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REVIEW

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From fundamentals to applications: recent developments in atmospheric pressure photoionization mass spectrometry

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Abstract Only five years after the first publication on atmospheric pressure photoionization (APPI), this technique has evolved rapidly as a very useful complement to established ionization techniques for liquid chromatography/mass spectrometry (LC/MS). This is reflected in a rapidly increasing number of publications in this field. On the one hand, thorough studies into the photoionization mechanism have provided deep insights into the roles and influences of the solvent, the dopant and other additives. On the other hand, a large number of new and attractive applications have recently been introduced. New instrumental developments have resulted in combined APPI/ESI (PAESI) and APPI/APCI sources and a microfabricated APPI source. In this review, the most important developments within the field are summarized, focusing in particular on the applications of the technique.

Keywords Photoionization · Atmospheric pressure photoionization · Liquid chromatography/mass spectrometry · Photoionization mechanism

Introduction

Atmospheric pressure photoionization (APPI), a relatively novel ionization technique that is used with liquid chromatography and mass spectrometry, is evolving rapidly as an important complement to other atmospheric pressure ionization (API) techniques like electrospray ionization (ESI) and atmospheric pressure chemical ionization (APCI). The rapidly growing number of publications in this field clearly demonstrates the high expectations that are now associated with this method (Fig. 1). Several general LC/MS review articles have already covered some areas of APPI development [1–7]; the first dedicated review was published by Raffaelli and Saba [8] in 2003. The current article therefore evaluates the results and findings from works published from 2003 up to now, which have not been reviewed previously.

API techniques enable both the LC effluent to be transferred into the gas phase and the analytes to be ionized. Due to different ionization systems, classical ESI and APCI sources cover different analyte polarity ranges. In ESI, where the ionization is mainly based on acid-base chemistry in solution, the LC eluent is sprayed from a capillary tip by means of a high voltage and rapid but gentle desolvation. It is suitable for ionizing polar compounds with masses of up to ~100,000 Da. In APCI, the analyte solution is introduced into a heated pneumatic nebulizer from which it is sprayed over a corona discharge needle that induces the ionization. The ionization conditions in APCI are considered to be somewhat "harder" than those in ESI [9]. It is therefore more suitable for less polar analytes, but acid-base reactions are still the most frequently observed ionization mechanism.

APPI, introduced by Bruins et al. in 2000 [10], is a complement to ESI and APCI and has been developed to broaden the range of ionizable analytes at atmospheric pressure. Bruins et al. however, stated in their initial paper [10] that "the range of compounds that can be efficiently ionized by APPI closely follows that of APCI" and that "the APPI source may find utility in many areas of application where the corona discharge APCI method is presently employed". However, certain groups of low- and nonpolar compounds, like polycyclic aromatic hydrocarbons [11], are indeed only made amenable to LC/MS analysis by applying photoionization.

The technical set-up for an APPI source is closely related to that of an APCI source, consisting of a heated nebulizer for spraying and partially desolvating the eluent and a VUV lamp that induces the ionization instead of the corona needle used in APCI. Two fundamentally different APPI sources are currently available. Built by an in-house universal machine shop, the first version of the APPI source, depicted in Fig. 2 was introduced by Bruins et al. in 2000

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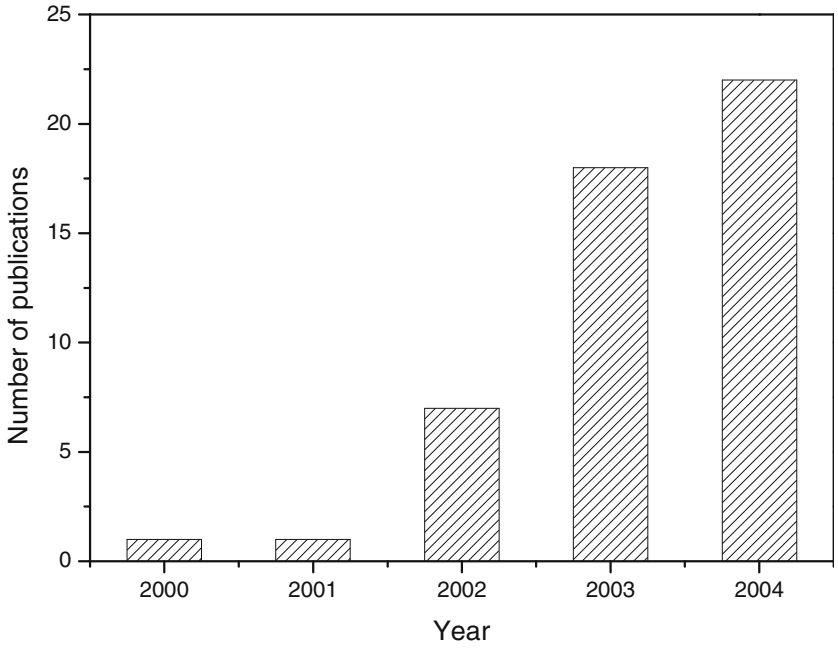


Fig. 1 Number of articles published in the literature (up to 2004) that are dedicated to APPI–MS

[10]. They used a closed axial source design to enhance dopant-assisted ion-molecule reactions. Here, the solvent stream is still in the heated nebulizer while being irradiated by the UV lamp. Therefore, the ions enter the MS sampling capillary linearly. This interface is currently available from Applied Biosystems/MDS Sciex as PhotoSpray. The second commercially available APPI source resembles the APCI set-up even more, as it is an open orthogonal source with the VUV lamp located where the corona needle is positioned in the APCI source (Fig. 3). This PhotoMate was developed by Syagen Technology for use with the mass spectrometers from many manufacturers, and is designed to enhance the direct use of APPI without a dopant (see below). This may be due to the fact that the ionization takes place in the spray chamber, where there is less probability of dopant–ion interactions.

For further information on the development and technical aspects of APPI sources, the reader is referred to [8].

Properties and mechanisms of APPI

It is apparent, from its range of applications, that an APPI source can handle a wide variety of compounds under very different LC conditions, making it the method of choice for the life sciences and biopharmaceutical industries, where

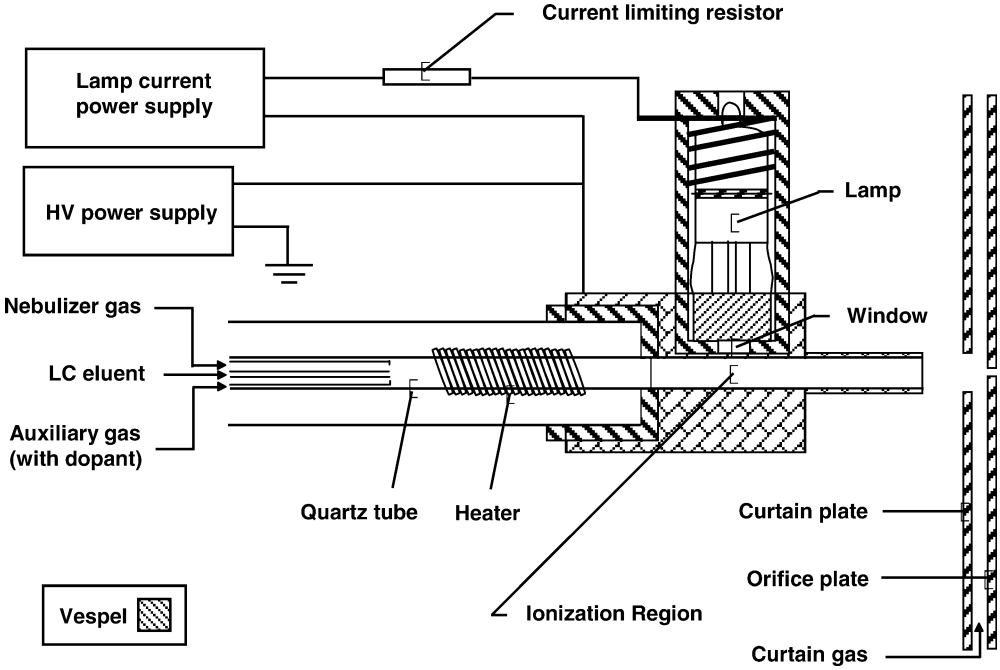


Fig. 2 Schematic drawing of the APPI source developed by Bruins and coworkers. Reproduced with permission from [10]. Copyright (2000) American Chemical Society

