Atmospheric Pressure Photoionization-Mass Spectrometry

by

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PREFACE

This work has been carried out in the Viikki Drug Discovery Technology Center (DDTC), Department of Pharmacy, University of Helsinki during the years 2001-2004. Part of the work in years 2002-2003 was performed at the Mass Spectrometry Core Facility of the University of Groningen in the Netherlands.

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ABSTRACT

Atmospheric pressure photoionization (APPI) is a novel ionization method for liquid chromatography-mass spectrometry (LC-MS), which was originally developed to widen the group of analytes that can be analyzed by LC-MS towards less polar compounds. This work seeks a higher understanding of the theoretical aspects of APPI, which is important to future development of the technique as well as to applications using it.

The formation of reactant ions in positive ion APPI was studied by recording the spectra of 13 different solvent systems with different proton affinities (PAs). Toluene, which formed a molecular ion through photoionization, was used as a dopant. The toluene molecular ions were observed to react through proton transfer with solvents and solvent clusters of high PAs. However, with solvents of low PA the toluene molecular ion stayed in the system and could be observed in the spectra. The effect of different reactant ions on the ionization of analytes was further studied by analyzing a group of naphthalenes with different functional groups and, therefore, also different PAs, in the same solvent systems. Analytes of high PA were seen to react through proton transfer with the protonated solvent molecules, whereas the analytes of low PAs were mainly ionized through charge exchange with the dopant molecular ion. In high PA solvents, the ionization efficiency for low PA analytes was poor because of the neutralization of the dopant molecular ion by proton transfer with the solvent. However, by using low PA solvents, the charge exchange and the ionization of low PA analytes could be enhanced as the toluene molecular ion stayed in the system. Ionization of analytes through proton transfer was deteriorated by the presence of high PA additives, as they depleted the protons from the system. The results showed that non-polar compounds that cannot be ionized by using electrospray and atmospheric pressure photoionization (APCI) can efficiently be ionized by using APPI, as long as the solvent system is carefully optimized.

A similar study was done to clarify the ionization process in negative ion mode: first, the formation of reactant ions was studied by recording the spectra of 17 solvent systems, after which seven analytes were analyzed in the same solvents. The spectrum of toluene as well as the spectra of neutral and basic solvents showed the same ions, which were assumed to originate from ionization of toluene, atmospheric gases and impurities. The introduction of acidic solvents or ammonium acetate changed the background radically as very abundant deprotonated molecules of the acids were formed. The analytes formed mainly deprotonated molecules through proton transfer, negative molecular ions through electron capture or charge exchange and phenoxide ions through substitution reactions. The best ionization efficiency for all analytes was obtained in neutral and basic solvents; whereas solvents that formed low PA reactant ions (acidic solvents and ammonium acetate) and solvents of positive electron affinity (EA) deteriorated the ionization efficiency of the analytes significantly. Oxygen was shown to play an important role as it could capture electrons and react with the solvents and analytes through charge exchange, proton transfer and substitution reactions.

Anisole was introduced as a new dopant for APPI and its use was demonstrated for a group of analytes. Anisole has higher PA than toluene and, therefore, the anisole molecular ion does not give up its protons as easily as the toluene molecular ion. Because of this, the anisole molecular ion is not neutralized by retention phase-liquid chromatography (RP-LC) solvents that possess relatively high PAs. Instead, the anisole molecular ion stays in the system even with these solvents, which makes possible the efficient charge exchange between the anisole molecular ion and low IE analytes. The use of anisole was shown to enhance the signal of low PA, low IE analytes even 100 fold in comparison to the situation when toluene was used. It

was also observed to promote the ionization of analytes through charge exchange instead of proton transfer.

