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Atmospheric pressure photoionization mechanisms
1. The case of acetonitrile

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Abstract

Experimental data show that acetonitrile, even though it has an ionization energy higher than the photon energy employed in atmospheric pressure photoionization (APPI) conditions, participates in the protonation of furocumarins. In order to clarify this unexpected behaviour, the processes activated by APPI on acetonitrile have been studied in detail. The formation of protonated molecules ($C_2H_4N^+$ cations), and of a complex between acetonitrile and water ($C_2H_6NO^+$ cations), is observed. To clarify the nature of this species, ^{13}C and ^{2}H labelling experiments and product ion spectra have been employed. The data suggest that photon irradiation leads to a first isomerization of acetonitrile molecules that leads to species that exhibit an IE < 10 eV and that consequently are able to generate photoionization products. The $C_2H_6NO^+$ species, already detected in stratosphere and produced in drift tube experiments, reasonably originates from the reaction of $C_2H_4N^+$ ions with water molecules. Both of these species can be considered as protonating agents.

Keywords: APPI; Ion/molecule reactions; Acetonitrile; Protonation; Photoionization

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1. Introduction

In recent years, the development of methods to ionize species of interest in atmospheric pressure conditions have completely changed the analytical power (and, in some extent, the general view) of mass spectrometry, and have allowed the direct analysis of analytes present in solution. Their development has been immediately reflected on the production of a highly effective LC-MS coupling [1]. Furthermore, the low-energy deposition, typical of these ionization methods, as well as the moderate or null thermal stress experienced by the analyte, reflects on their effective use in the structural characterization of labile molecules. As a matter of fact, atmospheric pressure chemical ionization (APCI) [2] and electrospray ionization (ESI) [3] represent the most widely employed methods in the biochemical and organometallic fields, in which the molecules of interest often exhibit a low stability.

More recently, a new atmospheric pressure ionization approach has been proposed; it is based on the interaction of a light beam with the vapours emanating from a nebulizer of the sample solution, and it is called atmospheric pressure photoionization (APPI) [4].

Photoionization (PI) has attracted mass spectrometrists from the early days of the technique; it

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exhibits some special theoretical advantages with respect to electron ionization, but it also suffers from some severe practical limitation [5]. Although the ion population in electron ionization conditions exhibits a wide internal-energy distribution, the ion population in photoionization conditions (due to the usually well defined photon energy and to the kind of photon–molecule interaction) shows a very homogeneous and narrow internal-energy distribution. The main disadvantages of PI are related to the yield of product ions, which is related to the sample vapour density and irradiation parameters (power, photon energy, irradiance). In the "classical" approach, in which the sample is vaporized inside an ion chamber at reduced pressure, the product ion yield is surely lower than that achieved by electron ionization with the same sample density. For these reasons, the use of photoionization in analytical mass spectrometry has been strongly limited.

Nevertheless photoionization was widely employed in chemical physical studies related to the photochemistry of gaseous species [5].

Nowadays APPI, due to the high sample density that is present in the region close to the nebulizer, leads to a very high sensitivity, which is better than that present in APCI conditions, where corona discharge represents the ionizing approach.

This point was well demonstrated by the paper from the Bruins and co-workers [4], that shows that by APPI, it is possible to obtain an analyte signal intensity eight times higher than that obtainable with a commercial APCI source. In that same paper, the operative conditions of the APPI source were simply described by considering that, in these conditions, direct photoionization can occur only for molecular species that exhibit an ionization energy lower than the photon energy ($h\nu$). Considering that the krypton discharge lamp emits 10.2 eV photons, only molecules with an IE $<$ 10.2 eV can be directly ionized with the formation of an odd-electron molecular cation $M^{\bullet +}$. For substances that have higher IE values, the APPI source can be validly and effectively employed by adding, in the analyte solution, a dopant (i.e., a compound with IE $<$ 10.2 eV), which once ionized activates charge-exchange or protonation reactions with the analyte, leading to its ionization. The method was employed for the LC-MS analysis of a series of samples, some of which were of pharmaceutical interest, and the obtained results were particularly exciting [4].