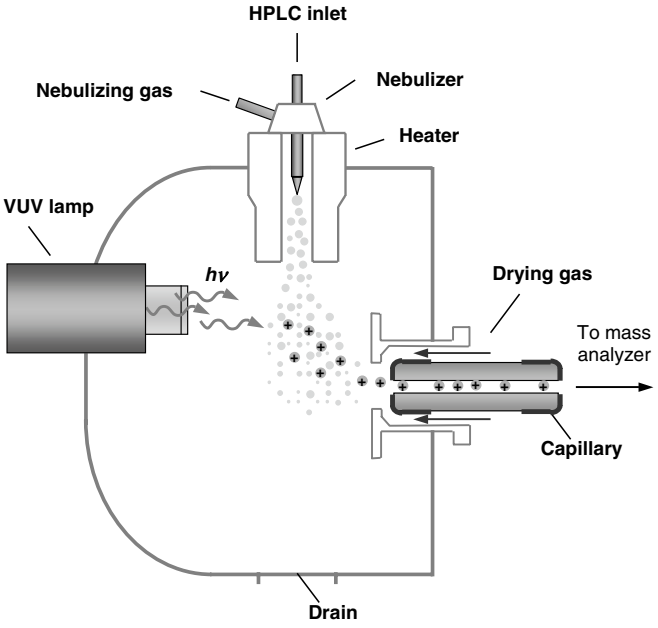


Fig. 3 Schematic drawing of the orthogonal, open-source type APPI interface

new products broaden the existing polarity range of target and/or product compounds.

As Syage et al. conclude from an investigation into the general properties of APPI [12], direct APPI has comparable sensitivity to APCI at high flow rates and superior sensitivity at low flow rates. It appears to be less susceptible to matrix-induced ion suppression and buffer-created chemical interference [12–19] than both APCI and ESI (Fig. 4), since sample handling is much easier as no intensive clean-up is required [20–22]. Furthermore, contamination of the APPI source does not significantly affect the ionization, which is in direct contrast to APCI [16]. These properties create excellent possibilities for high-throughput analysis [4, 6, 22, 23].

APPI has a large dynamic range (typically 3–4 orders of magnitude) [15–17, 22–24], which is comparable to APCI but larger than for ESI, and excellent sensitivity [21, 22, 24–26], extending down to low flow rates [12, 15]. Studies by Kostiainen et al. [27] have shown that APPI in the negative ion mode has better sensitivity than negative ion APCI, but this is dependent on the individual analyte. Furthermore, analyte sensitivity also depends on the choice of APPI source, as the ionization yield is influenced by the source geometry.

The mechanism

Positive ion formation

Photoirradiation does induce a couple of reactions prior to and simultaneously with the actual ionization of the analyte; initial explorations of these have already been reviewed by Raffaelli and Saba [8]. However, to obtain a complete overview of the mechanisms on which further studies are based, these findings will be briefly summarized.

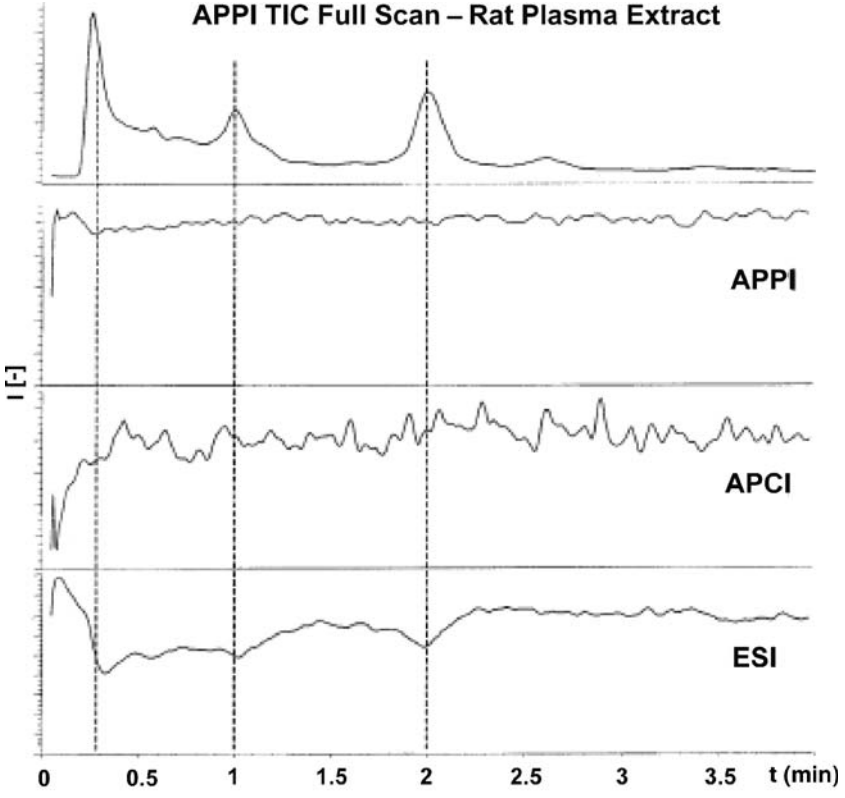


Fig. 4 Determination of ion suppression susceptibility for APPI, APCI and ESI by post-column addition of fluphenazine to a LC separation of rat plasma. Reprinted with permission from [12]. Copyright (2004) American Chemical Society

The basic principle of atmospheric pressure photoionization is the formation of a molecular radical ion, caused by the absorption of a photon by a molecule, followed by ejection of an electron (1). This is possible if the irradiating photon energy ($h\nu$) exceeds the ionization potential (IP) of the molecule. The statistical probability for direct ionization of an analyte molecule is low, which is especially valid for the closed axial PhotoSpray source, in which the 10 eV vacuum-UV photons have a short penetration depth in a dense mixture of gases and vapors at atmospheric pressure. Therefore, a large concentration of a substance that is easily ionized (the dopant) may be added to significantly increase the number of ions via initial photoionization of the dopant (2) and subsequent charge exchange with the analyte (3), provided that the electron affinity (EA) of the analyte is higher than the EA of the dopant. Furthermore, if the proton affinity (PA) of the analyte is higher than the PA of the deprotonated dopant ion, solvent molecules or clusters can act as intermediates between the dopant ion and the analyte by abstracting a proton from the dopant ion (4) and passing it on to the analyte (5).

$$M + h\nu \rightarrow M^{\bullet +} \quad \text{(general direct photoionization)} \tag{1}$$

$$D + h\nu \rightarrow D^{\bullet +} \tag{2}$$

$$D^{\bullet +} + AB \rightarrow D + AB^{\bullet +} \tag{3}$$

$$D^{\bullet +} + S \rightarrow [D - H]^{\bullet} + [S + H]^{+} \tag{4}$$

$$[S + H]^{+} + AB \rightarrow S + [AB + H]^{+} \tag{5}$$

Unexpected solvent behavior was observed by Traldi et al. for acetonitrile, where clusters of this solvent were found to be photoionized (6a,b), and so the solvent participates in the analyte ionization [28].

$$H_3C{-}C{\equiv}N + h\nu \rightarrow H_2C{=}C{=}NH^{\bullet +} \tag{6a}$$

$$H_2C{=}C{=}NH^{\bullet +} + H_3C{-}C{\equiv}N \rightarrow H_2C{=}C{=}NH_2^{+} + H_2C^{\bullet}{-}C{\equiv}N \tag{6b}$$

These findings were further supported by semi-empirical calculations [29].

Except for the latter findings, which have only been examined for acetonitrile, none of the abovementioned mechanisms provide an explanation for the formation of $[M+H]^+$ in dopant-free (or direct) APPI. Therefore, Syage set up a series of experiments to explore this [30], using a low-pressure PI source in order to be able to examine ion-molecule chemistry more systematically than is possible with an APPI source. The following observations were made:

a) For the headspace ionization of M in air, $M^{\bullet +}$ is formed almost exclusively
b) In the presence of protic solvents, $[M+H]^+$ has a significant relative abundance, which is not the case in aprotic solvents
c) Pressure induces equilibrium oscillations in the abundance of $[M+H]^+$ and $M^{\bullet +}$
d) The ratio $[M+H]^+/M^{\bullet +}$ correlates with the reaction length in the photoionization source

These observations provided evidence for the following reactions that form the mechanism of $[M+H]^+$ formation:

$$M + h\nu \rightarrow M^{\bullet +} \tag{7}$$

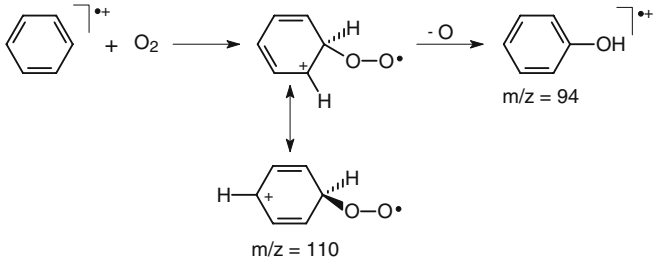
$$M^{\bullet +} + S \rightarrow [M + H]^{+} + [S - H]^{\bullet} \tag{8}$$

A simple thermodynamical model was drawn up, based on Eq. (8), that led to formula (9) for the enthalpy of hydrogen abstraction (ΔH):

$$\Delta H = IE(H) - IE(M) - PA(M) - D_{\mathrm{H}}(S) \tag{9}$$

where D_{H} is the H-atom bond dissociation energy. Predictions from the model agree qualitatively with experimental results for the ratio of the $[M+H]^+$ yield to the analyte concentration. However, the present form of this model is only elementary, and explains neither the observation of a stronger influence of PA than ΔH on the $[M+H]^+$ yield nor the efficiency of $[M+H]^+$ formation in the presence of water, which has a relatively high H-atom bond energy, indicating the importance of kinetics.

