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Atmospheric pressure photoionization☆ II. Dual source ionization

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Abstract

In this paper we describe results based on the combination of atmospheric pressure photoionization (APPI) with atmospheric pressure chemical ionization (APCI) and electrospray ionization (ESI). The main purpose of combining more than one ionizer is to extend the range of compounds that can be simultaneously analyzed. Three modes of operation are presented; use of either ionizer, simultaneous use of two ionizers, and rapid switching between ionizers during a single chromatographic run. The dual ionizer configurations only minimally affect the performance of either ionizer relative to the standard single-ionizer sources. However, it is observed that the operation of both ionizers together does not typically give the sum signal from either source operating alone. For APCI/APPI the signal can range from less than that of either source alone to the sum of the two individual sources. For ESI/APPI, we observed large suppressions of the ESI multiply-charged signal of proteins when the APPI source was on. These behaviors are presumed to be due to the interaction of the initially formed ions by both sources and attests to the importance of ion–molecule reactions that occur during and after the primary ionization events. We give examples of compounds that are preferentially ionized by either APPI, APCI or ESI and present thermochemical arguments based on molecular structure and functionality to explain this behavior. The dual source is also shown to be able to operate in negative ion mode opening up the potential to conduct wide ranging chemical analyses.

Keywords: Photoionization; Atmospheric pressure photoionization

1. Introduction

The use of atmospheric pressure photoionization (APPI) for LC–MS analysis has grown considerably over the last 2 years and is now an important tool for the analytical chemist. In this paper we report on the implementation of dual ionization sources using APPI. The main focus is on results recorded with a dual source involving APPI and atmospheric pressure chemical ionization (APCI). Preliminary results are also presented for a dual APPI/electrospray ionization (ESI) source. This work examines the potential for operating complementary ionization sources either simultaneously or in automated switched mode to broaden the range of compounds that may be ionized simultaneously. In this work we are also concerned with the interactions of the two sources with regard to the signals observed in dual-mode relative to the sum of the individual modes. A further goal of this work is to explain the preferential ionization efficiency of different compounds by these ionization methods based on a thermochemical model that takes into account the molecular structure and functional groups and the role of ion–molecule chemistry in the ionization region.

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The application of multiple ionization sources for mass spectrometry precedes LC–MS. The most widespread implementation is the combination of electron ionization (EI) and chemical ionization (CI) used primarily for GC–MS applications [1], although other combinations have been developed including an EI/PI source [2]. Dual ionization sources for LC–MS based on APPI, APCI and ESI have recently been reported by Horner et al. [3] Jackson et al. [4] and by Kavarik et al. [5], respectively. Fig. 1 summarizes the processes of ESI, APCI and APPI. In positive ionization mode both APCI and ESI rely on the proton affinity of the molecule. Characteristics of APCI and ESI include:

(i) Ionization efficiencies are sensitive to charge affinity and many compounds, particularly those that are non-polar, are only weakly detectable.
(ii) Ionization of target analyte can be suppressed by compounds that have higher charge affinities. This problem is especially acute with ESI.
(iii) Adducts (e.g., with Na^+) form readily with compounds during ESI and charge-bearing salt complexes can contribute to chemical background.

As illustrated in Fig. 1, photoionization (PI) is not based on charge affinity and therefore can be viewed as relatively orthogonal to ESI and APCI, particularly with regard to its propensity to ionize non-polar compounds. The general process of PI, including APPI, is a gas-phase ionization method that requires volatilization of analyte in the same manner as APCI. The primary event is production of a molecular radical cation $M^{\bullet+}$ (we drop the centerdot denoting radical from further discussion). The condition for photoionization is whether the photon energy exceeds the ionization energy, which explains the similar ionization efficiency for polar and non-polar compounds alike as well as evident tolerance of matrix additives that can interfere with the mechanism of ESI and APCI [6–8]. Because PI is not based on the charge affinity of molecules, its susceptibility to ion suppression is reduced relative to ESI and APCI. The presence of surrounding molecules (e.g., Y versus X in Fig. 1) does not directly impede the ionization efficiency of a particular analyte compound. It should be noted however, that APPI is not immune to ion suppression since a photoion once formed can undergo ion–molecule chemistry that could suppress the ion abundance. The common reaction of the initially formed molecular radical cation M^+ to MH^+ by hydrogen abstraction from abundant solvent is one example of subsequent ion–molecule chemistry [9], though this reaction is relatively innocuous because it still leads to an identifiable ion.

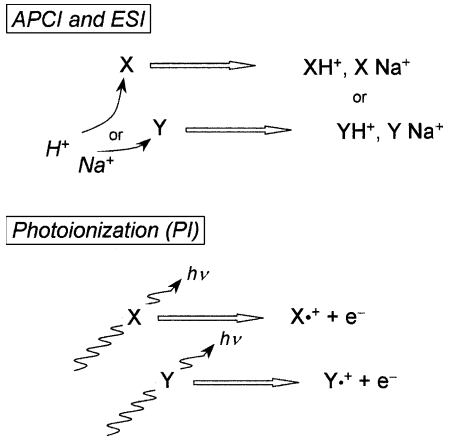

Fig. 1. Schematic diagram illustrating the ionization process for APCI and ESI vs. PI. Whereas APCI and ESI depend on the charge affinity of molecules, PI depends on ionization potential. The molecular ions $X^{\bullet+}$ and $Y^{\bullet+}$ are shown as radical ions (the centerdot is dropped through the remainder of the paper).

There are three main goals in this work. (1) Measure the properties and characteristics of dual ionization sources involving APPI (i.e., APCI/APPI and ESI/APPI). These measurements include the performance in dual-mode versus individual modes. (2) Examine the complementarities between pairs of ionizers for the choices of ESI, APCI, and APPI. The objective is to understand which pair offers the greatest orthogonality not just with regard to compound coverage, but for a wide range of instrument conditions such as flow rate. (3) Develop an understanding of the different ionization efficiencies that are observed for different compounds by ESI, APCI, and APPI. Some of these differences are flow rate and solvent dependent. We develop a thermochemical model as a starting point to better understand these differences.

Finally, we mention that the APPI work presented here is based on the "direct" method as compared to the "dopant" method introduced by Bruins and co-workers [10]. However, the APCI/APPI and ESI/APPI dual source configurations described here can presumably operate with dopants, provided that they do not interfere with the operation of APCI and ESI.

2. Experimental

2.1. APCI/APPI dual source and MS instrumentation

The dual APCI/APPI results were obtained using either a Thermo Electron LCQ Deca XP Plus ion trap MS or a Thermo Electron Quantum triple quadrupole MS. A Thermo Electron Surveyor autosampler and HPLC system were used for injection and solvent delivery. These dual sources include the Syagen PhotoMate APPI source. The APPI source is based on a radiofrequency (RF) discharge of a gas mixture consisting primarily of Kr and operates on the atomic emission lines at 10.0 eV and 10.6 eV. The gas composition and pressure were optimized for maximum radiant output. The RF driver and coil were designed and optimized for maximum and most efficient coupling of power into the plasma. A three-dimensional (3D) rendering of the APCI/APPI source is illustrated in Fig. 2 for both the LCQ and the Quantum instruments. The lamp is mounted to irradiate approximately the same volume that is ionized by the corona needle of the APCI source. As for the APCI source, the APPI source relies on vaporization of the analyte by the nebulizer/vaporizer unit.

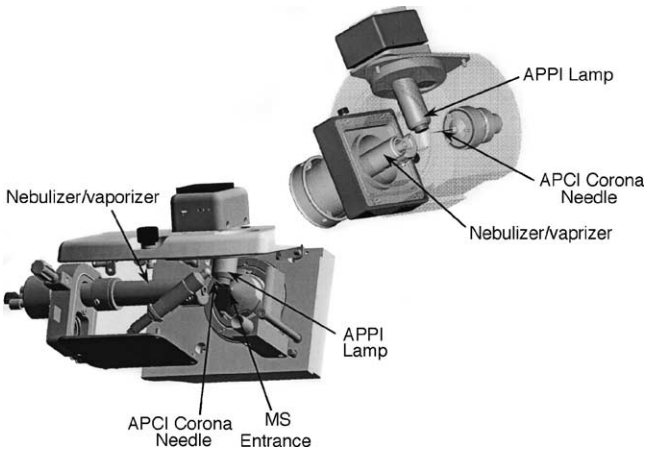

Fig. 2. Dual APCI/APPI source configurations for the Thermo Electron Quantum triple quadrupole MS (left) and the LCQ ion trap (right).