Those first data stimulated the interest of different research groups, as well as of the instrument producers, to make the APPI source commercially available. Many communications on APPI applications have been presented at international mass spectrometry meetings [6], and some interesting papers have been published [7–12].

Researchers agree that, at first sight, the mechanisms that operate in APPI seem to be particularly simple: for molecules with ionization energy $< h\nu$, a direct ionization is to be expected, with the formation of $M^{\bullet +}$; for molecules with an EI $> h\nu$, photoionization cannot take place and, consequently, the presence of a dopant is fundamental. For this purpose, benzene, toluene and acetone (IE = 9.24, 8.83 and 9.50 eV, respectively [13]) have been proposed.

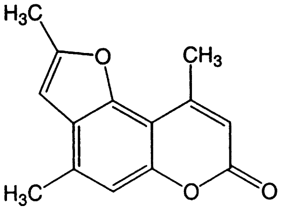
In order to verify these interesting hypotheses we performed experiments with different analytes that were dissolved in different solvents. The results were not so simple as expected; therefore an investigation was undertaken to clarify the role of the solvent in the atmospheric pressure photoionization mechanisms.

2. Experimental

The atmospheric pressure photoionization spectra were obtained with an Agilent 1100 Series MSD Trap (Agilent Technologies, Palo Alto, CA, USA).

Isomeric furocumarins (∼0.5 mg) were dissolved in 1 mL acetonitrile and that solution was further diluted (1:1000). Solutions of the samples were directly infused into the source (flow rate of 3 $\mu L\,min^{-1}$). The source was operating in the positive ion mode and was kept at 250 °C.

A CCl_4 solution of compound **1** and one of diphenylene (Sigma–Aldrich) 2×10^{-6} M were analysed under the same instrumental conditions.



Compound 1

Acetonitrile (CH_3CN, HPLC-grade, Carlo Erba Reagenti), stable isotope labelled acetonitrile (CD_3CN 99.95% and $^{13}CH_3CN$ 99%, Sigma–Aldrich) and CCl_4 (Carlo Erba Reagenti) were analysed in the positive and negative ion modes. MS/MS experiments were obtained by resonance activation of precursor ions.

3. Results and discussion

In an extensive study devoted to the characterization of a series of isomeric furocumarins [14] and considering that the ionization potential of these substances is <10 eV (e.g., the IE of furan is 8.88 eV [13]), the use of photoionization without any dopant was considered of interest to obtain odd-electron molecular ions with a low internal energy content. For such an aim, the acetonitrile solutions of these compounds were nebulized into the APPI source. On the basis of the mechanisms described in Section 1, the formation of the $M^{\bullet+}$ of furocumarin would be expected, and the possible participation of acetonitrile (IE = 12.20 eV [13]) for ionization would be excluded.

On the contrary, as shown by the spectrum of compound **1** (Fig. 1a), the most abundant peak is detected at *m/z* 229, corresponding to the protonated molecule, $[M+H]^+$, whereas the $M^{\bullet+}$ ion provided a peak with a relative abundance of only ca. 25%. This result, analogous to that obtained for the five other isomers of **1**, seems to indicate that, in the present case, the ionization mechanisms proposed for APPI are not operative.

In the spectrum of Fig. 1a, a further interesting behaviour is observed, i.e., the formation of a species at *m/z* 270. That ion might be a complex of the analyte and acetonitrile, and it is reasonable to assume that it is formed by the reaction of the MH^+ ion with neutral acetonitrile.

The main problem pertains to the origin of the MH^+ ion. In principle, acetonitrile cannot be considered the protonating medium: its high ionization energy value would indicate that, under irradiation, it cannot generate reactant ions. Consequently, a different proton source must be considered.