For the case of dopant-assisted photoionization, toluene and benzene usually lead to analyte ions via charge exchange reactions (3) and (4). However, Traldi et al. observed some protonation reactions as well and started investigating the underlying mechanism of this unexpected behavior [31]. Thorough examination and comparison of the spectra from the headspace benzene vapor and the nebulized benzene obtained with the open source type APPI interface confirmed that the initial photoionization product is the molecular benzene ion (m/z=78). At high partial benzene pressure, the benzene ion is suppressed and other ionization products are observed, including a phenol ion (m/z=94). Further investigations excluded water and proved the involvement of molecular oxygen in the formation of this species, as depicted in Scheme 1. Although some of the other ions formed also can act as effective protonating agents, the most important contribution to analyte ionization from proton transfer comes from the phenol species, suggesting effective ionization when using phenol in dopant-assisted APPI.



Scheme 1 Proposed mechanism for the formation of the toluene radical cation by reaction of the benzene radical cation with molecular oxygen in APPI. Reproduced from [31] with permission. Copyright (2003) John Wiley and Sons Ltd

Negative ion formation

Fortunately, the use of APPI is not limited to the positive ion mode. Negative ion APPI-MS has been thoroughly investigated by the groups of Kostiainen and Bruins [25]. In analogy to the mechanisms in the positive ion mode, ionization in negative ion APPI, assumed to be initiated by thermal electrons formed during the photoionization of toluene (2) [32] and subsequent solvent and oxygen reactions (10, 11), is achieved via proton transfer (12, 13a,b) and charge exchange (14), but additionally also via (dissociative) electron capture (15) and substitution reactions (16) [27].

$$S + e^- \rightarrow S^{\bullet -} \quad (10)$$

$$O_2 + e^- \rightarrow O_2^{\bullet -} \quad (11)$$

$$M + O_2^{\bullet -} \rightarrow [M - H]^- + HO_2^{\bullet} \quad (12)$$

$$S + O_2^{\bullet -} \rightarrow [S - H]^- + HO_2^{\bullet} \quad (13a)$$

$$M + [S - H]^- \rightarrow [M - H]^- + S \quad (13b)$$

$$M + O_2^{\bullet -} \rightarrow M^{\bullet -} + O_2 \quad (14)$$

$$M + e^- \rightarrow M^{\bullet -} \quad (15)$$

$$M + O_2^{\bullet -} \rightarrow [M - X + O]^- + OX^{\bullet} (X = H, Cl, NO_2) \quad (16)$$

Analytes possessing gas-phase acidity predominantly form deprotonated ions with additional in-source fragmentation and solvent adduction, whereas compounds of positive electron affinity (EA) tend to form molecular anions or substitution products instead.

Formation of the latter does not only apply for substitution of H, Cl or NO_2, as stated in Eq. 16, but also for other halogens like Br. The group of Traldi also experimented with the formation of negative ions, for which they used the open APPI source (PhotoMate) [33]. They state that the formation of negative ions can occur mainly by three different mechanisms: electron capture, dissociative electron capture and ion-pair production after electron capture. Slow electrons must be present in all cases, which can be ascribed to the direct photoionization of either analyte or dopant. In contrast to the findings of Kostiainen et al. [27], they argue that dissociative electron capture is the mechanism for the formation of both $[M-H]^-$ and $[M-Br+O]^-$ of tetrabromobisphenol. For the latter species, this occurs due to the initial formation of $[M-Br]^-$, which then immediately reacts with oxygen molecules present in the APPI source. However, it seems improbable that such a high yield of electron capture products can be obtained with such a low density of slow electrons produced by positive ion formation. Indeed, the observed high abundance of slow electrons must be ascribed to photoelectron emission from the metallic surface of the metal end plate (Fig. 3) at the MS entrance, for which evidence is provided. As they state and show [33], the stainless steel end plate is just in front of the krypton lamp and most of the lamp power is focused on this surface. However, this does not coincide with the real source design. As drawn in Fig. 3, the lamp points (almost completely) above the metal shield and so should not produce many photoelectrons. Furthermore, if so many photoelectrons are produced in the APPI source, compounds that are prone to undergo electron capture ionization in negative APCI should also be ionized via the same electron capture mechanism in negative APPI. However, unpublished experiments performed by the authors using the same type of APPI source proved otherwise for N-methyl-2,4-dinitrophenylhydrazine (MDNPH) and 4-methylhydrazino-7-nitro-2,1,3-benzoxadiazole (MNBDH) derivatives, as well as for 2,4-dinitrophenylazide (DNPA) [34]. These methylhydrazine derivatives do not have acidic protons to efficiently form $[M-H]^-$, but have sufficient electron affinity to capture electrons in negative APCI and subsequently dissociate at the N–N bond [35, 36]. The respective product ions are not observed (or are, but only to a very low extent) in negative APPI (Figs. 5, 6). Therefore, arguments for the mechanism of negative ion formation in APPI as described by Kostiainen et al. [27] appear to be more likely than the method described by Traldi and coworkers [33]. Reactions 12 or 13 would then lead to the formation of $[M-H]^-$ (with the intermediate formation of cresol, as described by the group of Traldi [31]), with reaction 16 being responsible for the observation of $[M-Br+O]^-$.

Nakahara et al. published several LC/APPI-MS applications using the PhotoMate in the negative ion mode and discussed their observations on the ionization mechanism and efficiency [15–18]. First, as the APPI source is essentially field-free (no voltages on the spray capillary or discharge needle), the voltage on the capillary inlet can be optimized independently. An optimum ion transfer was

