene	Hintikka et al. (2013)
ESI, APCI, **APPI** (+/−)	LC	Estradiol	Human serum and endometrial tissue	Toluene	Keski-Rahkonen et al. (2013)
ESI, APCI, **APPI** (+)	Direct infusion	Natural and synthetic corticosteroids		**None**, toluene, acetone	Greig et al. (2003)
APPI (+)	LC	Steroid profiles (immunoassay)	Serum and plasma	Toluene	Guo et al. (2009)
ESI, APPI (+)	LC	Cholesterol and cholesterol esters	Mouse plasma and tissue	Acetone	Castro-Perez et al. (2011)
Other endogenic compounds					
ESI, APCI, **APPI (+)**	LC	Free fatty acids and their esters, monoglyceride, diglyceride, triglyceride	E.g. fish oils	None	Cai and Syage (2006b)
ESI, APCI, APPI (+/−)	LC	Lipidomics	Leishmania donovani promastig-otes	Toluene, acetone	Imbert et al. (2012)
APPI (+/−)	RP-LC	Sphingolipids	Stratum corneum	Toluene	Muñoz-Garcia et al. (2006)
APPI (+)	LC	Vitamin D forms and metabolites	Serum	None, toluene, toluene/ acetone	Adamec et al. (2011)
ESI, APPI, MALDI (+)	flow-injection analysis (FIA) and RP-LC	Large apolar peptides		None, toluene, acetone	Delobel et al. (2003)
SR-APPI (+)	FIA	Protein palmitoylation		Toluene	Bagag et al. (2012)
ESI, APCI, APPI (+/−)	RR-LC	Endogenic metabolites	Human plasma	Acetone	Tian et al. (2013)

(*continued*)

Table 8.2 (Continued)

Ionization techniques	Sample introduction	Analytes/purpose of the study	Sample matrix	Dopant in APPI	References
Plant research					
ESI, APCI, and APPI (+/−)	LC	Flavonoids		None, toluene, tetrahydrofuran (THF)	Rauha et al. (2001)
APPI (−)	LC	Stilbenes and derivatives	Downy mildew-infected grapevine	Acetone	Jean-Denis et al. (2006)
ESI, APCI, **APPI** (+/−)	LC	Pentacyclic triterpenes	Tree extracts	Toluene, acetone, anisole	Rhourri-Frih et al. (2009)
Environmental analysis					
ESI, APCI, **APPI** (−)	LC	Fullerene aggregates	Environmental waters	**Toluene** (solvent), acetone, isooctane, heptanes, THF, benzene, pyridine, anisole	Chen and Ding (2012)
APPI (+)	LC	PAHs	Sediment	Toluene	Moriwaki et al. (2004)
APP (+)	UPLC	PAHs		Chlorobenzene	Cai et al. (2009a)
APPI (+)	UPLC	PAHs	Oyster	Chlorobenzene	Cai et al. (2012)
DAPPI (+)	Solid sample	PAHs	Spiked soil pellets	Toluene	Luosujärvi et al. (2010b)
ESI, APCI, **APPI** (+)	Direct infusion	PAHs and oxy-PAHs	Environmental and petroleum samples	Hexane (solvent)	Ghislain et al. (2012)
APPI (+)	LC	Azaaranes and azaarones	Dutch river sediments	Toluene	Brulik et al. (2013)
ESI, APCI, **APPI** (+/−)	RP- and **NP**-LC	Dinitropyrene and aminonitropyrene	Rat plasma	Toluene	Straube et al. (2004)
μAPCI, μAPPI (−)	GC	PCBs	Soil extract	Toluene	Luosujärvi et al. (2008)
APPI (+)	LC	DDT		Toluene	Moriwaki and Miyakoda (2007)

(*continued*)

Table 8.2 (Continued)