It must be emphasized that, considering the high-pressure regime in the APPI source, $[M + H]^+$ ions could be generated through complex mechanisms. The simplest mechanism to invoke would be the reaction of neutral furocumarin with H_3O^+, due to the presence, even if at a trace level, of water molecules in the nebulized gas. However, this process, requiring as a first step the ionization of water, is to be excluded, due to the high ionization energy (12.62 eV [13]) of water. The other molecular species present inside the source (i.e., N_2 and O_2), which could only be responsible for charge-exchange reactions, must also be excluded, due to their high IE values (IE = 15.58 and 12.13 eV, respectively [13]). Consequently, the most reasonable explanation for the $[M + H]^+$ formation lies in the involvement of the $M^{\bullet+}$ of furocumarin as the protonating medium.

To verify this point, and to exclude any possible role of acetonitrile, the same compound was injected in the APPI source with CCl_4 as solvent. Even in this case, as shown by Fig. 1b, the formation of protonated molecules of furocumarin is observed, but an increase of the relative abundance of $M^{\bullet+}$ becomes present. Worth noting is the presence of the CCl_3^+ cluster. Considering that IE $(CCl_4) = 11.47$ eV [13], this species reasonably originates by an ion-pair formation from electronically excited molecules. Considering that, aside from any traces of other compounds, the furocumarin has been nebulized in a non-protic medium, it follows that the formation of $[M + H]^+$ must be ascribed to a self-chemical ionization phenomenon; i.e., by reaction of the $M^{\bullet+}$ of furocumarin with neutral molecules, leading to the formation of $[M + H]^+$ and $[M - H]^\bullet$ species.