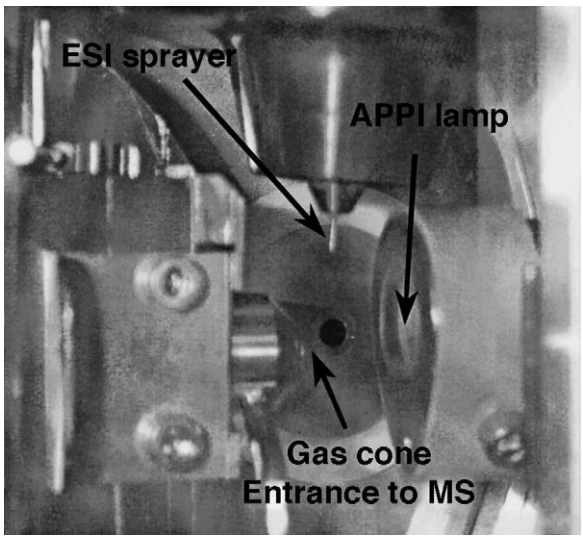

Fig. 3. Dual ESI/APPI source configuration for the Waters ZQ single quadrupole MS. This configuration allows rapid alternation of the PI lamp and ESI electrode voltage permitting switch times within the interscan delay time of 10–20 ms per scan.

Optimum ion abundance for the APPI source was observed by placing the lamp upstream of the mass spectrometer inlet in the direction of the vaporizer. For the Thermo Electron LCQ and Quantum, the lamp was aligned orthogonal to the plane defined by the MS inlet axis and the nebulizer/vaporizer axis.

2.2. ESI/APPI dual source and MS instrumentation

The dual ESI/APPI results were recorded on a Waters ZQ quadrupole and a Micromass LCT MS instrument. The ESI/APPI source does not use an APCI nebulizer/vaporizer unit, but rather relies on the vaporization of analyte achieved by the heated, nebulized ESI source on the Waters ZQ. A heated sheath flow of nitrogen is provides the evaporative assist. A picture of the configuration is shown in Fig. 3. A principal difference between the Thermo Electron and the Waters version of the APPI source is that the later source has a ring electrode around the lamp window that assists the transmission of ions toward the MS inlet aperture.

2.3. Dual ionizer switching

One of the main utilities of a dual source is the opportunity to switch sources during a chromatographic run. Three modes of operation are available; APPI only, APCI only, and APPI and APCI operating simultaneously (ESI instead of APCI for the dual ESI/APPI source). For the APCI/APPI source used here on the Thermo Electron instruments, the APPI source was switched manually and the APCI source was computer controlled. The experimental ESI/APPI source used here had complete software control. The ESI source could be switched on and off by switching the ESI capillary voltage. APPI was switched by turning the lamp on and off. Furthermore voltage for the APPI ring electrode was provided by the APCI corona needle source (APCI was disengaged for this study). The circuitry for switching between ESI and APCI in the stock source was then used to switch the ESI and APPI source in the present configuration.

Unless otherwise noted all results in this paper were obtained by direct, rather than dopant APPI. The structures of the compounds reported in this paper are given in Fig. 4. The structures of compounds DHPE, PDP-Ac, and TBPA are proprietary and are not shown.

3. Results and discussion

3.1. APCI/APPI

3.1.1. Results

In this subsection we present a body of data comparing ionization efficiencies of a broad range of compounds by APPI versus APCI under various solvent conditions. In the following subsection we provide a thermochemical basis for understanding the differing ionization efficiencies for these compounds by the two ionization methods.

The main benefit of a dual ionization source is to expand the range of compounds that can be analyzed without changing sources and more preferably with automatic switching during a single chromatographic run. APPI generally has better ionization efficiency for non-polar compounds and other classes of compounds compared to APCI and ESI. Fig. 5 shows an example of this for the compound DHPE recorded on the Thermo Electron LCQ using flow injection. The extracted ion chromatogram (XIC) traces for the parent ion MH^+ at m/z 202 show considerably better signal/noise (S/N) ratios for APPI relative to APCI. The ion abundance is about an order of magnitude greater as seen in the mass spectra in Fig. 5. It is also evident that mass spectral S/N is higher for APPI due to the strong signal of the target compound and not

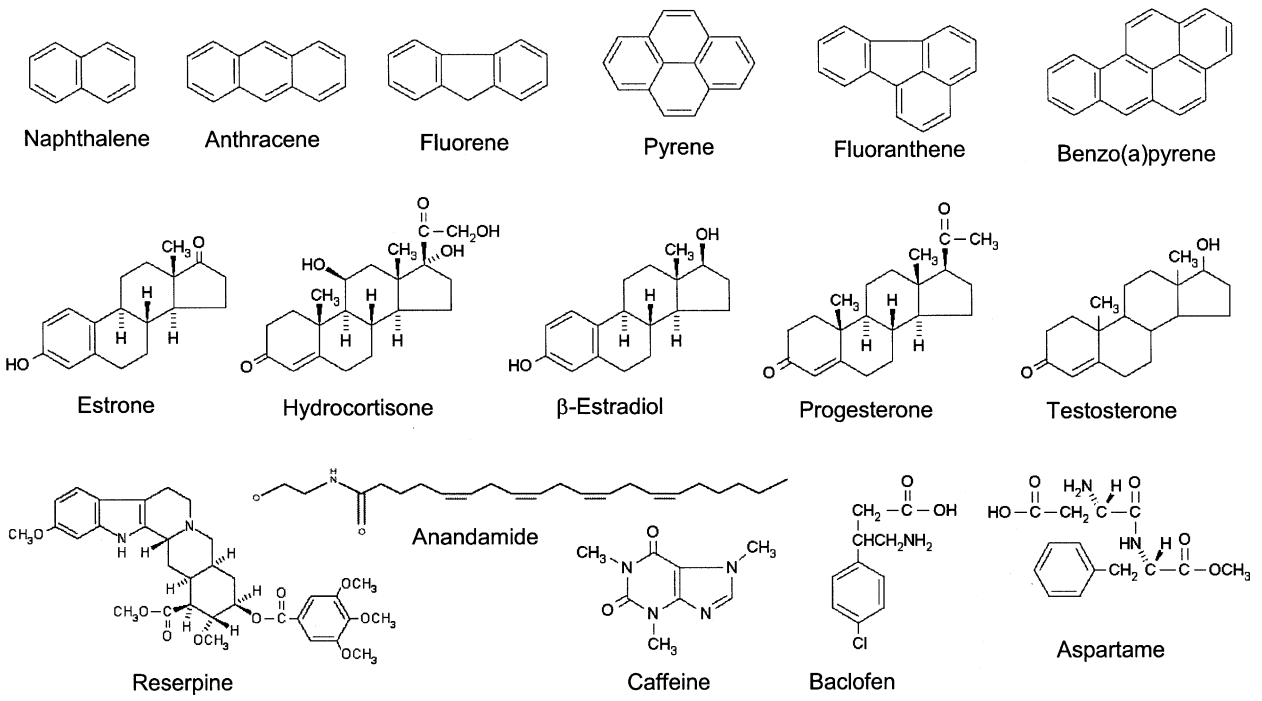

Fig. 4. Structures of compounds studied in this work.

because of diminution of the background compound signal that is evident in the APCI mass spectra.