Ionization techniques	Sample introduction	Analytes/purpose of the study	Sample matrix	Dopant in APPI	References
APPI (−)	LC	PBDEs	House dust	Toluene	Lagalante and Oswald (2008)
APPI (−)	LC	PBDE	Sediments and earthworms	Isooctane	Jhong and Ding (2007)
APPI (−)	LC	Brominated flame retardants	Environmental water and industrial effluents	Acetone (solvent)	Bacaloni et al. (2009)
APPI (−)	LC	TBBPA derivative flame retardants	Great lakes herring gull eggs	Acetone	Letcher and Chu (2010)
APPI (−)	LC	Brominated flame retardants	Seepage water, sewage wastewater, sewage sludge, and sediments	Acetone	Nyholm et al. (2013)
APPI (−)	LC	Achiral and chiral methylsulfonyl PCB metabolites	Animal tissue extracts	Toluene	Cooper et al. (2012)
ESI, APCI, APPI, APCI/APPI (+/−)	UPLC	Estrogenic chemicals	Environmental waters	Toluene	Lien et al. (2009)
ESI (−), APPI (+)	LC	Steroid hormone profiles	Urban and tidal rivers	Toluene	Yamamoto et al. (2006)
APPI (+)	LC	Testosterone, estradiol, ethinyl estradiol, and 11-ketotestosterone	Fathead minnow fish plasma	Toluene	Zhang et al. (2009)
APPI (−)	LC	Fluorotelomer alcohols and perfluorinates sulfonamides	Biota samples	Toluene	Chu and Letcher (2008)
Heated ESI, APCI, **APPI** (+/−)	LC	Pharmaceuticals and personal care products, steroid hormones, sterols	Aqueous matrices	Toluene, acetone, anisole, chloro-benzene	Wang and Gardinali (2012)
LPPI	Direct syringe/ capillary injection	Chemical weapons	Water	none	Syage et al. (2006)
ESI, **APCI**, APPI (+)	LC	Aliphatic and aromatic amines	Atmospheric particles	n.a.	Ruiz-Jiménez et al. (2012)

(*continued*)

Table 8.2 (Continued)

Ionization techniques	Sample introduction	Analytes/purpose of the study	Sample matrix	Dopant in APPI	References
Oil and petrol					
ESI-, APPI-FTICR (+/−)	Direct infusion	Molecular characterization	Oil sand process water	Toluene	Barrow et al. (2010)
APPI-FTICR	Direct infusion	SARA-fractionation: saturates, aromatics, resins, and asphaltenes	Crude oil	Toluene (solvent)	Cho et al. (2012)
APPI-FTICR (+/−)	Direct infusion		Crude oil	Toluene (solvent)	Purcell et al. (2006)
ESI-, APPI-FTICR (+)	Direct infusion	Sulfur speciation	Petroleum	Toluene (solvent)	Purcell et al. (2007a)
ESI (+/−), APPI (+)	Direct infusion	Speciation of nitrogen containing aromatics	Petroleum	Toluene (solvent)	Purcell et al. (2007b)
APPI (+)	Direct infusion		Bitumen	Toluene	Tachon et al. (2011)
Food analysis					
APPI, APCI (+)	LC	Carbamate pesticide residues	Vegetables and fruits	Acetone	Takino et al. (2004)
APCI, APPI (−)	LC	Chloramphenicol residues	Fish meats	Acetone	Takino et al. (2003)
μAPPI (+)	capLC	Carbamate pesticides	Tomato	**Toluene**, acetone and anisole	Kruve et al. (2011)
ESI (+/−), APPI (+)	RP- and NP-LC	Aflatoxin	Cow milk	Toluene, acetone, anisole (solvent)	Cavaliere et al. (2006)
APPI (+)	LC	PAHs	Schrimps	Toluene	Smoker et al. (2010)
APP (+)	UHPLC	PAHs	Oysters	Chloro-benzene	Cai et al. (2012)
ESI, APPI (+)	FIA	Anthocyanin profiles (fingerprinting)	Wine	Toluene	Gómez-Ariza et al. (2006)

Bold formatting is used for better visibility of the superscript period, which otherwise would be very small. It signals an odd electron in the molecular species, and is therefore not without meaning.

higher than that with ESI or APCI. The dopant (acetone) was added to the mobile phase, which was reported to cause much less noise than delivering the dopant from a separate syringe pump via the auxiliary gas line as usually. The method was applied to determine the pharmacokinetic profiles of the three analytes in serum after inhalation of ciclesonide and fluticasone propionate.