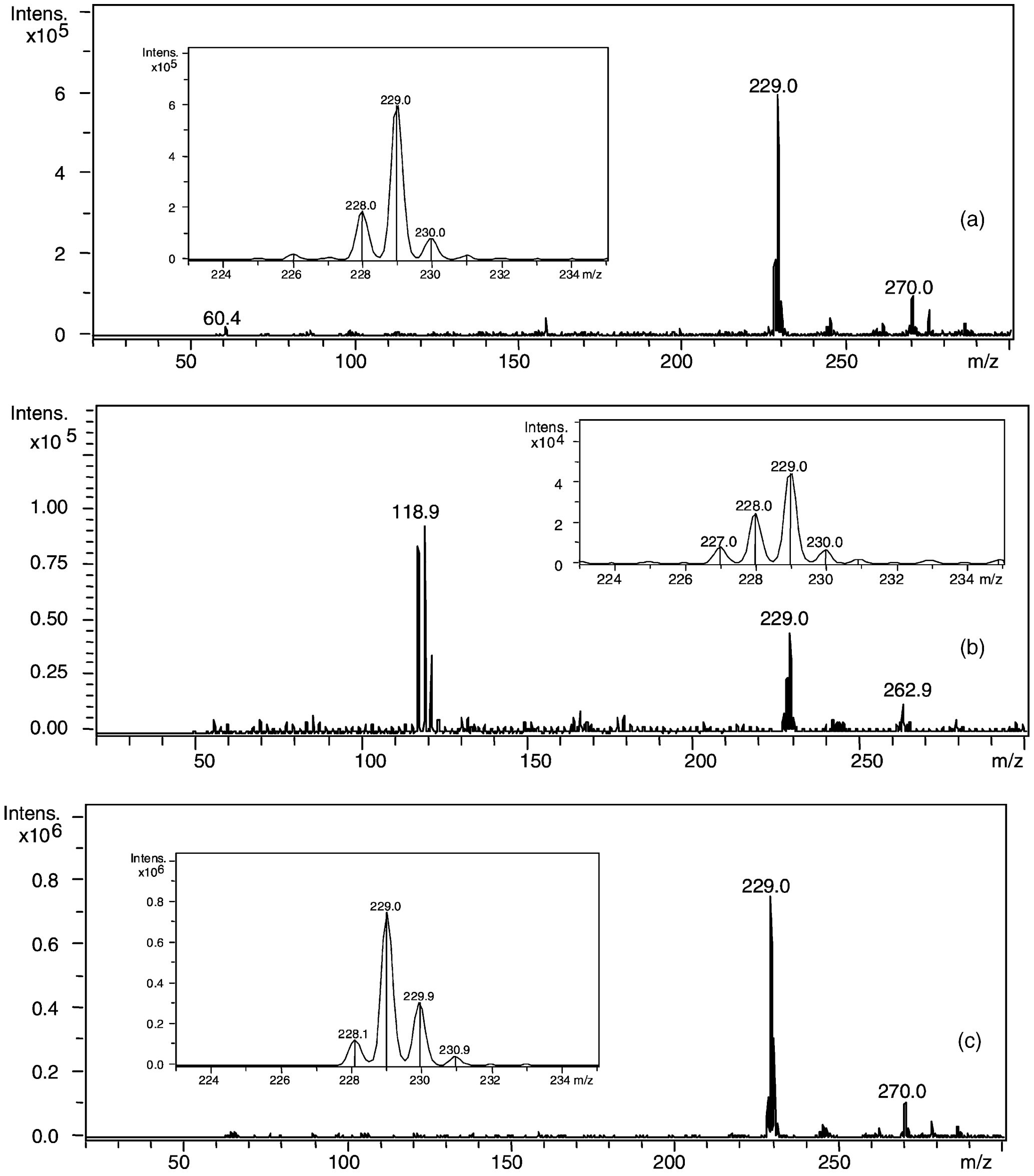


Fig. 1. (a) APPI mass spectrum obtained by the nebulization of a CH_3CN solution of compound **1**. (b) APPI mass spectrum obtained by the nebulization of a CCl_4 solution of compound **1**. (c) APPI mass spectrum obtained by the nebulization of a CD_3CN solution of compound **1**.

To further confirm this hypothesis, we have injected in the same experimental conditions a CCl_4 solution of diphenylene. As can be seen in Fig. 2, only the production of $M^{\bullet +}$ (*m/z* 152) is observed.

However, the higher ratio of $[MH^+]/[M^{\bullet +}]$ of the molecular species of **1** obtained by using acetonitrile (for Fig. 1a and b) would suggest a, at least partial, participation of this solvent (or ionic species generated by it) in the ionization phenomenon that leads to $[M + H]^+$. To verify this hypothesis, the furocumarin was injected in deuterated acetonitrile (CD_3CN). The spectrum reported in Fig. 1c shows a clear increase

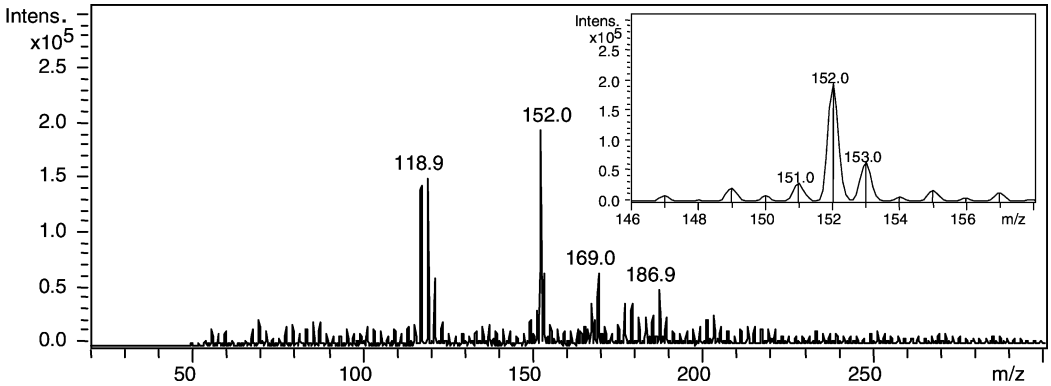


Fig. 2. APPI mass spectrum obtained by the nebulization of a CCl_4 solution of diphenylene.

of the species at *m/z* 230, corresponding to $[M + D]^+$ cations. This proof is unequivocal for the implication of acetonitrile in the $[M + H]^+$ production.

Considering the high ionization energy of acetonitrile (12.20 eV [13]), its direct implication in protonating reactions, requiring its photoionization as an intermediate step, seems to be excluded. Consequently, the photoinitiated reactions of acetonitrile itself were studied.