Though Fig. 5 shows an example where APPI would be the preferred ionizer for DHPE, it is also the case that APCI will provide better signal than APPI for certain compounds. Fig. 6 shows XIC traces for two compounds injected as a mixture, PDP-Ac (top) and TBPA (bottom) obtained in three different modes of operation: APPI-only (first set of peaks), dual-mode (second set of peaks), and APCI-only (last set of peaks). The APPI source is turned on and off using a manual switch on

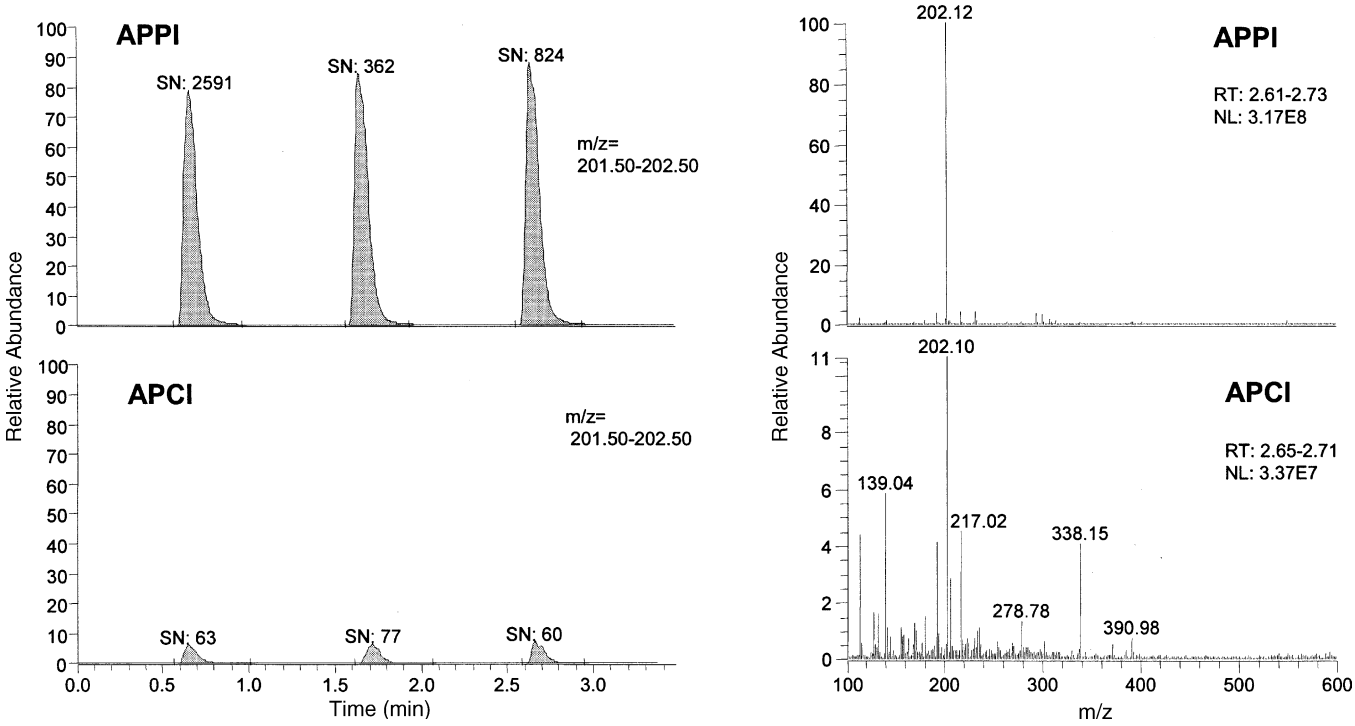

Fig. 5. Extracted ion chromatograms (XIC) for three successive injections of DHPE recorded by APPI and APCI (recorded on the LCQ). The corresponding full scan mass spectra for the third injection are also shown. Conditions were 278 ppm, 10 μL injections, 500 μL/min flow rate, mixture of MeOH–water (90:10).

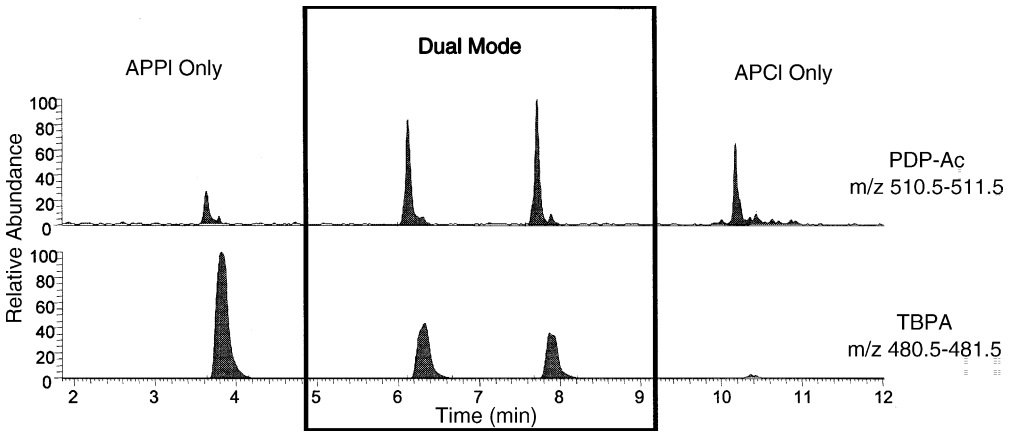

Fig. 6. XIC traces for PDP-Ac and TBPA for APPI only, dual-mode, and APCI only mode (recorded on the LCQ). This example shows the utility of the dual-mode for simultaneously detecting both compounds. Conditions were 13.7 ppm TBPA and 86 ppm PDP-Ac, 10 μL injections, 400 μL/min flow rate, mixture of MeOH–water (50:50).

the ionizer driver box. The APCI source is turned on and off through the existing software. By APPI only, TBPA is efficiently detected, but PDP-Ac is relatively weakly detected. By APCI only, PDP-Ac is efficiently detected, but TBPA is relatively undetected. These results suggest that APPI and APCI are complementary sources for the efficient detection of these compounds. When operated in dual-mode both compounds are efficiently detected. It is interesting to note, however, that although the PDP-Ac signal in dual-mode appears to be the sum of the signals in single-mode, the dual-mode signal for TBPA is less than the sum of the single-mode signals. In some cases the dual-mode signal is greater than the sum of the single-mode signals. This suggests that there may be some interference in the operation of APPI and APCI simultaneously. This is not unexpected since the generation of ions by both mechanisms can create new possibilities for ion–molecule chemistry and ion–electron recombination. We have not attempted to elucidate the details of these competing mechanisms at this time.

The detectability of steroids by APPI is relatively more efficient than by APCI or ESI. For example the relative signal strengths of XIC traces for a flow injection of 500 pg of estrone was recorded by the three ionization methods (not shown). Recorded on a Thermo Electron LCQ, APPI yielded an MH^+ signal that was about five times stronger than by APCI and more than an order of magnitude stronger than by ESI.

Fig. 7 tabulates results for ion abundance by APPI-only, APCI-only, and dual-mode operation as a function of solvent conditions for four disparate compounds (naphthalene, caffeine, reserpine, and hydrocortisone). These results were recorded on a Thermo Electron LCQ and we expect some instrument dependence. When some of these conditions (compounds and solvent conditions) were run on other instruments (e.g., Agilent 1100 Series LC/MSD or Waters ZQ), different behavior was observed. For example, reserpine detection under MeOH–water conditions generally gives strong signal by APCI and APPI. However, the trends in Fig. 7 are of qualitative importance and lead to some useful observations:

(1) APPI generally excels over APCI for detection of nonpolar compounds such as naphthalene. APPI also performs well over a wider range of solvent conditions for naphthalene.
(2) The dual-mode of operation does not generally give the sum signal of APPI-only and APCI-only. Apparently the ionizers interact with each other presumably by forming a different distribution of ions and electrons that can change the distribution of ions that are formed by either ionizer alone. This interaction can be destructive as evidenced by the results for naphthalene in hexanes. Whereas APPI is effective at forming naphthalene ions (M^+ in this case), the presence of the APCI corona discharge not only fails to form significant abundance of naphthalene ions, but actually suppresses the APPI contribution in the dual-mode of operation. The mechanism for this interaction is not known, but may have to do with the greater number of free electrons present with the corona discharge on that could neutralize molecular ions such as M^+ of naphthalene.
(3) Solvent conditions interact with either the ionization process (certainly for APCI, if not so much for APPI) or the subsequent survival of analyte ions (through ion–molecule chemistry or other charge transfer mechanism). A good example of this is AcCN, which is observed to suppress ion signal for APPI and APCI in many cases [11]. This behavior has been observed on other instruments as well.