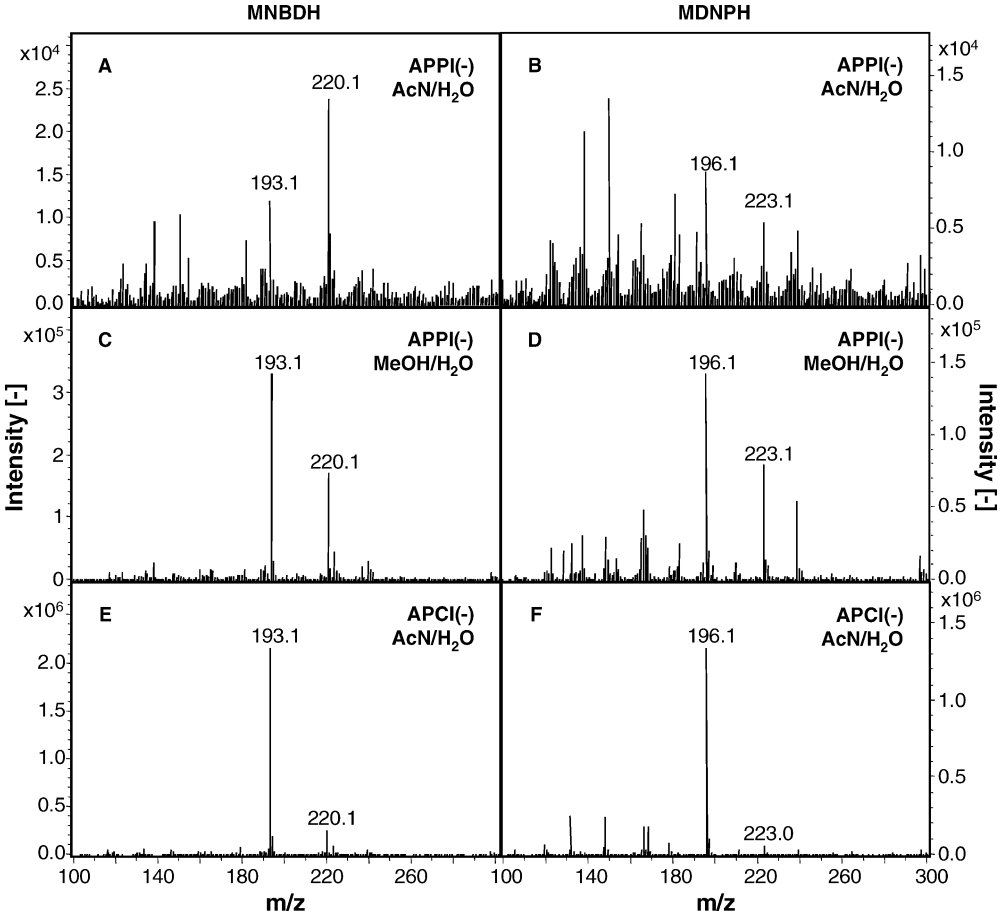


Fig. 5A–F MS spectra of MNBDH and MDNPH in **A**, **B**) acetonitrile/water and **C**, **D**) methanol/water, obtained with negative ion APPI, and in **E**, **F**) acetonitrile/water, obtained with negative ion APCI

observed for capillary voltages of 1000–1500 V, with a strong signal decrease at higher voltages. However, when using a dopant (acetone), this signal decrease was not observed; instead a rather slight signal enhancement was sometimes seen [15, 16]. This gave rise to the statement that "acetone as the dopant could generate enough electrons to improve the ionization efficiency and the excess amount of electrons could compensate for signal decrease

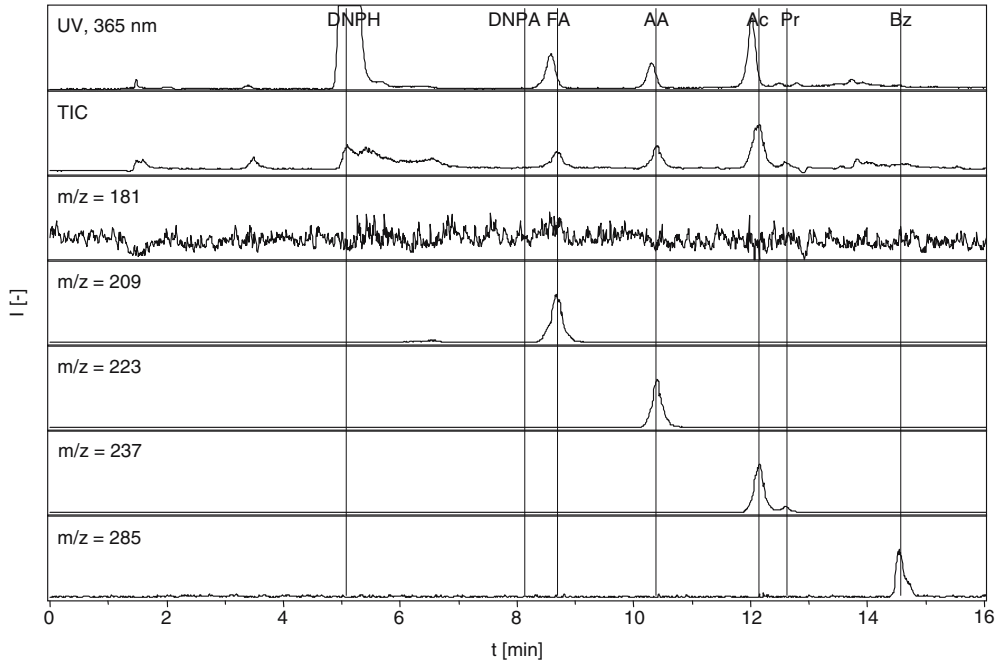


Fig. 6 LC/APPI-MS chromatograms of car exhaust, showing DNPH derivatives of formaldehyde (FA), acetaldehyde (AA), propanal (Pr) and benzaldehyde (Bz). Reprinted from [34] with permission. Copyright (2004) Elsevier B.V.

as result of the capillary voltage" [16]. The generation of electrons, however, does not correspond to Kaupilla's studies on the ionization mechanism [27]. Furthermore, with respect to the "ion-assisted ion transmission" [15], not all compounds have ion intensities that are positively influenced by the addition of a dopant [17]. The latter can even lead to a much lower absolute maximum intensity than when no dopant is used, although the signal itself (obtained with dopant) is stable as function of capillary voltage [18]. Therefore, eliminating capillary voltage effects on the ion intensity does not provide general assistance to ion transfer; instead just a dopant-assisted improvement in ionization efficiency, which is strongly analyte dependent.

In summary then, for both negative and positive ion formation in APPI, the analyte is mostly analyzed indirectly, via chemical reactions that are photo-initiated. Although dopants do not have a similar effect in APCI [27] (due to different ionization initiation process(es)), analyte ionization in the APPI interface is as much a chemical process as so-called atmospheric pressure chemical ionization. The major difference is the initiation of the ionization process by corona discharge (APCI) or absorption of vacuum UV photons (APPI).

Effect of solvent composition and dopants

It is evident that dopants and solvents play an important role in the ionization mechanism, and the choice of dopant and solvent strongly affects the ionization efficiency [12]. Therefore, as Raffaelli and Saba stated in their review (and they, in turn, were referencing a similar statement from Bruins et al. [10]), as a general rule, the solvent used in APPI–MS must be selected carefully, because it can profoundly affect the limits of detection of the compounds under investigation [8].

Toluene has been widely used as dopant, especially in the PhotoSpray source, but is not suitable for the ionization of compounds with low PA and IE, as the toluene radical cation tends to transfer its proton to the high PA solvent instead, which is the case for reversed-phase solvents as acetonitrile and methanol [32]. Bruins et al. introduced the use of anisole as a better choice for the photoionization of samples that have low ionization energies and low proton affinities [37]. A high intensity can be achieved for the anisole molecular ion even in the presence of reversed-phase solvents, leading to a signal increase of as much as a hundredfold for the mentioned samples, compared with the signal intensity obtained using APPI with toluene as the dopant. However, not all applications guarantee the successful use of a dopant, and dopant-free photoionization is in certain cases the better choice, especially when using the PhotoMate [21, 34].

With respect to the influence of the solvent, as studied for the PhotoSpray source, the relative deprotonation decreases when the solvent exhibits higher gas-phase acidity than the analyte. Solvents that possess positive electron affinity generally deteriorate the ionization by capturing thermal electrons and thus depleting the source for further analyte ionization [27]. Although it may be questionable as to whether these electrons are so important in the analyte ionization (as discussed above in "Negative ion formation"), they still may play a role in intermediate ionization steps. Otherwise, quenching of the ionization by solvents with positive EA must occur in another way. A further effect of the solvent is observed in the oxygen substitution of certain analytes, the amount of which is dependent on the solvent composition [27].

Furthermore, as already mentioned by Raffaelli and Saba [8], addition of buffers such as ammonium acetate, ammonium hydroxide or acetic acid may suppress the ionization of solvents in APPI [32], which was also observed by Yoshioka et al. [38]. Again, this effect strongly depends on the analyte and the solvent. However, it is a general observation that the effect of buffer ions on the ionization is much less than observed in APCI and especially ESI [39, 40].

Recent source developments and coupling issues

Recently, further investigations have been performed by several groups with respect to the development and modification of APPI sources and to coupling them with different types of separation systems and mass spectrometers. Starting from the two basic concepts of APPI described above, innovative ideas have lead to extensions of the instruments, techniques and the application range of APPI.

Since no APPI sources were commercially available for Fourier-transform ion cyclotron resonance mass spectrometry (FTICRMS), Greig et al. built their own photoionization interface by mounting a Cathodeon PKS100 photoionization lamp into the drain of a regular APCI source [21].

In order to extend the range of compounds that can be simultaneously ionized, different ionization sources can be combined. First, McIndou et al. developed a so-called photoassisted electrospray ionization (PAESI) source [41]. This is a combination of APPI and ESI, based on the principle of a PI lamp and an ESI source. In this set-up, the analytes are still in solution during the photoionization, which weakens the PI effect. For the studied compounds, any compound that can be analyzed by ESI–MS can also be analyzed by PAESI–MS. The positive effect of PI on the ionization was mainly observed for some positively or negatively charged coordination or organometallic compounds, giving higher ion currents and hence improved signal-to-noise ratios. Characteristic photolysis products such as carbonyl dissociation products were not observed.