The gas-phase reactivity of acetonitrile has been widely studied due to its implication in high-stratosphere chemistry [15] and to its power as a chemical ionization reactant [16,17]. Briefly, in the former case, a series of experiments was performed with a temperature-variable drift-tube mass spectrometer to study the reactivity of H_2O and CH_3CN mixtures with H_2^+ ions. Different protonated clusters of CH_3CN and water were detected, and good agreement was found between the laboratory-generated species and the stratospheric positive ions that were detected an altitude of ca. 36 km [15]. When introduced into an ion trap mass spectrometer and ionized by interaction with an electron beam, acetonitrile mainly generated four different ions at *m/z* 40, 41, 42 and 54, due to the $[M-H]^+$, $M^{\bullet+}$, $[M+H]^+$ and $C_3H_4N^+$ ions, respectively. The last ionic species is generated, as proven by ^2H and ^{13}C labelling experiments, by the reactions of $[M - H]^+$ with neutral acetonitrile, and with the further loss of HCN [17]. $[M - H]^+$ and $C_3H_4N^+$ ions are particular effective as reactant species for the analysis of alkanes and alkenes in CI conditions [17].

In stratospheric and electron ionization environments, highly ionizing media are present. In the present case, as discussed above, the photon energy (10 eV) and the ionization energy of acetonitrile (12.20 eV [13]) seem, on the basis of the ionization mechanism proposed for APPI, to exclude its implication in any ionization phenomenon. However, by nebulizing only acetonitrile, ions are generated under irradiation, and a quite complicated spectrum is obtained, with signals of relatively high abundance (2×10^4 digital-analogic converter units). Fig. 3a contains a typical spectrum obtained in these conditions. The most abundant ion is detected at *m/z* 60, and a weak peak due to protonated acetonitrile is present at *m/z* 42. Interestingly, neither $[M-H]^+$ nor $C_3H_4N^+$ ions, of high abundance in the CI spectra of acetonitrile, are present in this case. The formation of ions at *m/z* 60 and 42 necessarily implies the occurrence of ionization phenomena. By switching off the Kr lamp and leaving constant all the voltages applied to the source, *m/z* 60 and 42 completely disappear; these result proves that they originate from photochemical reactions.

In our opinion, three different mechanisms can be invoked to justify this unexpected ion production:

(i) ionization of a complex of acetonitrile with some neutral species present in the source environment, exhibiting an IE < 10 eV;