Theron et al. used LC-APPI-MS to analyze venlafaxine and its major metabolite *O*-desmethylvenlafaxine from human plasma (Theron et al. 2007). APPI was chosen as the ionization method because the linear range with ESI was insufficiently low, and ion suppression by coeluting compounds in the biological matrix was observed. In both ESI and APPI, elevated concentrations of venlafaxine were observed to cause suppression of ionization, but the effect was much less pronounced in APPI, which could cover a range of 5–1250 ng/ml, whereas ESI could only cover 5–625 ng/ml. Elimination of the sample suppression by using APPI instead of ESI was suggested to save valuable method development time compared to chromatographic alterations or extensive sample cleanup procedures. The LC-APPI-MS method was used to monitor the changes in concentration of venlafaxine and its major metabolite in plasma after the oral administration of venlafaxine.

Chiral LC-APPI-MS was used in a study of the metabolic breakdown products of omeprazole and its main metabolites in human serum (Martens-Lobenhoffer et al. 2007). APPI was chosen because of its good compatibility with normal-phase (NP) solvents that are required for the chiral separation of the omeprazole metabolites. 2-Propanol-hexane mobile phase used in the assay has self-doping properties, which made the addition of dopants unnecessary. The signals for the omeprazole metabolites were reported to be approximately 1.5 times the signal obtained with APCI. Matrix effect was studied by injecting human serum samples to a constant analyte flow, but no obvious matrix effect was observed. The validated quantitative method was used to study the predictability of omeprazole therapy efficacy in gastroesophageal reflux disease with respect to CYP3A4 and CYP2C19 activity.

Although APPI has been found to be well suited to analysis of phase I drug metabolites, phase II metabolites are more fragile, and therefore, in their case, ESI as a softer ionization techniques is usually the method of choice. This was shown by Keski-Hynnilä et al., who compared ESI, APCI, and APPI for the LC-MS identification of apomorphine, dobutamine, and entacapone phase II metabolites from biological samples (Keski-Hynnilä et al. 2002). As a more gentle ionization method, ESI was able to identify the highest number of conjugates (22) compared to APCI or APPI (12 and 14 conjugates, respectively), including fragile glucuronide and sulfate conjugates.

Wang et al. compared ESI, APCI, and APPI for the quantitative analysis of 17 illicit drugs and their metabolites from oral fluid using isotope dilution and ultra-high pressure liquid chromatography-mass spectrometry (UHPLC-MS; Wang et al. 2010). The studied compounds were four opiates and their metabolites, five amphetamines, flunitrazepam and its two metabolites, and cocaine and its four metabolites. In case of APPI, direct and dopant-assisted APPI were compared, and direct APPI was found more efficient. In a study by Marchi et al., LC-APPI-MS was used for the quantification of the commonly consumed benzodiazepines alprazolam and flunitrazepam and their main metabolites in authentic toxicological hemolyzed blood samples (Marchi et al. 2009b). APPI was chosen over ESI because of its wider dynamic range and lower sensitivity to signal alteration with biological samples.

8.4.2 Steroids

Steroids are challenging compounds in LC-MS because of their neutral nature and the lack of readily ionizable groups. Because of their low polarity, ESI does not necessarily give the desired sensitivity; in addition, it often suffers from matrix suppression effects because steroids typically are to be analyzed from biological fluids (Keski-Rahkonen et al. 2013). APPI has become a popular ionization method for the analysis of synthetic and endogenic steroids from biological matrices, both by LC-MS and by GC-MS. Leinonen et al. compared ESI, APCI, and APPI for the LC-MS analysis of the free anabolic steroid fraction in human urine samples (Leinonen et al. 2002). All methods were found suitable, but ESI gave the lowest limits of detection. None of the three methods showed disturbance by the matrix, but APPI required column-switching when batches of urine samples were analyzed. APPI and APCI were observed to be more energetic than ESI. Keski-Rahkonen et al. compared ESI, APCI, and APPI in both polarities for the analysis of estradiol from female serum and endometrial tissue (Keski-Rahkonen et al. 2013). Negative ion APPI gave the lowest quantification limits and was found to have the most potential for estradiol analysis. ESI was found to be highly susceptible to ion suppression when endogenous serum and tissue homogenate extracts were analyzed, whereas APCI and APPI were largely unaffected by the sample matrix. Greig et al. compared ESI, APCI, and APPI for the analysis of natural and synthetic corticosteroids (Greig et al. 2003). APPI was shown to be more sensitive than ESI or APCI and requires less heat for desolvation, resulting in less thermal degradation of the analytes and higher S/N than APCI. The introduction of dopants in this study was shown to be advantageous only to some of the compounds, while for others, they completely suppressed the analyte signals.