Based on the results of Fig. 7, it would appear, at least for the Thermo Electron LCQ, that operating in dual-mode may not always be the best mode of operation, but rather it may be desirable to operate in APPI-only and APCI-only in switched mode. This conclusion is also borne out in the qualitative comparison of the three modes of operation for a wider range of compounds summarized in Table 1. In almost

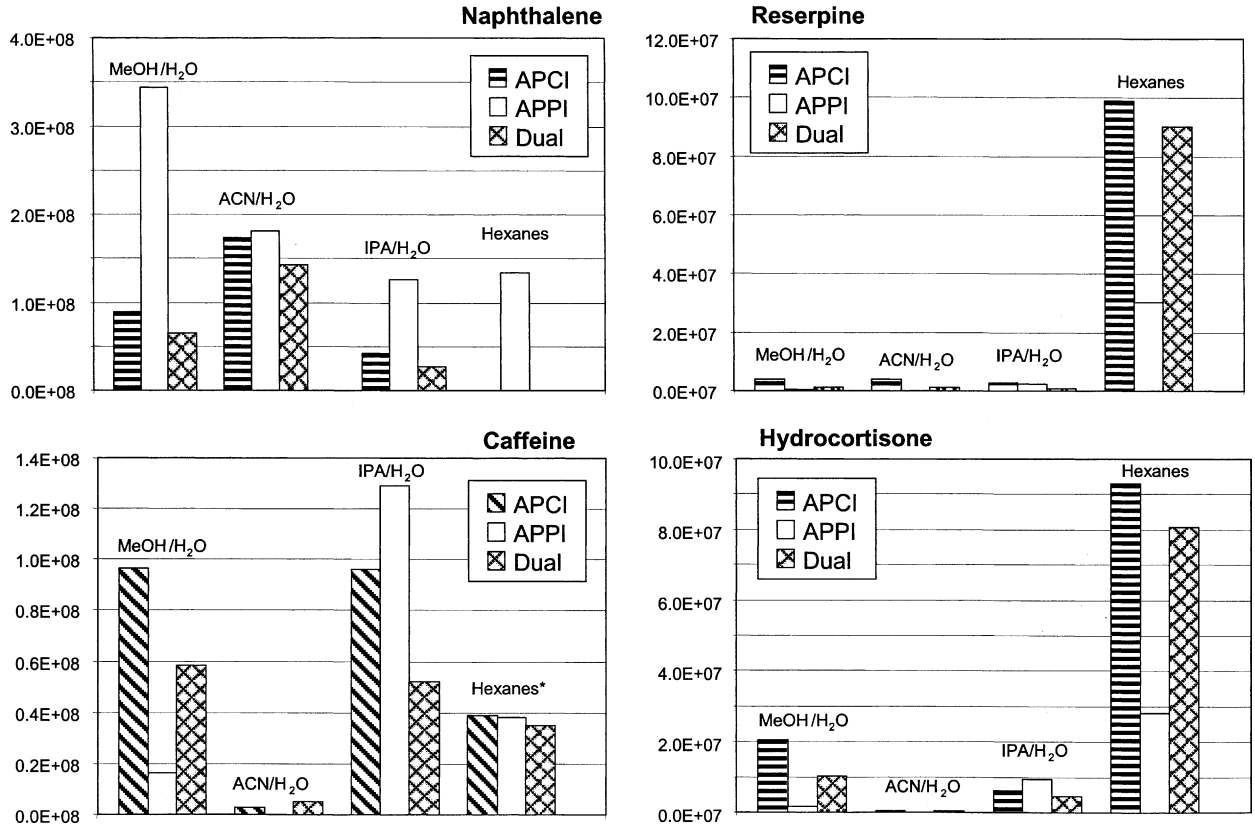

Fig. 7. Relative signal strengths for various compounds under different solvent conditions as measured by APPI-only, APCI-only, and dual-mode on the LCQ. Solvent mixtures are in a ratio of 50%:50% and flow rate was 200 μL/min. Injected quantities were 1 ng analyte in 10 μL solutions.

all cases, either APPI or APCI gives the strongest signal. The dual-mode case often gives comparably strong signal, but it is not typical for the dual signal to significantly exceed the signal from either APPI or APCI. On the other hand, it is also true that whereas either APPI or APCI may give the weakest signal of the three modes, the dual-mode seldom gives the weakest signal. A strategy to operate in dual-mode as a general practice may therefore prove successful. It should be noted that the results in Table 1 are strictly qualitative as the relative signal strengths of the different modes of ionization vary widely with solvent condition, flow rates, and instrument type. The guidance offered here is more to recognize the variation of behavior and anticipate this when operating with a specific instrument for a particular set of compounds. However, it should be clear that operating in either APCI/APPI switched-mode or in dual-mode greatly expands on the range of compounds that can be reliably analyzed.

Table 1
Qualitative comparison of sensitivity for APPI, APCI, and dual ionization[a]

Compound	Sensitivity comparison	
	100 μL/min	200 μL/min
Aspartame	APPI > dual > APCI	APPI ~ APCI > dual
Anthracene	APPI ~ APCI ~ dual	APCI ~ dual > APPI
Anandamide	APPI > dual ~ APCI	Dual > APCI > APPI
Baclofen	APCI > dual > APPI	APPI ~ dual > APCI
Benzopyrene	APCI ~ dual > APPI	APCI ~ dual > APPI
Caffeine	APCI ~ dual > APPI	APCI ~ dual > APPI
Fluoranthracene	APCI > dual > APPI	APCI ~ dual > APPI
Fluorene	APCI ~ dual > APPI	APCI ~ dual > APPI
Naphthalene	APPI ~ APCI > dual	APPI ~ APCI ~ dual
Pyrene	APPI ~ APCI ~ dual	APCI ~ dual > APPI
Reserpine	APPI > dual > APCI	APPI > dual ~ APCI

[a] Conditions: gradient 10–90% MeOH in 4 min, column, 50 mm × 2.1 mm 3 μm BDS Hypersil, injection volume 10 μL, concentration 100 pg/μL, flow rate 200 μL/min, solvents 10 mM NH_4OAc; MeOH, detection by XIC of dominant ion mass peak (typically M^+ or MH^+).

The linearity and reproducibility of the three modes of operation of the dual APCI/APPI source were investigated for flow injection of hydrocortisone and reserpine. Ion trap MS instruments (e.g., the Thermo Electron LCQ) exhibit reasonable linearity, but are not the ideal choice for quantitation. Nonetheless, the linearity for the APCI-only, APPI-only and dual APCI/APPI modes are comparable with χ^2 values ranging from about 0.95–0.997 for 1 pg to 1 ng injections at different flow rates. For the hydrocortisone measurements, the signal by APPI was slightly stronger than by APCI and interestingly the combined signal in the dual-mode was greater than the sum of the individual source signals; however, not by an amount that we would consider statistically significant. The relative standard deviations (R.S.D.s) for different injected quantities of hydrocortisone are summarized in Table 2. The point of these measurements is to show that the dual-mode of operation gives comparable linearity to either mode alone, however, the LCQ ion trap may not be the ideal

Table 2
Reproducibility for APPI, APCI, and dual ionization for hydrocortisone[a]

Injected mass (pg)	Reproducibility (R.S.D., %)		
	APCI	APPI	APCI/APPI
100 μL/min flow rate			
1	30.8	60.8	26.4
10	11.1	10.2	7.3
100	7.6	7.4	3.1
1000	5.1	6.3	2.4
200 μL/min flow rate			
1	17.0	55.5	13.3
10	12.6	12.5	3.4
100	3.0	2.4	2.7
1000	1.4	2.5	1.9

[a] Conditions: gradient 30–100% MeOH in 3 min, column, 100 nm × 1 nm, 5 μm BDS Hypersil C_{18}, injection volume 10 μL, solvents water; MeOH, detection by XIC of MH^+.

instrument for demonstrating linearity. In other work, it has been shown that APPI has an inherent linearity of at least four orders of magnitude [7].