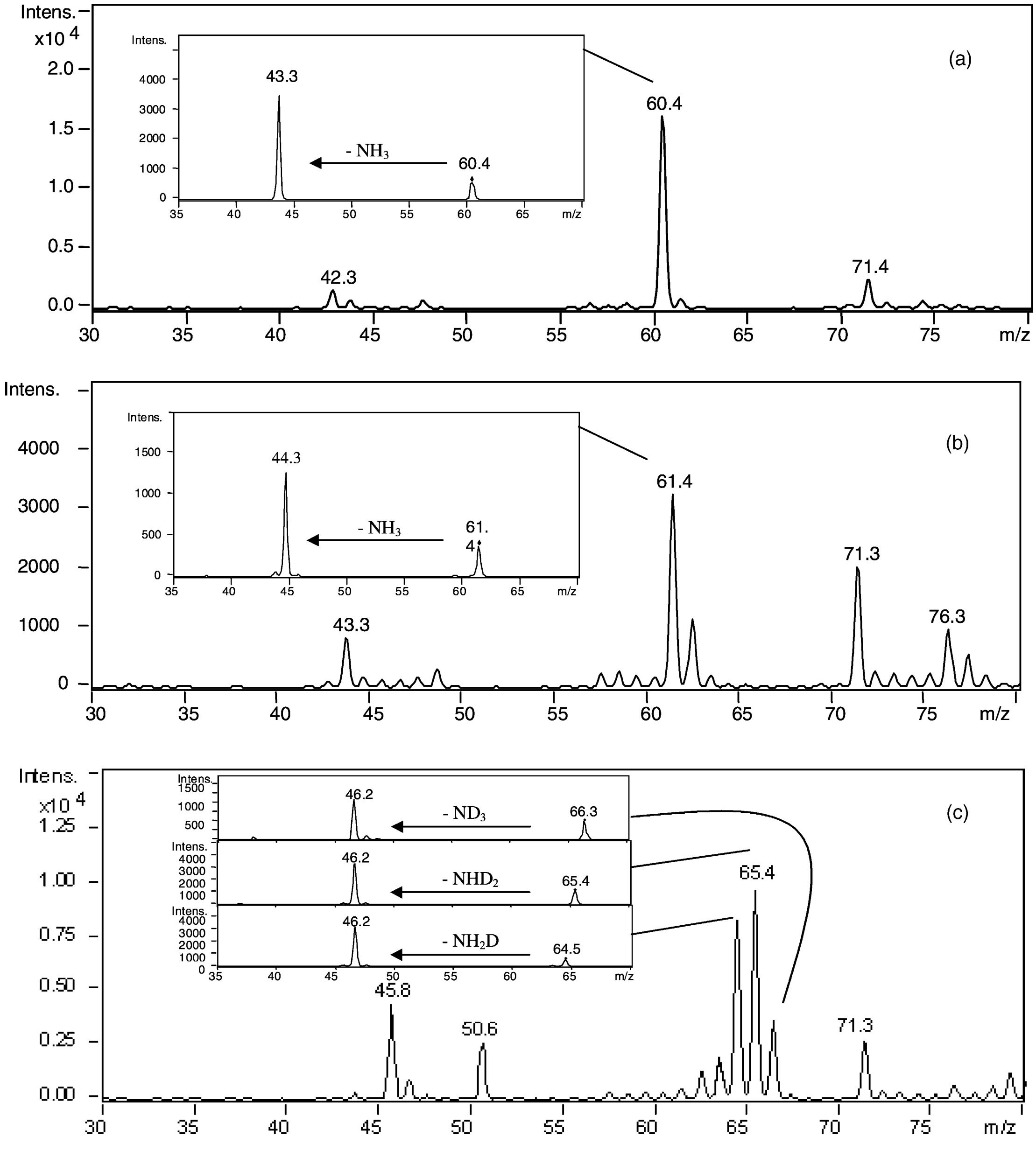


Fig. 3. (a) APPI mass spectrum obtained by nebulizing CH_3CN. In the inset, the MS/MS spectrum of the ion at *m*/*z* 60 is shown. (b) APPI mass spectrum obtained by the nebulization $^{13}CH_3CN$. In the inset, the MS/MS spectrum of the ion at *m*/*z* 61 is shown. (c) APPI mass spectrum obtained by nebulizing CD_3CN. In the inset, the MS/MS spectra of the ions at *m*/*z* 66, 65, 64 are shown.

(ii) photon-induced isomerization of acetonitrile that leads to neutral species that exhibit an IE $<$ 10 eV;

(iii) photon-activated formation of an ion-pair (as detected in the CCl_4 APPI spectrum).

Point (iii) is to be excluded, due to the complete lack of signals in the negative ion mode.

Point (i) must be considered. In fact, the most abundant ion in the spectrum of Fig. 3a, detected at *m*/*z* 60, is due to the protonated complex of CH_3CN and

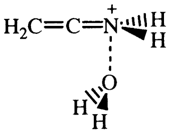
H_2O. It must be hypothesized that the $[CH_3CN{\cdot}H_2O]$ complex, already present in the solution, is nebulized and, if exhibiting an IE value lower than 10 eV, is ionized. The $[CH_3CN{\cdot}H_2O]^{\bullet+}$ ion could protonate neutral complexes, leading to the ion at *m*/*z* 60. Unfortunately, IE data on the $[CH_3CN{\cdot}H_2O]$ complex are not available and, consequently, experimental evidence for the validity of point (i) cannot be given.

Point (ii) assumes that the interaction photon-acetonitrile leads to an excited state that is able to activate isomerization reactions (see Scheme 1). Structures **a**–**d** reported in Scheme 1 would represent, in our opinion, the most reasonable isomers generated by this phenomenon. Interestingly, the ionization energy of structure **a** [18], is 10.1 eV very close to the photon energy employed in the APPI source. Hence, structure **a** could lead, by irradiation, to production of ionic species. Unfortunately, for structures **b**–**d** experimental data on the ionization energy are not available, but, in our opinion structure **c** could be assumed as the most reasonable photoionization product of acetonitrile.