8.4.3 Other Endogenic Compounds

Besides steroids, other endogenic compounds, including different lipid groups, neurotransmitters, endogenic metabolites, and even large peptides and proteins, have also been widely analyzed by APPI. Cai et al. compared ESI, APCI, and APPI in the analysis of free fatty acids and their esters (Cai and Syage 2006b). Because of the low solubility of the lipids in aqueous RP-LC solvents, NP-LC was used. The linear range in APPI and APCI was four to five decades, whereas in ESI, the linearity was dramatically reduced because of the use of mobile-phase modifiers that were required to reach the required sensitivity range. APPI was observed to be two to four times more sensitive than APCI and also more sensitive than ESI without the mobile-phase modifiers. The NP-LC-MS method utilizing APPI was successfully applied to the analysis of fish oils. In another study by Tian et al., ESI, APCI, and APPI were compared for the analysis of a wide range of endogenic metabolites in plasma (Tian et al. 2013). Each technique was found to have its own advantage over the other two techniques for certain types of metabolites. ESI was found to be the best method for detecting glycerophosphocholines, glycerophosphoethanolamines, acyl carnitines, bile acids, and sulfates; APCI for cyclic alcohols, fatty acids, and linoleic acids; and APPI for steroids, sphingolipids, some amino acids, nucleosides, and purines.

Because APPI uses high temperature for the vaporization of the sample, it is best suited for small, easily vaporizable compounds. Large biomolecules are usually ionized by ESI, which does not require heating for the formation of gas-phase ions. However, there are some examples where APPI has been used for the successful analysis of proteins or peptides or other large biomolecules. For example, Delobel et al. used APPI for the analysis of large hydrophobic peptides (Delobel et al. 2003). Although the peptides could not be analyzed as intact molecules, their in-source fragmentation was observed to produce mainly B- or C-type fragments, which can be utilized for peptide sequencing (Bagag et al. 2012).

8.4.4 Plant Research

Many interesting plant compound groups, such as flavonoids, stilbenoids, and triterpenes, ionize efficiently in APCI and APPI (Rauha et al. 2001; Jean-Denis et al. 2006; Rhourri-Frih et al. 2009). In a study by Rauha et al., ESI, APCI, and APPI were compared for a group of flavonoids in positive and negative ion modes (Rauha et al. 2001). Negative ion ESI was found to give the best sensitivity for the acidic flavonoids. Rhourri-Frih et al. compared ESI, APCI, and APPI for the analysis of pentacyclic triterpenes (Rhourri-Frih et al. 2009). APCI and APPI were found to be better suited for the triterpenes than ESI: APPI was found to be the most sensitive ionization method in positive ion mode, whereas APCI showed the greatest sensitivity for acidic triterpenes in negative ion mode. Finally, LC-APPI-MS was utilized for the analysis of pentacyclic triterpenes from plane and birch bark tree extracts.