More aspects of dual-source ionization were explored by Syage et al. [42], focusing on the combination of APPI with both APCI and ESI. The latter combination of these is the more complementary. Three operation modes are possible for the dual ionization source: the use of only one ionizer,

the use of both simultaneously and a rapid switching between the two ionizers, which can be applied in both the positive and the negative ion modes. Although dual ionizer configurations only minimally affect the performance of either ionizer relative to the standard single-ionizer sources, the operation of both ionizers together does not typically give the sum of the signals of the two individual sources and may result in interference and unexpected results. For ESI/APPI, ESI multiply-charged protein signals were strongly suppressed by the activated APPI source, presumedly due to the interaction of the ions formed initially by both sources [42]. Similarly to [43, 44], where the results from APCI performed with and without corona discharge were compared for proteins, the suppression of the protein signals may be due to degradation caused by the photon energy. In APCI/APPI, signals for various compounds were observed, ranging from less than the signal of either source alone to the sum of the two individual sources. A simple thermodynamic model does not fully predict these ionization effects [42] and a deeper understanding of the underlying mechanisms is needed to allow better methods to be developed.

Different APPI source developments were presented by Kostiainen et al., who developed a microfabricated heated-nebulizer chip for APPI–MS [45]. A nebulizer chip, containing fluid inlets, flow channels for the liquid sample and nebulizer gas, a mixing chamber, an integrated microheater and an exit nozzle, is positioned as close as possible to a krypton discharge lamp, which again is positioned as close as possible to the MS inlet (Fig. 7), the respective positions being critical to efficient ionization. A repeller is required to efficiently direct the ions formed towards the mass spectrometer. Dopant is delivered as part of the sample solution and the ionization mechanisms that take place in the positive and negative ion modes are mainly similar to those observed in conventional APPI. The microAPPI, handling flow rates of 0.05 to 5 μL/min, exhibits stable performance and can be used for weeks.

Closely related to APPI is APLI, atmospheric pressure laser ionization. An APLI source for the coupling of LC and MS was recently developed by Benter et al. [46]. Where a one-step VUV approach is used in APPI, the APLI technique is based on resonant or near-resonant two-photon ionization. Utilizing this resonantly enhanced multiphoton ionization (REMPI) as the primary ion production mechanism, APLI strongly enhances the selectivity of the ionization process. Furthermore, the photon flux in APLI is much higher than with APPI, leading to detection limits in the femtomolar range. As the APLI operates primarily directly on the analyte, efficient ionization of even nonpolar compounds is obtained, but the current method is also limited to the analysis of simple, polymeric or polycyclic aromatic hydrocarbons. Therefore, future directions of APLI, which can easily be fitted on most AP mass spectrometers, include multicolor excitation, dopant-assisted APLI, and electron attachment ionization [46].

The implementation of APPI–MS is not only limited to conventional LC. The development of μ-APPI [45], for example, enables the coupling of APPI–MS to micro- or nano-LC and microfluidic devices. The flexibility of SFC/MS systems has also been extended with the integration of APPI, since they can be used with other nonpolar compounds, and the signal-to-noise ratio is enhanced [47]. Implementation of APPI in capillary electrophoresis/mass spectrometry (CE/MS) is another option [39]. The APPI process is less affected by nonvolatile salts such as phosphates, which are frequently used for CE separations. This leads to better results than those obtained with ESI–MS, because of less spectral background and enhanced signal-to-noise ratios. CE/APPI-MS was studied in more detail by Somsen et al. [40]. Using an adapted APPI source with a sheath-liquid sprayer designed for CE/ESI–MS, the influence of different types of dopants, the effect of background electrolyte and the ionization efficiencies of both polar and nonpolar compounds were investigated. With the ability to ionize nonpolar compounds, an ionization efficiency unaffected by nonvolatile buffers, and a significant signal enhancement due to the use of dopants, the APPI turned out to be, although less sensitive than ESI, a much more generally applicable ionization method for CE/MS. Since even sodium dodecyl sulfate (SDS) has no negative effect on the photoionization efficiency, APPI may provide a good solution for improving the compatibility of on-line micellar electrokinetic chromatography (MEKC)/MS.

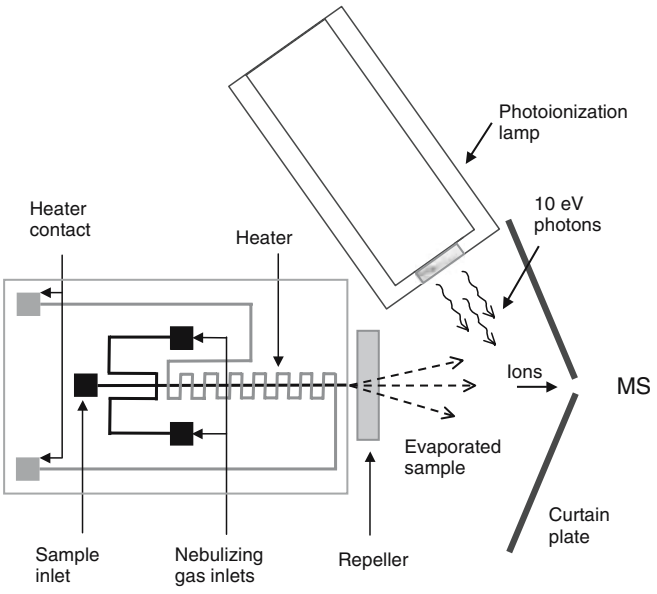


Fig. 7 Schematic drawing of the microAPPI. Reprinted with permission from [45]. Copyright (2004) American Chemical Society

Applications of LC/APPI–MS

In the period since the appearance of Raffaelli and Saba's review (May 2003) [8], the rapid growth of APPI–MS in analytical chemistry has been reflected by the publication of more than thirty application papers in different fields,

and the inclusion of the technique in several reviews for pharmaceutical [4–7] and environmental [3] analysis.

Originally, the APPI source was thought to expand the polarity range in which analytes are amenable to LC/MS analysis [2, 8, 32], although in the first APPI paper [10], Bruins et al. stated that "the range of compounds that can be efficiently ionized by APPI closely follow that of APCI" and "the APPI source may find utility in many areas of application where the corona discharge-APCI method is presently employed". And indeed, with the exception of some compound classes that are only poorly ionized by ESI or APCI, most applications are dedicated to species that are sufficiently ionized by ESI or APCI, and they often compare the different ionization techniques. However, implementing APPI instead of APCI (or ESI) often produces an improvement in sensitivity, due to the lower susceptibility of the APPI source to ion suppression.

Although most applications can be assigned to the categories of pharmaceutical analysis or environmental chemistry, APPI–MS has found its way into other fields of analysis as well. The selection of analytes displayed in Fig. 8 provides a selection of compounds that have been analyzed by APPI–MS within the last three years. As the two different types of commercially available APPI sources have somewhat different ionization properties, and as the ionization mechanism strongly depends upon the solvent conditions, all application data are summarized in Tables 1 and 2, providing an overview of the analyte, sample matrix, ion source used, dopant used, the ionization mode and hence the ions observed.

Pharmaceutical and clinical analysis

In the fields of pharmaceutical and clinical analysis, most published applications are directed at determination of steroids [21, 22, 25, 48–50]. As investigated by Soldin et al., LC/APPI–MS analysis enables the simultaneous determination of nine steroids in serum samples with limits of detection that are sufficient for clinical chemistry and a selectivity that cannot be obtained with established immunoassay methodologies [48]. For the latter reason,