However, the odd-electron molecular ion **c** cannot be invoked as a protonating medium: the further hypothesis that this ion reacts with some neutrals, giving rise to an acidic species, is needed. In this frame, the reaction of **c** with neutral acetonitrile (reaction (1)) seems to be the most probable one.

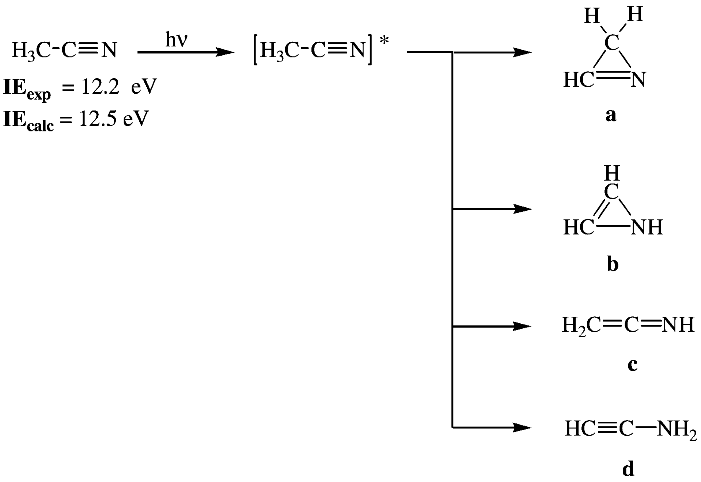
$$H_2C{=}\underset{\mathbf{c}}{C}{=}NH^{\bullet+} + H_3C{-}C{\equiv}N$$
$$\rightarrow H_2C{=}\underset{\mathbf{e}}{C}{=}NH_2{}^{+} + H_2C^{\bullet}{-}C{\equiv}N \qquad (1)$$

The even-electron ion **e** is particularly stable: in this structure, the positive charge can be stabilized either by the nitrogen doublet or by the π-electron cloud. The presence of ion **e** can rationalize the presence of the ion at *m*/*z* 42 in the spectrum of Fig. 3a, as well as the formation of $[M + H]^+$ of compound **1** discussed above; however, for the abundant ion at *m*/*z* 60, further hypotheses must be proposed. It could be assigned to the formation of a complex structure **f** of ion **e** with the H_2O, that is present at a trace level inside the source.



f

It must be emphasized that structure **f**, constituted by protonated acetonitrile and water, formally corresponds to the same ionic species discussed in point (i). However, in this case its formation is justified by



Scheme 1.

a reaction that occurs after the generation of ion **c**. Structure **f** represents an ionic species already discussed as present at stratospheric level and generated in drift-tube experiments. Even in the softer ionization conditions present in APPI with 10 eV photons, its formation is observed, giving account for its high stability, reasonably due to the strong interaction between the electronegative water oxygen and ammonium cation.

In order to investigate the validity of the proposed structure, a series of further experiments, based on collisional activation and isotope labelling, were undertaken.

The ion at *m*/*z* 60, when collided, generates only one product ion at *m*/*z* 43, corresponding to the loss of a neutral species of 17 u (see Fig. 3a). Considering the chemical composition of the precursor, two different neutral species can be considered, i.e. $^{\bullet}OH$ and NH_3. Accurate mass measurement would immediately lead to the identification of the neutral species lost, but unfortunately they cannot be performed on the available instrument. Hence, in order to verify all the above proposals (at this stage, only reasonable hypotheses) we performed a series of analogous experiments with stable isotope-labelled compounds.

When $^{13}CH_3CN$ was nebulized inside the APPI source, the spectrum reported in Fig. 3b is obtained. The protonated acetonitrile, originally at *m*/*z* 42, is shifted to *m*/*z* 43 and the ion **f**, originally at *m*/*z* 60, is shifted to *m*/*z* 61. These shifts prove the implication of only one acetonitrile molecule in the formation of ion **f**. The MS/MS spectrum of the ion at *m*/*z* 61 (see inset in Fig. 3b) shows the formation of a fragment at *m*/*z* 44, which corresponds to the loss of the 17 u species.