8.4.5 Environmental Monitoring

Many environmentally interesting compounds are neutral and nonpolar and therefore difficult to ionize by ESI. APPI has therefore found wide use in environmental monitoring applications. PAHs originate from combustion of fossil fuels and tend to gather in the environment and biosystems. Because many of them have been found carcinogenic, they are important targets in environmental and toxicological analysis. As completely nonpolar, PAHs are poorly ionized with ESI and APCI, but they give intense signals with APPI. APPI has therefore become the most important ionization method for PAHs in LC-MS, and it has been used for the analysis of PAHs from water, sediment and polluted soil, and oysters (Moriwaki et al. 2004; Cai et al. 2012; Ghislain et al. 2012). In a study by Straube et al., ESI, APCI and APPI, and NP- and RP-LC separation were tested for the analysis of dinitropyrenes (DNP), aminonitropyrenes (ANP), and diaminopyrenes, which can be used as biomarkers for monitoring human exposure to diesel engine emissions (Straube et al. 2004). With ESI, DNP could not be efficiently ionized and diaminopyrene showed mainly $M^{+\cdot}$ ions, whereas with APCI and APPI, ANP and diaminopyrene could be analyzed in positive ion mode as $[M+H]^+$ and DNP in negative ion mode as $M^{-\cdot}$. The best precision and sensitivity for ANP and DNP were obtained using NP-LC and APPI. NP-LC-APPI-MS was used for analysis of ANP and DNP in rat plasma after oral

ingestion of DNP, and it was shown that orally ingested DNP metabolized to ANP because only ANP was detected in the plasma at elevated levels.

Fullerenes are completely nonpolar compounds, which have recently found use in nanomaterial technology. Development of analysis methods for fullerenes from complex biological or environmental matrices has become increasingly important in their risk assessment. As completely nonpolar, they are difficult to ionize by ESI or APCI. However, fullerenes are highly conjugated molecules and as such have positive EAs and can be efficiently ionized by electron capture. Negative ion APPI has been tested for the analysis of fullerenes with better performance than with ESI or APCI (Li et al. 2012). Because fullerenes are likely to end up in the environment, they have been analyzed from environmentally important matrices, such as municipal influent and effluent, industrial wastewater, and surface waters (Chen and Ding 2012; Núñez et al. 2012).

Many synthetic halogenated compounds are toxic or carcinogenic and have a tendency to accumulate in the environment and in bioorganisms. Such compounds include polybrominated flame retardants, such as polybrominated diphenyl ethers (PBDEs) (Lagalante and Oswald 2008) and tetrabromobisphenol-A (Letcher and Chu 2010), PCBs (Luosujärvi et al. 2008), and dichlorodiphenyltrichloroethane (DDT) (Moriwaki and Miyakoda 2007). Because halogenated compounds have positive EAs, they can be efficiently ionized by NI-APPI. The studied matrices include soil, house dust, environmental waters, industrial effluents, sewage wastewater, sludge and sediments, earthworms, bird eggs, fish, and animal tissue (Jhong and Ding 2007; Lagalante and Oswald 2008; Luosujärvi et al. 2008; Bacaloni et al. 2009; Letcher and Chu 2010; Zhou et al. 2010; Cooper et al. 2012; Nyholm et al. 2013).

Feminizing chemicals that originate from human and animal medication and end up in environment have been observed to disrupt the endocrine systems of aquatic organisms and are thus a major environmental concern. These compounds include natural and synthetic steroids and pharmaceuticals (e.g. bisphenol A). Sensitive and specific methods for identification and controlling the levels of these chemicals in ecosystems are therefore highly important. Because APPI is well suited for analysis of steroid hormones, it has also been used in many studies, where steroid residues have been analyzed from environmental sources. Yamamoto et al. compared ESI and APPI for the LC-MS analysis of estrogens, androgens, and their conjugates from estuarine and river water samples (Yamamoto et al. 2006). APPI was found to be more sensitive toward the unconjugated steroids, whereas ESI was more sensitive toward the conjugated steroids. The methods were used to determine the steroid levels in Osaka City rivers and estuaries, and local accumulation of steroids in the waters was observed. In another study by Wang and Gardinali, ESI, APCI, and APPI were compared for the LC-MS analysis of pharmaceuticals, personal care products, steroids, and sterols from environmental waters (Wang and Gardinali 2012). APPI was found to be the most sensitive ionization method for the majority of analytes, which unlike ESI and APCI allowed even the detection of two difficult-to-ionize fecal related sterols, coprostan-3-ol and coprostan-3-one. Different results were obtained in a study by Garcia-Ac et al., who compared ESI, APCI, and APPI for the analysis of five target pharmaceuticals from municipal

wastewater (Garcia-Ac et al. 2011). In their study, ESI was found to be the most suitable ionization technique because, unlike APPI and APCI, it allowed the detection of all the target compounds and generated the highest peak areas and S/N ratios. APCI and APPI were reported to suffer from background interference by the matrix, which partly explains their poorer performance. Besides water, APPI has been used to study the accumulation of steroids in marine organisms, as in a study by Zhang et al., where sex steroids and their metabolites were studied from fathead minnow fish plasma (Zhang et al. 2009).