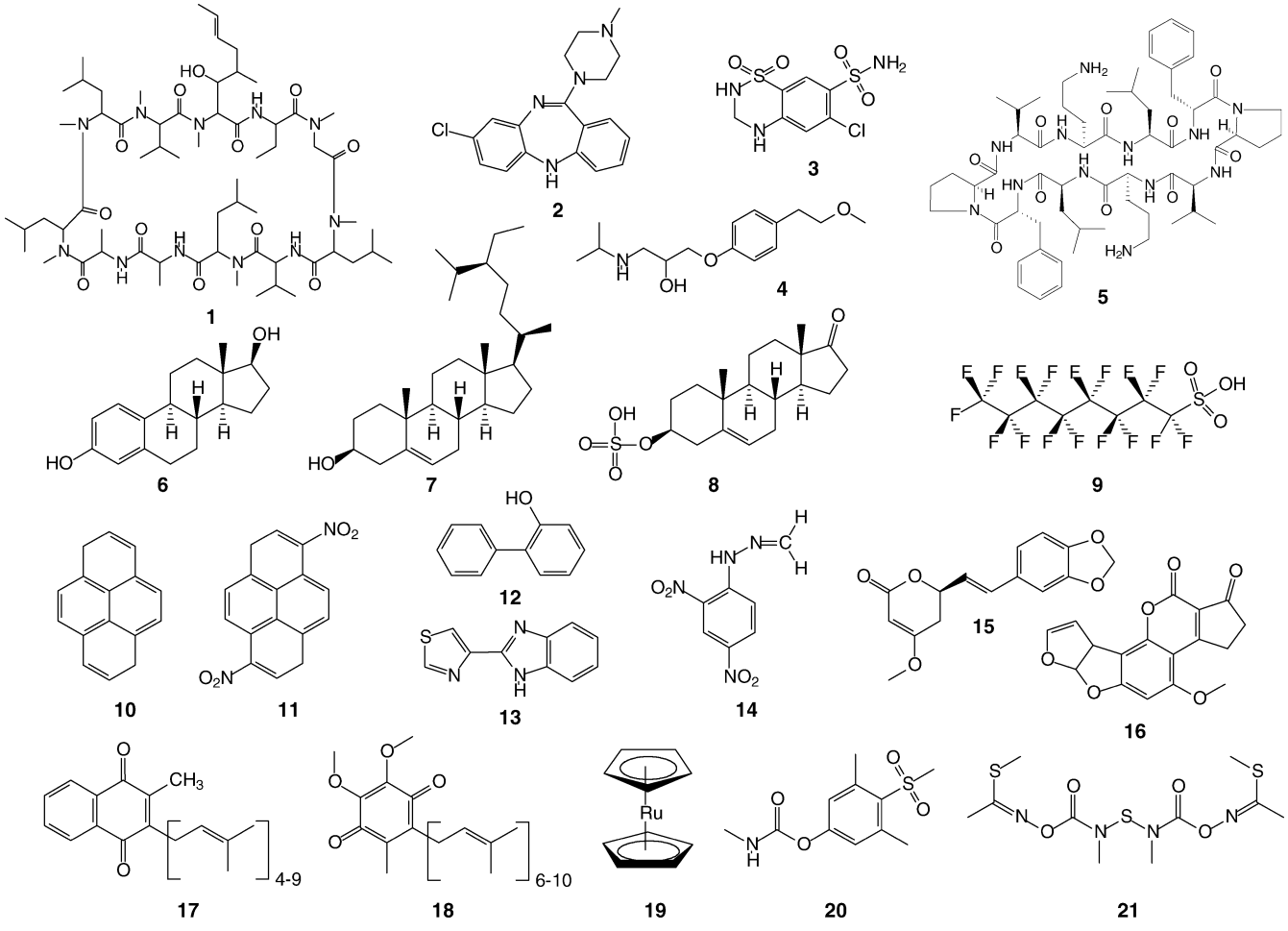


Fig. 8 Structures of representative compounds in current LC/APPI-MS applications. 1) cyclosporin A, 2) clozapine, 3) hydrochlorothiazide, 4) metoprolol, 5) gramicidin S, 6) estradiol, 7) sitosterol, 8) levonorgestrel, 9) perfluorooctane sulfonic acid, 10) pyrene, 11) 1,6-dinitropyrene, 12) *o*-phenylphenol, 13) thiabendazole, 14) 2,4-dinitrophenylhydrazone of formaldehyde, 15) methysticin (kava lactone), 16) aflatoxin B, 17) menaquinone$_{4-9}$, 18) ubiquinone$_{6-10}$, 19) methiocarb sulfone (carbamate pesticide) and 20) thiodicarb (carbamate pesticide)

Table 1 Experimental conditions and results from pharmaceutical and biochemical applications of LC/APPI-MS

Analyte	Matrix	Source[a]	Dopant	Ion mode	Observed ion[b]	Reference
(Small) drug molecules						
Ketoprofen	Caco-2 cells	PS	toluene	pos/neg	$[M+H]^+/[M-H]^-$	[23]
Fluorescein	Caco-2 cells	PS	toluene	pos/neg	$[M+H]^+$	[23]
Cephalexin	Caco-2 cells	PS	toluene	pos/neg	$[M+H]^+$	[23]
Midazolam	Caco-2 cells	PS	toluene	pos/neg	$[M+H]^+$	[23]
Propanolol	Caco-2 cells	PS	toluene	pos/neg	$[M+H]^+$	[23]
Metoprolol	Caco-2 cells	PS	toluene	pos/neg	$[M+H]^+$	[23]
Hydrochlorothiazide	Caco-2 cells	PS	toluene	pos/neg	$[M-H]^-$[c]	[23]
Ranitidine	Caco-2 cells	PS	toluene	pos/neg	$[M+H]^+/[M-H]^-$	[23]
Antipyrine	Caco-2 cells	PS	toluene	pos/neg	$[M+H]^+$	[23]
Verapamil	Caco-2 cells	PS	toluene	pos/neg	$[M+H]^+$	[23]
Lonafarnib	Rat plasma	PS	toluene	pos	$[M+H]^+$	[13, 14, 51]
Clozapine	Rat plasma	PS	toluene	pos	$[M+H]^+$	[13, 14]
Cyclosporin A	Rat plasma	PS	toluene	pos	$[M+H]^+$; $[M+NH_4]^+$	[51]
Steroids						
Estriol	Serum/plasma	PS	toluene	neg	$[M-H]^+$	[48]
Androstenedione	Serum/plasma	PS	toluene	pos	$[M+H]^+$	[48]
Testosterone	Serum/plasma	PS	toluene	pos	$[M+H]^+$	[48]
Dehydroepiandrosterone	Serum/plasma	PS	toluene	pos	$[M-H_2O+H]^+$	[48]
DHEAS	Serum/plasma	PS	toluene	pos	$[M-H_2SO_4+H]^+$	[48]
Cortisol	Serum/plasma	PS	toluene	pos	$[M+H]^+$	[48, 49]
	Standard sol.	PM[d]	toluene	pos	$[M+H]^+$	[21]
11-Deoxycortisol	Serum/plasma	PS	toluene	pos	$[M+H]^+$	[48]
Progesterone	Serum/plasma	PS	toluene	pos	$[M+H]^+$	[48]
17α-Hydroxyprogesterone	Serum/plasma	PS	toluene	pos	$[M+H]^+$	[48]
Estradiol	Serum/plasma	PS	toluene	pos	$[M-H_2O+H]^+$	[48]
Cortisone	Serum/plasma	PS	toluene	pos	$[M+H]^+$	[49]
	Standard sol.	PM[d]	toluene	pos	$[M+H]^+$	[21]
Levonorgestrel	Plasma	PS	toluene	pos	$[M+H]^+$	[22]
17α-Methyltestosterone	Plasma	PS	toluene	pos	$[M+H]^+$	[22]
Lanosterol + metabolite	Inhibition buffer	PS	toluene	pos	$[M+H-H_2O]^+$	[25]
p-Sitosterol	Serum	na	na	na	na	[50]
Campesterol	Serum	na	na	na	na	[50]
Brassicasterol	Serum	na	na	na	na	[50]
Stigmasterol	Serum	na	na	na	na	[50]
Tetrahydrocortisone	Standard sol.	PM[d]	toluene	pos	$[M+H]^+$	[21]
Tetrahydrocortisol	Standard sol.	PM[d]	toluene	pos	$[M+H]^+$	[21]
Tetrahydro-11-dehydrocorticosterone	Standard sol.	PM[d]	toluene	pos	$[M+H]^+$	[21]
3α,5α-Tetrahydrocorticosterone	Standard sol.	PM[d]	toluene	pos	$[M+H]^+$	[21]
Hydrophobic peptides						
Gramicidin S	Standard	PS	toluene/acetone	pos	$[M+H]^+$, $[M+Na+H_2O]^+$	[52]
Gramicidin A	Standard	PS	toluene/acetone	pos	$[M+H]^+$, fragm	[52]
Tetrapeptide YPLG-NH_2	Standard	PS	toluene/acetone	pos	$[M+H]^+$, $[M+Na+H_2O]^+$	[52]
E1-Nter	Standard	PS	toluene/acetone	pos	$[M+H]^+$, fragm	[52]
TME1	Standard	PS	toluene/acetone	pos	fragm, ($[M+H]^+$)	[52]