The APPI and APCI sources give relatively similar reproducibilities as measured by the %R.S.D. values (the larger value for APPI at 1 pg is not considered significant as the signal strength is very weak in both cases). It is worth noting that the reproducibility of the combined APCI/APPI signal is generally better than for the individual sources. This coupled with the greater signal strength and generally improved S/N ratio argues for considering the dual-mode of operation as the preferred method for certain types of analyses, although as mentioned earlier, this may not always be the best mode of operation.

The dual APCI/APPI may also be operated effectively in negative ionization mode. Fig. 8 compares the mass spectral signal for the $[M–H]^-$ ion of β-estradiol recorded in negative ion mode on the Thermo Electron Quantum triple quadrupole MS. The mass spectra are shown for APCI only and by the simultaneous application of APPI and APCI. In the latter case, the signal was about a factor of 100 stronger, though this level of enhancement is atypical for general classes of compounds.

3.1.2. Thermochemical model and discussion

Though the mechanism of direct PI is straightforward, the subsequent ion–molecule chemistry that can occur at atmospheric pressure can be complicated. Likewise the ionization mechanism by APCI and subsequent ion–molecule chemistry is relatively complex. In order to develop a basis for understanding differences in ionization efficiencies as a function of molecular structure and to begin addressing the issue of ion suppression we start with a simple model for APPI and APCI.

The primary processes of direct PI of analyte molecule M at elevated pressures (e.g., atmosphere) are:

$$M + h\nu \rightarrow M^+ + e^-, \quad \Delta H_{PI} = IE(M) - h\nu \tag{1}$$

$$M^+ + S \rightarrow MH^+ + S(–H),$$
$$\Delta H = IE(H) - IE(M) - PA(M) + D_H(S) \tag{2}$$

where IE is ionization potential, $h\nu$ the photoionization energy, PA proton affinity and D_H is hydrogen bond energy. Reaction (2) is the mechanism for production of the MH^+ ion by PI and is treated in detail in another paper [9].

The primary process for positive ionization by APCI is given by:

$$M + SH^+(\text{or } S_2H^+) \rightarrow MH^+ + S\,(\text{or } 2S),$$
$$\Delta H_{CI} = PA(S \text{ or } S_2) - PA(M) \tag{3}$$

where we assume that the dominant charge carrier is protonated solvent SH^+. Because of the high abundance of solvent and the generally strong ion–molecule bond energies (typically 1 eV for hydrogen bonded molecules), we also consider the importance of the protonated solvent dimer S_2H^+ (larger complexes are considered significantly less abundant and does not warrant consideration here).

Table 3 contains the pertinent thermochemical data to estimate ionization efficiencies based on enthalpy. We include commonly used solvents. Unfortunately, there is insufficient data on proton affinities and ionization potentials for large molecules. We have therefore chosen a representative set of different types of molecules in order to obtain meaningful understanding of the ionization processes at atmospheric pressure. Our purpose is to assess the relative efficiencies of ionizing a variety of analyte molecules by APPI and APCI and to explore potential ion suppression effects. We consider two contributions to ion suppression (i) processes that interfere with the ionization step and (ii) subsequent ion–molecule chemistry that would diminish the analyte ion abundance.

Table 3
Compilation of thermochemical data and calculation of enthalpies for ionization by APPI and APCI[a]

Compound	PA	IE	ΔH_{PI}[b]	ΔH_{CI}, $n = 1$[c]	ΔH_{CI}, $n = 2$[c]
MeOH	7.89	10.85	0.9	0.0	1.5
$(MeOH)_2$	9.34	10.38[d]	0.4	−1.5	0.0
Water	7.36	12.61	2.6	0.5	2.0
Acetonitrile	8.17	12.19	2.2	−0.3	1.2
DMSO	9.16	9.01	−1.0	−1.3	0.2
Anthracene		7.45	−2.6		
Naphthalene	8.44	8.14	−1.9	−0.6	0.9
Benzene	7.86	9.25	−0.8	0.0	1.5
Phenol	8.51	8.47	−1.5	−0.6	0.8
Aniline	9.09	7.72	−2.3	−1.2	0.3
m-Chloroaniline	8.98	8.09	−1.9	−1.1	0.4
1-Aminonaphthalene	9.41	7.1	−2.9	−1.5	−0.1
Toluene	8.23	8.82	−1.2	−0.3	1.1
TNT		10.59	0.6		

[a] Unless otherwise stated, values are from (a) ref. [28], (b) ref. [29].
[b] Based on photon energy of 10 eV.
[c] $n = 1$ and $n = 2$ refer to charge carriers $(CH_3OH)H^+$ and $(CH_3OH)_2H^+$, respectively.
[d] Estimate based on determination in ref. [30].

First we examine APPI. Table 3 shows that all compounds chosen are exothermic with respect to ionization with the exception of TNT. We chose this latter compound to dramatize the effect of electron withdrawing groups on raising the ionization potential of molecules. In general, though, as molecules are larger, the IE values are lower as seen by the values for the series anthracene, naphthalene, and benzene. In effect, this means that a wide range of large molecules, including almost all classes of drug compounds should be ionizable by PI. With regard to ion suppression, the simple model for PI does not predict any processes that would interfere with the primary ionization step. However, ion suppression, can occur subsequent to photoionization by ion–molecule chemistry. Reaction (2) involving solvent is a form of such a reaction, however, the product ion MH^+ is readily identifiable and the reaction is not considered a suppression effect. Some solvents can induce ion suppression; an example is dimethyl sulfoxide (DMSO) due to its low IE value. For analyte M with IE(M) > IE(DMSO) (i.e., >9.16 eV) could undergo charge exchange whereby M^+ + DMSO → M + $DMSO^+$. However, most larger molecules will have IE values less than 9.16 eV and therefore not suffer this fate. Because DMSO has a high proton affinity, it can also cause ion suppression of protonated ions by the reaction MH^+ + DMSO → M + $(DMSO)H^+$. (Another potential ion suppression mechanism is the deprotonation of molecular ion M^+ by molecules R with high proton affinity to give M(–H) + RH^+. We plan to examine this mechanism in future work.)

The primary ionization mechanism for APCI is proton transfer as represented by reaction (3). This is a competitive process whose efficiency depends on the proton affinity of the analyte molecule M being greater than that of the charge carrier or any other compounds that might compete for charge. Table 3 considers the enthalpy of APCI (ΔH_{CI}) assuming monomer and dimer CH_3OH charge carriers. The calculated values of ΔH_{CI} are exothermic for $(CH_3OH)H^+$ charge carrier, but endothermic for $(CH_3OH)_2H^+$ charge carrier. Given the high abundances of $(CH_3OH)_2H^+$ and larger complexes generally seen in typical mass spectra, it is plausible that solvent ion complexation may pose an impediment to efficient APCI. We now consider ion suppression of APCI assuming DMSO as our model suppressor. Unlike for APPI, DMSO can interfere with the primary APCI ionization step. From Table 3 it is clear that ΔH_{CI}(DMSO) < ΔH_{CI}(M) is a common condition and because DMSO is generally in much greater abundance than analyte M, the charge carriers are far more likely to transfer the proton to DMSO than to M. For analyte ions MH^+ that do form, subsequent collisions with DMSO can lead to parasitic proton transfer MH^+ + DMSO → M + $(DMSO)H^+$.