When CD_3CN is nebulized in the APPI source a quite complicated spectrum is obtained (see Fig. 3c). The $C_2D_4N^+$ ion is detected at *m*/*z* 46. Its presence is a confirmation of the validity of the hypothesis reported in reaction (1); i.e., that ion **c** originates from reaction of two acetonitrile species. In this case, the peak cluster due to ions **f** (in the unlabelled compound at *m*/*z* 60) becomes quite complicated and shows abundant peaks at *m*/*z* 64, 65 and 66. The first ion just corresponds to the complex of $C_2D_4N^+$ and H_2O, whereas the second and the third ions correspond to the complex of $C_2D_4N^+$ ion with HDO and D_2O, respectively. It must be emphasized that the presence of proton exchange in a high-pressure source is a phenomenon that easily occurs and that explains well the presence of HDO and D_2O.

The MS/MS spectra of the species at *m*/*z* 66, 65 and 64 are reported in Fig. 3c. They all show the formation of the same product ion at *m*/*z* 46. Losses of neutral species of 20, 19 and 18 u, respectively, are observed. The first two processes can be explained by only invoking the loss of ND_3 and ND_2H and/or $^{\bullet}OD$. Considering that the $^{\bullet}OD$ radical loss would be an exception to the even-electron rule [19], the loss of ammonia, with a different degree of deuteration, due

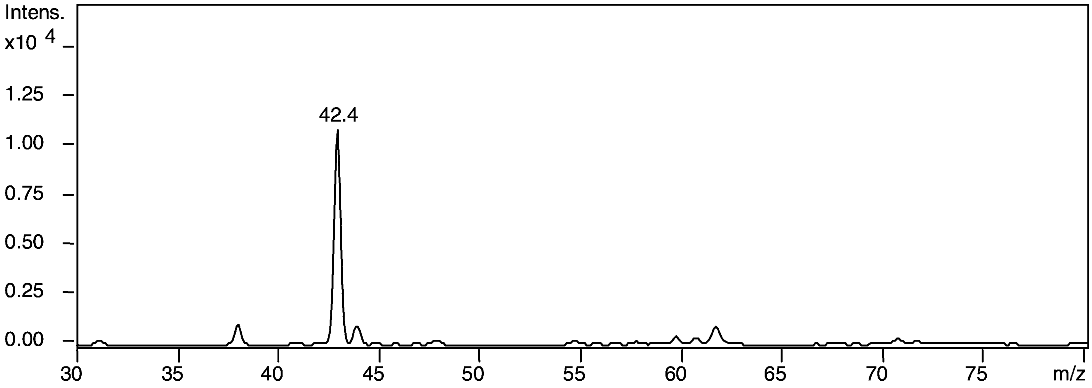


Fig. 4. APCI mass spectrum obtained by nebulizing CH_3CN.

to D/H exchange with the water molecules present in ion **f**, results to be the most reasonable one.

In order to be confident that the ionic species discussed above originate by photoinization reactions, a further series of measurements was performed under APCI conditions. In this case, as shown by the spectrum reported in Fig. 4, the production of protonated acetonitrile is highly favoured, and leads to the ion at *m*/*z* 42. The ion at *m*/*z* 60, the base peak in the APPI spectra of acetonitrile, has practically disappeared; that result demonstrates its photochemical origin.

In conclusion, the experimental data show that the ionization mechanisms present in an APPI source are not only those due to simple and direct photoionization phenomena, but to a series of unexpected photon-induced reactions that produce leading to reactive species. Ions **c** and **f** can both be considered responsible for the protonation of furocumarins when their acetonitrile solutions are nebulized in an APPI source. These results do not impair the high analytical power of the APPI method, but rather can be considered as a standing point to rationalize the behaviour of analytes that are examined in these conditions. Works are now in progress to clarify the ionization mechanisms that operate when a dopant is employed in APPI measurements.

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