[a]PS=PhotoSpray (Applied Biosystems/MDS Sciex/Machine shop, University of Groningen, The Netherlands); PM=PhotoMate (Agilent/Syagen)
[b]Most abundant
[c]Strongly dependent on solvent system
[d]Home-made, by inserting a krypton lamp into the drain of an APCI source (Apollo, Bruker); therefore mostly resembling PM
na=data not available

Kushnir et al. developed an LC/APPI–MS method for the determination of cortisol and cortisone [49]. Besides superior selectivity towards immunoassay-based methods, the APPI–MS technique offers better limits of detection and easier sample handling than ESI- or APCI-based methods. Similar results were obtained for other cortico-

Table 2 Experimental conditions and results of environmental and other applications of LC/APPI–MS

Analyte	Matrix	Source[a]	Dopant	Ion mode	Observed ion[b]	Reference
Environmental						
Estrogens (synthetic, endogenous)	Env. water	PM	na	na	na	[53]
Polycyclic aromatic hydrocarbons (PAHs): Phe, Ant, Flu, Py, B*a*A, Chr, B*b*F, B*k*F, B*a*P, B*e*P, B*ghi*P, IP	Sediment	PS	toluene	pos	$M^{\bullet+}$	[11]
1,6-Dinitropyrene	Rat plasma	PS	toluene[c]	neg	$M^{\bullet-}$	[20, 56]
1,6-Aminonitropyrene	Rat plasma	PS	toluene[c]	pos	$[M+H]^+$	[20, 56]
1,6-Diaminopyrene	Rat plasma	PS	toluene[c]	pos	$[M+H]^+$	[20, 56]
Aldehydes and ketones, as their DNPH derivatives	Car exhaust, cigarette smoke	PM	–	neg	$[M-H]^-$	[34]
o-Phenylphenol	Citrus fruits	PM	–	neg	$[M-H]^-$	[38]
Diphenyl	Citrus fruits	PM	–	pos	$M^{\bullet+}$	[38]
Perfluorooctane sulfonate	River water	PM	acetone	neg	$[M-H]^-$	[15]
2-Phenylbenzotriazoles (PBTAs)	River water	PS	toluene	pos	$[M+H]^+$	[24]
Chloramphenicol	Fish meat	PM	acetone	neg	$[M-H]^-$	[16]
Carbamate pesticides	Vegetables, fruits	PM	acetone	pos	$[M+H]^+$; $[M+NH_4]^+$	[17]
Thiabendazole	Citrus fruits	PM	–	pos/neg	$[M+H]^+/[M-H]^-$	[38]
Imazalil + metabolite	Citrus fruits	PM	–	pos/neg	$[M+H]^+/[M-H]^-$	[38]
Patulin	Apple juice	PM	–	neg	$[M-H]^-$	[18]
Kava lactones (yangonin, kawain, methysticin, desmethoxyyangonin, dihydrokawain, dihydromethysticin)	Solid food, beverages	na	na	pos	na	[55]
Ubiquinones ($UQ_{6–10}$), menaquinones ($MK_{4–9}$)	Env. samples, cell cultures	PS	toluene/ acetone	pos	$[M+H]^+$	[26]
Aflatoxin B1, B2, G1, G2	Peanuts, corn, nutmeg, red pepper	PM	na	na	na	[19]
Avermectin, moxidectin	Milk	PM	–	pos/neg	$[M+H]^+/[M-H]^-$	[54]
Other						
Metal connected porphyrin oligomers	Standard solution	na	na	na	na	[57]
UG8 asphaltene	Serum/plasma	PS, PM	na	pos	na	[58]
Metallocenes, phosphane compounds, metal-carbonyl complexes, bi- and tripyridine complexes	Standard solution	PM	toluene[c]	pos	$M^{\bullet+}$	[41]

[a]PS=PhotoSpray (Applied Biosystems/MDS Sciex/Machine shop, University of Groningen, The Netherlands); PM=PhotoMate (Agilent/ Syagen)
[b]Most abundant
[c]Normal-phase chromatography/flow injection/direct infusion
na=data not available

steroids [21]. Here, APPI was found to require less heat for desolvation, giving rise to less thermal degradation of the analytes and higher signal-to-noise than APCI [21]. Similarly, LC/APPI–MS/MS was found to be superior to LC/ESI-MS/MS for levonorgestrel determination. With a dynamic range of 3–4 orders of magnitude, lower background noise, and four times better detection limits than ESI–MS/MS, the APPI-based method enables high sample throughput and simple sample preparation [22]. For lanosterol and its demethylated metabolite, APPI–MS provides 10–500-fold lower detection limits than can be obtained by APCI–MS [25]. LC/APPI–MS/MS also has significant advantages over GC/MS for the analysis of free and esterified phytosterols, exhibiting a 150-fold decrease in limits of detection and a reduction in analysis time from three hours to 15 minutes [50].

Next to steroid analysis, several pharmaceutical applications of APPI–MS can be found with respect to other (small) drug molecules. For instance, APPI–MS can be very useful for determining a variety of drugs (see Table 1, Fig. 8) in biological matrices like cell cultures and plasma [13, 14, 23, 51]. Kotiaho et al. [23] compared the results from LC/APPI–MS with those from LC/ESI–MS, finding a broader linear dynamic range for APPI–MS and lower detection limits for ESI–MS, but concluding that both are excellent methods for high-throughput analysis of small molecules. In a comparative study of LC/APPI–MS and LC/APCI–MS analyses applied to two model compounds and a number of drug development species, Hsieh et al.

found similar analytical results for both methods [13, 14], with APPI–MS having the advantage due to less ionization suppression effects by the matrix. Hsieh et al. compared all three ion sources for the analysis of cyclosporin A in rat plasma, taking into account the implementation of both normal-phase (NP) and reversed-phase (RP) chromatography for APCI– and APPI–MS [51]. In normal-phase LC/APPI–MS, no dopant is required to achieve maximum ionization efficiency, and the limit of detection is equivalent to that obtained in RP chromatography (with both APCI and APPI). However, although all methods are found to be equivalent in analytical accuracy, the LC/ESI–MS/MS method proved to have the lowest limit of detection.

In the field of biochemistry, hydrophobic peptides are a group of compounds that are not easily amenable to common types of ionization for LC/MS purposes. While APCI failed completely (except for gramicidin S), APPI appears to be a complementary tool to ESI, demonstrating a sensitivity almost as good and additionally providing sequence information because of highly specific in-source fragmentation [52].

Environment

Applications of APPI–MS for environmental analysis include the determination of various contaminants in water samples. Since they have high potency and severe biological impacts, determining estrogens in waters is mandatory but difficult to achieve at trace levels. However, both synthetic and endogenous estrogens have been succesfully determined by APCI– and APPI–MS/MS [53].

The simultaneous analysis of eight phenylbenzotriazole-type (PBTA) mutagens extracted from river water was performed by LC/APPI–MS/MS with toluene as dopant. APPI provided higher signal intensities than measurements with an ESI interface. River water analysis of PBTAs at the ultratrace level should be possible with an APPI interface [24].

Perfluorooctane sulfonate (PFOS) is another important contaminant in river water. This compound can be determined by reversed-phase LC/MS with APPI by automated on-line extraction using turbulent-flow chromatography (TFC). This method is fast, sensitive and simple, and extraction, separation and selective detection could be achieved with satisfactory detection limits and selectivity within an analysis time of only 18.75 minutes. APCI is not at all suited to determining PFOS. Using an ESI interface results in similar LODs/LOQs to APPI, but an important advantage of APPI over ESI is that no matrix effects are observed [15].

Because of health concerns, the use of chloramphenicol (CAP) is banned as veterinary drug for animals that are used in food production, both in the US and the EU. The determination of CAP in fish meat with LC/APPI–MS and LC/APCI–MS resulted in similar limits of detection and a lower matrix effect for APPI–MS [16]. Because of this, there was no need to use matrix-matched standards in APPI, and the measurements all showed excellent recovery and repeatability. The intensities of the additional peaks in APPI were lower than in APCI and did not cause any interference. Contamination of the APPI source did not affect the ionization, while contamination of the APCI source resulted in decreased ionization efficiency.

The advantage of low matrix effects in APPI compared to APCI was also apparent when analyzing residues from 22 different carbamate pesticides in vegetables and fruits. In the positive ion mode, the limits of detection obtained for carbamates with both ionization techniques are similar [17]. The simultaneous determination of four aromatic post-harvest fungicides in citrus fruits (and the major metabolite of one of them) using dopant-free LC/APPI–MS resulted in results comparable to those achieved when using an APCI source [38]. The results for one of the fungicides were worse than with APCI. This compound could only be measured in the positive ion mode, while another fungicide could only be measured in the negative ion mode. However, the APPI method was found suitable for routine analysis of these fungicides in citrus fruits.