3.1.3. Interpretation of results using thermochemical model

We now examine whether the observed results can be qualitatively explained by the simple thermochemical model above. First we should note that there are many factors besides the thermochemistry that contribute to ionization efficiencies, such as (i) APPI photon flux versus APCI charge flux, (ii) individual instrument characteristics, (iii) kinetic-driven versus thermodynamic driven processes and overall equilibrium considerations, and (iv) photon absorption by solvent, which is a function of flow rate, etc. Still, it is possible to discern qualitative trends and understand them from the context of the thermochemistry.

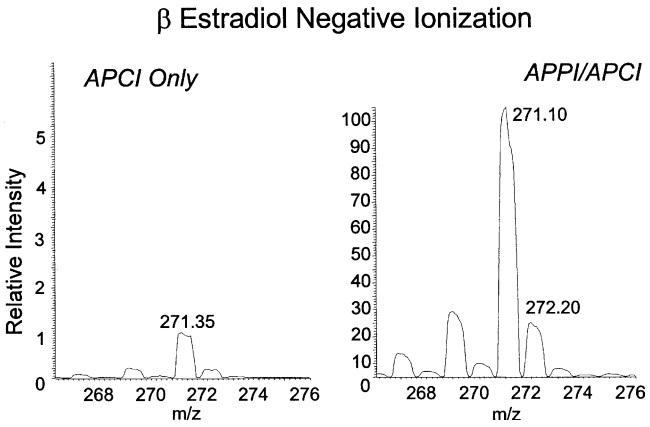

Fig. 8. Negative ion mass spectra of β-estradiol recorded by APCI-only and by APCI/APPI dual-mode qualitatively showing a 100-fold improvement in signal intensity by the latter mode (recorded on the Quantum).

APPI is favored by analyte compounds M that have low ionization potentials [Eq. (1)] and APCI by M that have high proton affinity [Eq. (3)]. For APPI the condition is a step function and we expect a similar ionization efficiency over a wide range of compounds, whereas for APCI the ionization efficiency will depend on the proton affinity. We expect APCI < APPI for non-polar compounds as well as polar compounds with functional groups that decrease proton affinity (e.g., electron withdrawing groups) and we expect APCI > APPI for compounds with functional groups that increase proton

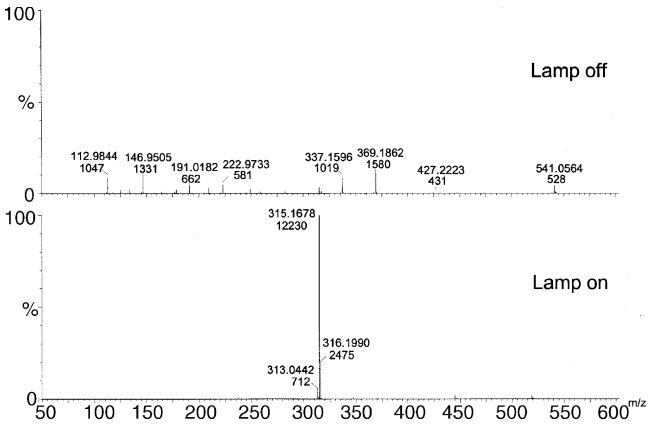

Fig. 9. Positive ion mass spectra of progesterone (100 ng/μL) recorded for the dual ESI/APPI source on the Waters LCT. The top spectrum is in ESI-only mode and the bottom spectrum is in dual-mode. Spectra were recorded by flow injection analysis (5 μL sample injection) for mixture of methanol–toluene (95:5) solvent. Both mass spectra are on the same absolute intensity scale.

affinity (e.g., base groups and electron donating groups). The importance of the electron donating/withdrawing properties of substituents can be seen in Table III for aromatic compounds. For example *m*-chloroaniline has proton affinity that is lower than that of aniline by 0.11 eV/molecule (2.5 kcal/mol). The physical explanation is that electron withdrawing groups leave a net positive charge on the remainder of the molecule that can destabilize the bonding of a proton, whereas electron donating groups provide negative charge that can stabilize a proton.

We are now equipped to evaluate the differences in ionization efficiency for APPI and APCI. Though the structures in Figs. 5 and 6 are proprietary, it can be stated that DHPE is a non-polar compound and TBPA has multiple bromine substituents. That the ions of these compounds are weakly observed by APCI and strongly observed by APPI is consistent with the above qualitative predictions. The next set of compounds studied were steroids, which are characterized by lack of strong base groups such as amines. This class of compounds is often difficult to ionize by APCI and ESI. The general observation in the results in this paper are that these compounds are efficiently ionized by APPI (Figs. 7–11), though not as efficiently ionized as other types of compounds (Fig. 10). In comparison with APCI, it is generally observed that APPI > APCI in ionization efficiency (e.g., estrone, Section 3.1.1). Finally we refer to Table 1 for more comparative ionization efficiencies for non-polar and other classes of compounds. We consider the results for the lower flow rate (100 μL/min) in order to minimize effects due to absorption of photons by the solvent. At the most non-polar end of the scale are naphthalene and anthracene. As anticipated, their proton affinities are relatively low and the enthalpy of ionization by APCI are unfavorable as tabulated in Table 3. Indeed, in terms of ionization efficiency, APPI > APCI as is also the case for pyrene. In other work on another instrument, we have noted benzo[a]pyrene to be more efficiently ionized by APPI than by APCI [7], but not for the LCQ for unknown reasons. It is more difficult to predict comparative ionization efficiencies for the intermediate cases of proton affinity because of inadequate thermochemical data and other factors as noted above. We comment instead on selective cases where APPI > APCI. This includes aspartame, anandamide, and reserpine. We have noticed a general trend that compounds with phenolic and carbonyl groups are not efficiently ionized by APCI (unpublished) and this might explain the results for aspartame and anandamide. Phenol is a weak acid and therefore expected to be less efficiently ionized relative to a more basic compound such as aniline (Table 3). It is not clear why APPI > APCI for reserpine; however, we have observed this relationship to be reversed for other instruments, so the result here may be due to factors other than the thermochemistry.

It is not reasonable to expect full predictability from a simple thermochemical model. However, it is important to begin developing a foundation for understanding the relative efficiencies of APPI and APCI in order to optimize the techniques and to better design methods based on them.

3.2. ESI/APPI source

3.2.1. General properties

APPI and ESI are relatively orthogonal ionization sources with regard to the range of compounds to which each source is most suited. This makes APPI and ESI potentially a more complementary combination of sources than APPI and APCI. This potential was explored by integrating an APPI source to an ESI source on a Micromass LCT and on the Waters ZQ. In this arrangement, the heated ESI source is used to deliver and vaporize the analyte. As mentioned earlier, APPI is a gas-phase ionization technique whereas ESI is a liquid-phase ionization technique. It is therefore necessary to thermally assist the ESI evaporative mechanism to achieve operation at reasonably high flow rates.

The ESI results presented here are for the dual ESI/APPI source with the lamp off so that the only functioning ionization mechanism was ESI. On the other hand the APPI signal was originally thought to require the ESI source on to successfully volatilize the particles. The ESI voltage however was reduced from 3800 V to 2800 V from ESI to APPI mode. Fig. 9 compares the spectral shape for progesterone for ESI (upper frame) to ESI/APPI (lower frame). The ESI/APPI sig-

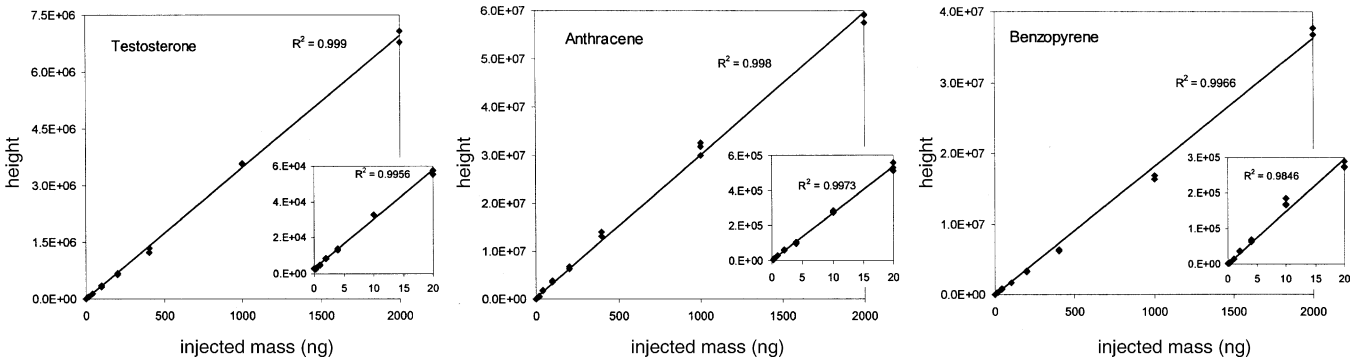

Fig. 10. Linearity plots for three compounds recorded in APPI-only mode using the standard Waters thermally-assisted ESI source for sample delivery and volatilization (recorded on the ZQ). Sample delivery was by flow injection analysis using 2 μL injections at 100 μL/min flow rate. Solvent was a mixture of MeOH–water (90:10).