8.4.6 Oil Analysis

Characterization of hydrocarbons and other compounds from oil and related samples is important for quality control and method development of petroleum industry, as well as studying the effect of oil spills in wildlife. Oil samples are highly complicated, containing easily over 100 000 polar and nonpolar compounds, and they are typically analyzed by using high-resolution mass spectrometry, usually Fourier transform ion cyclotron resonance (FT-ICR), which can provide elemental formulas for the detected compounds. In most applications of this area, the sample is introduced via direct infusion, without chromatographic separation, but often the sample is fractioned according to the compound polarities and solubilities to saturates, aromatics, resins, and asphaltenes (SARA) before the MS analysis. The aim is usually to characterize the proportions of compounds containing certain elements, functional groups, or number of conjugations rather than to clarify the complete molecular structures of all the compounds. Because of its suitability for both polar and nonpolar compounds, APPI has been utilized for characterization of compound classes from crude oil, petroleum, bitumen, and oil sands processing water (Purcell et al. 2006, 2007a,b; Barrow et al. 2010; Tachon et al. 2011; Cho et al. 2012).

8.4.7 Food Analysis

APPI has also been used for analyses related to food safety, quality control, and health-effective food components, such as vitamins. Takino et al. compared APCI and APPI for the analysis of carbamate pesticide residues in vegetables and fruits (Takino et al. 2004). The spectra obtained with APPI were found to be simpler and thus more easily interpreted, with mainly $[M+H]^+$ ions and less fragmentation or adduct formation than in the spectra obtained with APCI. Similar S/N levels were obtained with both ionization methods. In the analysis of carbamates from vegetable and fruit samples, although the matrix was observed to cause alterations in the chromatogram, the appearance of additional peaks in the chromatogram did not interfere with the analyte ionization because the peaks were well separated. In another study by the same authors, chloramphenicol, which is an antibiotic with severe health effects that make it necessary to control its residual levels in human food, was analyzed from fish meat using APPI and APCI (Takino et al. 2003). APPI was found to be superior in terms of S/N, thanks to its lower chromatographic baseline. APPI was also less affected by injection of the fish meat extract than APCI. The long-term performance and possible contamination of

the APPI source was tested by repeated injections for two days, but the chloramphenicol signal intensities were observed to stay on the same level, giving no sign of contamination. In another study by Cavaliere et al., ESI and APPI were compared in the LC-MS analysis of aflatoxin M1 from cow milk (Cavaliere et al. 2006). For APPI, both RP- and NP-LC were tested. Similar results were obtained with RP- and NP-LC, but RP-LC was preferred because of the more harmful solvents in NP-LC. ESI showed lower LOD than APPI for the studied compounds, but because of higher possible injection volume and minor matrix suppression effect, better method quantification limits (MQLs) could be achieved with APPI. The MQL with both ESI and APPI were four times lower than the maximum allowed level of aflatoxin M1 in milk products.

8.5 Conclusions

Photoionization is finding its way into a greater diversity of applications. Although APPI was originally developed and adopted as a key ionization option for LC-MS, it is now finding its way into a greater diversity of applications, such as ambient analysis, GC-MS, and direct injection analysis. Compared to other popular ionization sources, such as ESI and APCI, APPI has the benefits of ionizing a greater range of compounds, particularly nonpolar compounds, a wider linear range, and lower susceptibility toward complex matrix components. The unique advantages and benefits of photoionization are well established, and further advances are limited only by the creativity and ingenuity of analytical chemists seeking better solutions to their applications.

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