Similarly, negative ion APPI has shown to be a viable alternative to positive ion no-discharge APCI for the determination of anthelmintic compounds [54]. Although the latter method, which is effectively coordination ion spray (CIS) with sodium, has overall better limits of detection, APPI dominates when measuring in the negative ion mode.

Another comparison of APPI and APCI has been carried out by Nakahara et al. [18]. They determined patulin, a mycotoxin in apple juice. From this study, APPI again appeared to give lower chemical noise and signal suppression while showing the same limits of detection as APCI. Furthermore, reproducibility and repeatability were good and the linearity of patulin in apple juice spanned three orders of magnitude. No significant differences have been found for mass spectra obtained with dopant-free and acetone dopant-assisted photoionization. Nakahara et al. also determined aflatoxins in food [19]. A comparison has been made between the results from aflatoxin measurements performed with LC/APPI/MS and LC/ESI/MS. APPI showed less chemical noise and less signal suppression than ESI and also had lower RSDs and LODs, thus confirming their earlier study for another group of analytes.

Furthermore, LC/APPI-MS has been compared to LC/UV by Diachenko et al. [55]. Six kava lactones in foods and beverages were determined by both methods, which resulted in comparable results for most analytes. The relatively low MS sensitivity can probably be ascribed to the low polarity of the lactones.

The ability of microbes to adapt to their environment is a direct result of their capacity to form different respiratory quinones. Geyer et al. have carried out the simultaneous mass spectrometric analysis of microbial respiratory ubiquinones and menaquinones [26]. Quantification of these compounds by means of chemical and photoionization MS in positive ion mode, making use of SRM detection, gave limits of detection that were at least three times better for APPI. Electrospray ionization was only possible for ubiquinones. The detection method used by Geyer et al. was succesfully applied to quinone quantification in a variety

of environmental samples and cell cultures. Quantification was possible due to the high selectivity of quinone detection using the SRM mode.

Dinitropyrene and aminonitropyrene, which result from diesel engine emissions, were determined in rat plasma by ESI, APCI and APPI normal-phase HPLC/MS/MS. They were detected with the highest precision and best detection limits by APPI [20]. Diaminopyrene and aminonitropyrene were quantified in positive ion mode, while dinitropyrene was quantified in negative ion mode. Selectivities were higher than for fluorescence detection (FLD) or chemiluminescence detection (CLD). Compared to GC/MS, the LC/APPI–MS method developed by Volkel et al. is more selective and does not require exhaustive sample preparation, but its instrumental limits of detection are lower. The same group also studied the ionization of these ambiphilic compounds in ESI, APCI and APPI, after derivatization with pentafluorobenzoyl chloride or acetic anhydride, in comparison with GC/MS analysis [56]. Only acetylation of the analytes allowed for unequivocal identification in the biological matrix and resulted in improved detection limits and the best selectivity. For acetylated diaminopyrene, electrospray ionization yielded the lowest limit of detection. Ionization by APPI resulted in the lowest limit of detection of the less polar acetylated aminonitropyrene. Although the optimum ionization technique is different for each compound, the LC/MS/MS method presented permits the direct detection of acetylated diaminopyrenes in urine and may also be applied to toxicokinetic studies and human biomonitoring.

Besides the analysis of water samples and biological materials, air monitoring gives rise to other environmental applications of APPI–MS. Polycyclic aromatic hydrocarbons (PAHs), caused by incomplete combustion of fuels and wood, are strong environmental contaminants. Because of their low polarities, PAHs are unsuitable for ionization in ESI or APCI interfaces, but since MS is a more selective detection method for their determination than UV/Vis or fluorescence spectroscopy, MS is still the desired solution. A determination of 12 different PAHs consisting of 3–6 aromatic rings by LC/APPI–MS has been carried out by Alary et al., and this is shown to be a powerful approach for the analysis of PAHs in the environment [11].

For aldehydes and ketones from exhaust samples, derivatized with 2,4-dinitrophenylhydrazine (DNPH), determination by dopant-free LC/APPI–MS has shown to be an attractive alternative to APCI–MS (Fig. 6) [34]. LODs are typically slightly lower in APPI–MS and more carbonyls can be detected at low levels in real samples. However, the linear range obtained with APPI is slightly reduced compared to APCI.

Other applications

Besides the use of LC/APPI–MS for several pharmaceutical, clinical or environmental applications, APPI-MS was also found to be a very powerful tool for characterizing metal-connected porphyrin oligomers [57].

The molecular weight of asphaltene is an important factor when setting up an efficient and economically viable heavy oil refining strategy. In order to unambiguously measure the molecular weights of asphaltenes, APCI– and APPI–MS were chosen by Mullins et al. to analyze UG8 asphaltene. These techniques were found to be suitable for determining the molecular weight accurately [58].

Organometallic and coordination compounds, including metallocenes, phosphane compounds, metal-carbonyl complexes and bi- and tripyridine complexes, were determined by means of PAESI [41]. In some cases, mainly for some positively and negatively charged compounds, higher ion currents and signal-to-noise ratios were obtained than when using conventional ESI.

Conclusions and perspectives

Although there have only been five or so years of APPI development, this ionization technique has evolved rapidly to become a very useful complement to established ionization techniques in LC/MS. Based on the closed axial PhotoSpray model developed by Bruins et al. and the open orthogonal PhotoMate, further developments of APPI sources have lead to the implementation of dual source APPI/ESI (PAESI) and APCI/APPI, microAPPI, and the coupling of APPI–MS to different separation techniques such as (besides classical LC) SFC, CE and even MEKC.

Thorough studies have been performed to obtain deeper insight in the mechanism behind photoionization, and these have found that, most of the time, the final ionization of the analyte is effectively chemical ionization with major solvent and dopant contributions to the overall reaction. Observations of protonation made when using benzene or toluene as dopant (or solvent) are due to the formation of phenol and cresol (respectively) molecular cations, rather than direct proton transfer from the dopant molecular cations to the analyte. The influence of solvent and dopant on the ionization efficiency is strongly analyte-dependent. Anisole has been added to benzene, toluene and acetone in the list of effective dopants and, based on the mediation of phenol in benzene-assisted photoionization, phenol will be subject to study due to further dopant possibilities. Ionic additives in the solvent may have some effect on the ionization, but the APPI source is recognized as being far less susceptible to ion suppression than APCI and especially ESI. Furthermore, APPI may not reach detection limits that are as low as those afforded by ESI, but it does exhibit a large linear range of typically 3–4 decades and (compared to APCI) it works well at low flow rates, while still having excellent limits of detection.

With many and diverse applications in the fields of pharmaceutical analysis, clinical chemistry, environmental science and others (this list is constantly growing), APPI offers a good alternative and complementary technique to APCI and ESI in the hyphenation of LC and MS. For many

groups of compounds, the analytical figures of merit in APPI are similar or slightly worse than those in APCI or ESI. However, especially for steroid compounds and quinones, APPI turned out to be beneficial with respect to limits of detection. Although the overall range of compounds that can be ionized by APPI is not so different from the range of compounds amenable to APCI, APPI has been shown to be especially advantageous with respect to ion suppression by matrix components. Therefore, it is likely that APPI–MS will be used in a rapidly increasing number of applications, especially in the fields of pharmaceutical and environmental chemistry. Furthermore, the milder conditions used in APPI and the possibility of operating the source at lower temperatures than in APCI makes the technique attractive for thermally labile or otherwise instable compounds that suffer from in-source fragmentation in APCI.

APPI has also been used for some less common applications, such as the characterization of metal-connected porphyrin oligomers. These kinds of applications will not have a significant impact on the total number of APPI applications, but the technique will certainly be explored for more analyses of this kind in the coming years.

Although APPI was expected to broaden the range of ionizable compounds on the low polarity side, very few applications have been reported for these kinds of analytes. The detection limits achieved by the method in these cases are often still not as good as can be obtained for more polar compounds. The ionization mechanism is probably the main issue here. The solvent plays a much larger role in the analyte ionization, whereas the "electron-ejection" and "electron-capture" theory is less applicable than first expected, making the method less promising for low polarity analytes.

The underlying mechanisms in photoionization have been studied thoroughly and systematically and several new aspects have been revealed. However, our understanding of the ionization mechanism is not yet complete and, although we may never fully understand this mechanism, future investigations will certainly face this challenge and bring deeper insights into APPI.

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