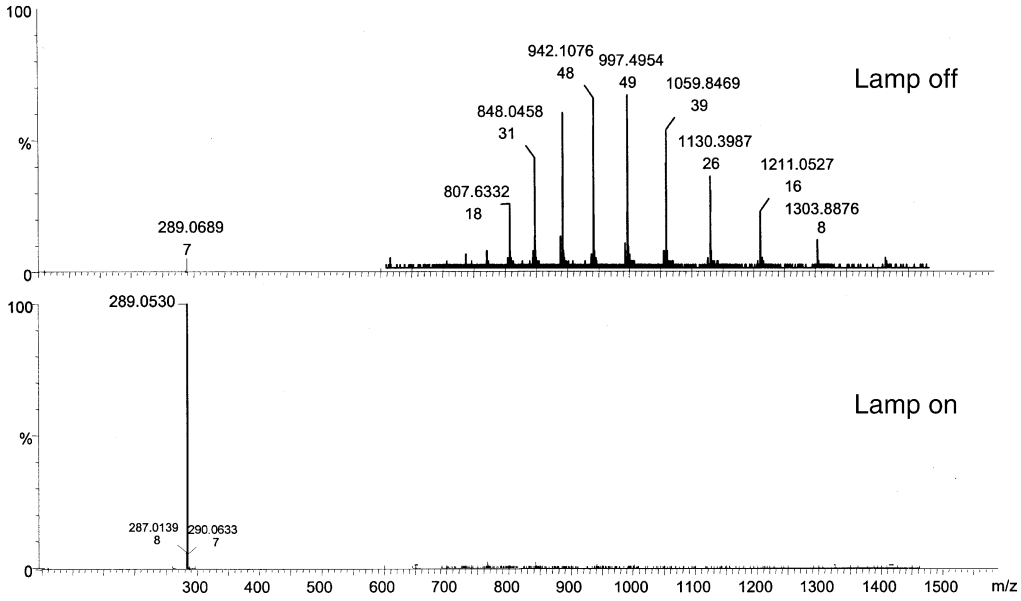

Fig. 11. Positive ion mass spectra of a mixture of testosterone (10 pg/μL) and myoglobin (10 nmol/μL) recorded for the dual ESI/APPI source on the Micromass LCT. The top spectrum is in ESI-only mode and the bottom spectrum is in dual-mode. Spectra were recorded by flow injection analysis (5 μL sample injection) using a mixture of MeOH–toluene–formic acid (95:5:0.1). The ESI capillary voltage was 3.8 kV for the top spectrum and 2.8 kV for the bottom spectrum. This difference in voltage is not enough to eliminate the ESI-only signal; the loss of the protein multiply-charged signal is correlated with turning the lamp on.

nal leads to strong $[M + H]^+$ ion signal while the ESI shows several ions and a very weak parent ion. Similar results were obtained for testosterone. The ESI data show Na^+ adduct signal. However, the mystery is why the many peaks that appear in both ESI spectra fortuitously disappear from the spectrum when the lamp is turned on. This intriguing phenomenon needs further study and is not entirely explainable by the reduction in the ESI voltage.

The linearity of the APPI-only mode of the dual source was measured for testosterone, anthracene, and, benzopyrene on the Waters ZQ system. The results in Fig. 10 show good linearity for these compounds over a four-decade range of injected masses. This attests to the quantitation potential for APPI. The body of data for APPI indicates that linearity and reproducibility are comparable to APCI and generally better than ESI. With regard to performance as a function of flow rate, the APPI sensitivity (defined as signal per unit mass) peaked at about 100–200 μL/min and drops to about half at 500–1000 μL/min. More systematic measurements are reported elsewhere [5].

3.2.2. Combined protein/drug analysis

A promising application of the ESI/APPI source is the potential to simultaneously detect drug and protein compounds, which could have important benefits for clinical diagnostics. To demonstrate this capability, a 5 μL sample of 10 nmol/μL myoglobin (from horse heart) and 10 pg/pL testosterone in methanol was injected using flow injection (no chromatography). The mobile phase used was 0.1% formic acid in 95:5 methanol:toluene mix. The formic acid was added to facilitate positive ESI ionization of the myoglobin. The data in Fig. 11 show the results of combining the ESI and PI source to allow one to take this data without changing sources. ESI is not effective in ionizing testosterone, whereas, APPI gives strong signal. Conversely, APPI is not effective at ionizing myoglobin, or at least does not produce a multiply-charged ion that lies within a reasonably low *m/z* range. Hence the combination of APPI and ESI offers simultaneous detection of these two very disparate compounds. Interestingly the ESI signal for myoglobin is greatly reduced with the lamp on. We speculate that PI is generating low energy electrons that are neutralizing the high charge states of the myoglobin, thus putting the *m/z* out of range of the spectral acquisition.

Fig. 12 shows results for a mixture of Melittin and an analyte compound [2,7-dibromo-9,9-bis(2-ethylhexyl)fluorene]. Again ESI is not successful at detecting the analyte, whereas APPI is. In this case the ESI capillary voltage is turned off. This greatly suppresses the ESI signal while not apparently compromising volatilization significantly, at least at the 100 μL/min flow rates used in these measurements. Fig. 12 therefore shows clear separation of protein and analyte signal by the APPI and ESI modes of operation.

3.2.3. Rapid switching

The switching strategy used to record the data in Fig. 11 was to leave the ESI source operating with high voltage (3800 V for ESI and 2800 V for APPI detection) and turning the APPI lamp on and off. A more recent implementation allowed us to toggle the ESI voltage on and off as was done for the data in Fig. 12. We tested the switching circuit on the Waters ZQ ES/PI configuration. The circuit was also used to switch the ESI capillary voltage between 0 V and 3000 V for APPI and ESI detection, respectively. The APPI source independently has a high voltage electrode to “push” the ions to

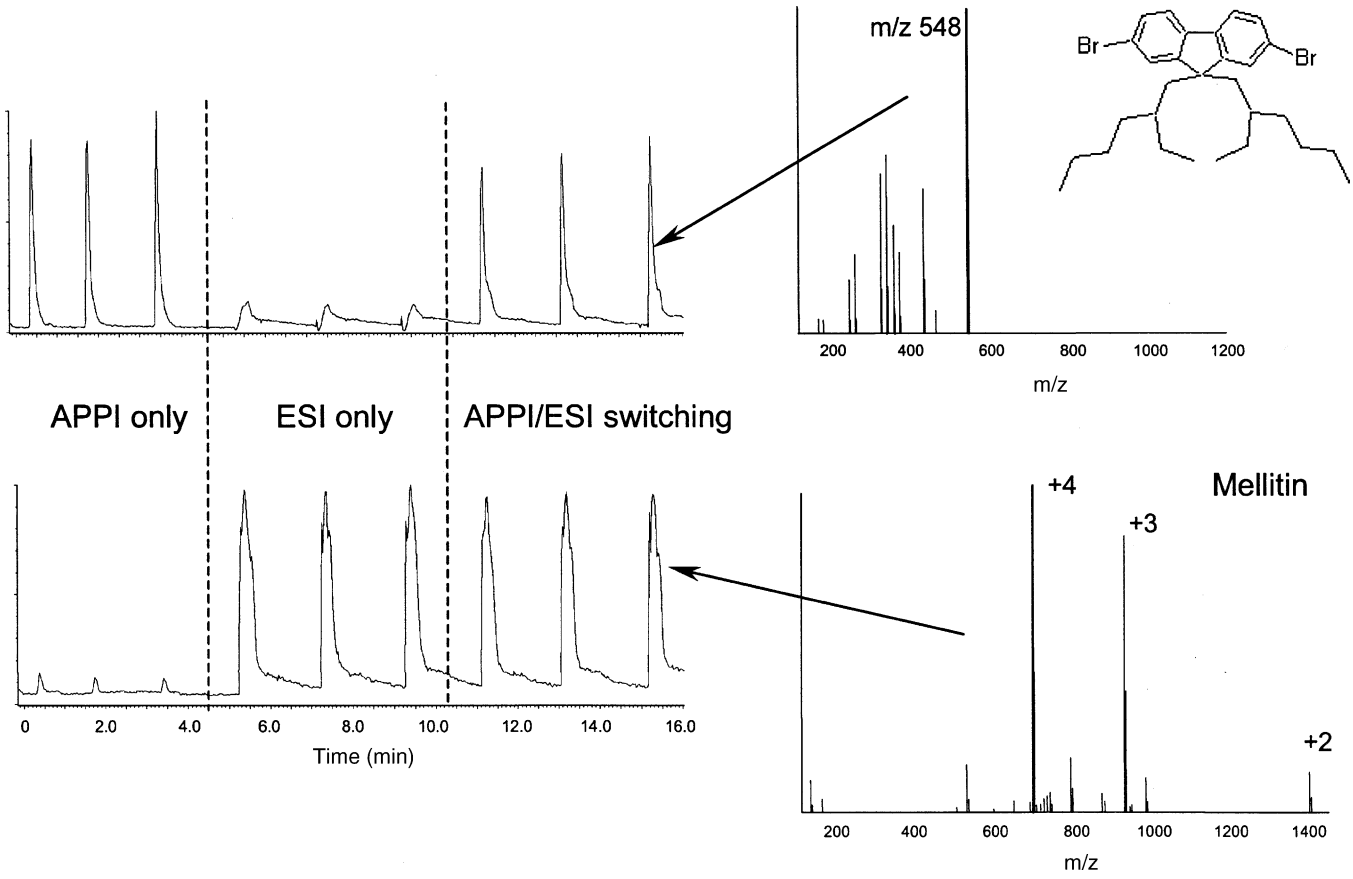

Fig. 12. Chromatograms (TIC) and mass spectra demonstrating the ESI/APPI rapid switching mode for a mixture of melittin and an analyte compound (recorded on the ZQ). The APPI source was on for the first three injections and the ESI source for the second three injections. In the final three injections, each source was rapidly switched enabling the detection of both the analyte and melittin.

the MS entrance cone and this source of voltage was provided by the pirated APCI circuitry.

In Fig. 12 the analyte/melittin sample was introduced by flow injection analysis. Two sets of total ion chromatograms were recorded because the dual source was set for switched mode. However, the first three APPI-only chromatograms were recorded by leaving the lamp on and turning the ESI source off. Furthermore only analyte sample was injected. In the next set of ESI-only chromatograms, the APPI lamp was turned off and the ESI voltage was turned on. In this set of runs only melittin sample was injected. In the final set of ESI/APPI switching chromatograms, the APPI lamp and ring electrode and the ESI capillary voltage were rapidly switched and the injected sample was a mixture of analyte and melittin. The switching takes place within the 100 ms interscan time of the Waters ZQ and the full scan mode transpired for 900 ms giving a sampling rate of 1 Hz (0.5 Hz for each ionizer).

The mass spectra for each set of TICs show distinctly different signal. During the APPI cycle, the analyte appears specifically with no trace of melittin signal. During the ESI cycle, melittin appears specifically with no trace of analyte signal. The mass spectra shown on the right of Fig. 12 correspond to the TICs in the ESI/APPI switching mode, however, essentially identical spectra were observed in the APPI-only and ESI-only mode. This indicates that the APPI and ESI ionizers fast switching mode achieves stability equivalent to that the APPI-only and ESI-only modes.

3.2.4. Potential applications

The ESI/APPI source may provide new capabilities for clinical diagnostics. For example in a well-publicized study it was reported that the presence of C-reactive protein (CRP) might be a better predictor of heart disease than the current method of testing LDL cholesterol [12]. It was also reported that because C-reactive protein and LDL cholesterol measurements tended to identify different high-risk groups, screening for both markers might provide a better prognosis screening than either marker alone. ESI/APPI analysis would appear to be a favorable way to achieve combined drug/protein detection. ESI/APPI detection of macromolecules and small molecules may open up new strategies for affinity-based mass spectrometry. Ligand–receptor interactions can be studied where the receptors are proteins, nucleic acids or carbohydrates and the ligands are drug compounds or proteins [13]. Jorgensen et al. showed that ESI/MS could be used to determine association constants KA for complex between glycopeptide antibiotics and several peptide ligands [14]. The peptide

ligands of low mass were not measured. In a related study Griffey et al. used ESI-MS to determine binding sites for small molecules to RNA targets [15]. Again only the bound and unbound RNA targets were detected. The ESI/APPI combination may allow all three entities to be measured in a single analysis. In a similar manner, ESI/APPI may also prove well-suited for high-throughput applications such as screening combinatorial libraries against protein targets [16]. In this application the protein, drug compound and protein/drug complex may be simultaneously detected with relative abundances representing a measure of binding interaction. This could then lead to high-throughput screening involving multiple drug compounds or target compounds.

4. Summary and conclusions

Developing multiple ionization sources has a history going back to gas chromatography where the combination of chemical ionization (CI) and electron ionization (EI) is commonly used. In reporting on dual ionization sources for LC–MS we considered combinations involving APPI and their relative merits when combined with APCI or ESI for different applications. We also focused on switching strategies to enhance the analysis of existing applications and to lead potentially to new applications.

The implementation of a compact, sensitive PI source for commercial analytical MS instruments includes subatmospheric pressure (e.g., low pressure photoionization, LPPI) [17–20]. APPI is a more common implementation owing to the widespread use of LC–MS and review articles are now appearing that describe these recent developments [21,22]. The literature is growing on the properties and applications of APPI. In addition to the work cited above, studies have been reported on the dependence of APPI ionization efficiency on solvent and eluent conditions [23,24] and applications where APPI methods are preferred over other methods, such as in detection of drug samples in biological matrices [25,26] and characterization of hydrophobic peptides [27].

Dual sources for LC–MS have the practical benefit of expanding the range of ionizable compounds that can be analyzed simultaneously without the inconvenience of manually switching spray chambers as is conventionally done. When ionization sources are thoughtfully combined and operated, new applications may result. In terms of the optimal combination of two sources, we believe that the ESI/APPI combination offers the greatest benefits relative to other dual combinations. APPI and APCI are similar in terms of being limited to compounds that can be vaporized. ESI and APCI are similar in terms of their ionization efficiencies being based on the charge affinity of the compounds. Furthermore APCI and ESI are most efficient for different flow regimes (high and low, respectively). The ESI/APPI combination provides broad coverage of vaporizable compounds and the ESI coverage of large molecules. Furthermore evidence indicates that compared to APCI, APPI operates with high sensitivity over a wider range of flow rates, particularly at lower flow rates where ESI excels [8].

Much understanding still needs to be gained. For example, the operation of both ionizers simultaneously can interfere and lead to unexpected results. The APCI/APPI source results in combined signal that is not the sum of the individual ionizer signal and in some cases can suppress signal relative to a single-ionizer. The ESI/APPI source showed the disappearance of ESI ions and ESI multiply-charged ions when the APPI source was switched on. Some of this unexpected behavior can be beneficial and an understanding of the underlying mechanisms might allow better methods to be developed.

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