Effect of the solvent flow rate on the ionization efficiency of the analytes was studied by measuring the signal of two different analytes at variating solvent flow rates. To promote proton transfer, acridine was analyzed in acetonitrile, and, to promote charge exchange, 2-ethylnaphthalene was analyzed in chloroform. Toluene was used as the dopant. The signal of the analytes and the total ion current inside the ion source were measured. Two different APPI sources were used to test the possible effect of the ion source construction. The total ion current inside the ion source was shown to decrease as the solvent flow rate was increased, although the proportion of the dopant was kept constant. In addition, the signals of the analytes saturated or decreased at high solvent flow rates, although the analyte mass flow increased. The effect of the solvent flow rate was more serious for the low PA 2-ethylnaphthalene than for the high PA acridine. The major reason for the signal loss was thought to be the neutralization of the dopant molecular ions as the number of neutralizing species in the ion source increased. Other possible explanations, which could not be ruled out, were neutralization of analytes and reactant ions by recombination reactions and the loss of photons in collisions with gas-phase particles and ion source surfaces.

The miniaturization of the APPI source was realized by combining the krypton discharge lamp used in the conventional APPI source with a heated nebulizer microchip, developed for miniaturization of APCI. The nebulizer chip consisted of fluidic and gas inlets, a mixer and a nozzle etched onto a silicon wafer, which was anodically bonded to a Pyrex glass wafer, on which an aluminum heater was sputtered. The use of the micro-APPI in the analysis of four analytes was demonstrated and the spectra were compared to the spectra obtained with conventional APPI. Ionization in positive and negative ion modes was successfully achieved and the obtained spectra were largely similar to those obtained with conventional APPI, indicating that the ionization in micro- and conventional APPI sources takes place by the same mechanisms. Ionization was achieved at all flow rates experimented (0.05-5 µl/min), being most efficient at 1-5 µl/min. A stable signal was obtained throughout a five-hour measurement, which proved the excellent stability of the micro-APPI. The micro-APPI can in future be used to combine microfluidic separation systems or nano-LC with mass spectrometer.

LIST OF ORIGINAL PUBLICATIONS

I Tiina J. Kauppila, Tiia Kuuranne, Eduardo C. Meurer, Marcos N. Eberlin, Tapio Kotiaho, Risto Kostiainen: Atmospheric Pressure Photoionization Mass Spectrometry (APPI-MS) - Ionization Mechanism and the Effect of Solvent on the Ionization of Naphthalenes. Analytical Chemistry 2002, 74, 5470-5479.

II Tiina J. Kauppila, Tapio Kotiaho, Andries P. Bruins, Risto Kostiainen: Negative Ion-Atmospheric Pressure Photoionization-Mass Spectrometry. Journal of the American Society for Mass Spectrometry 2004, 15(2), 203-211.

III Tiina J. Kauppila, Risto Kostiainen, Andries P. Bruins: Anisole, a New Dopant for Atmospheric Pressure Photoionization-Mass Spectrometry of Low Proton Affinity, Low Ionization Energy Compounds. Rapid Communications in Mass Spectrometry, 2004, 18, 808-815.

IV Tiina J. Kauppila, Risto Kostiainen, Andries P. Bruins: Effect of the Solvent Flow Rate on the Ionization Efficiency in Atmospheric Pressure Photoionization Mass Spectrometry. Journal of the American Society for Mass Spectrometry 2004, submitted.

V Tiina J. Kauppila, Pekka Östman, Seppo Marttila, Raimo Ketola, Tapio Kotiaho, Sami Franssila, Risto Kostiainen: Atmospheric Pressure Photoionization-Mass Spectrometry with a Microchip Heated Nebulizer. Analytical Chemistry 2004, accepted for publication.

These publications will be referred to in the text by their Roman numerals.

ABBREVIATIONS

ΔG_{acid}	gas-phase acidity
ACN	acetonitrile
APCI	atmospheric pressure chemical ionization
API	atmospheric pressure ionization
APPI	atmospheric pressure photoionization
CE	capillary electrophoresis
CI	chemical ionization
D	dopant
$D^{+\cdot}$	dopant molecular ion
$[D-H]^{\cdot}$	deprotonated dopant molecular ion
EA	electron affinity
EC	electrochemistry
ESI	electrospray ionization
e^{-}	electron
eV	electron volt
F	fragment
GC	gas chromatography
IE	ionization energy
IMS	ion mobility spectrometry
HPLC	high performance liquid chromatography
LC	liquid chromatography
LPPI	low pressure photoionization
M	analyte
$M^{+\cdot}$	molecular ion
$M^{-\cdot}$	negative molecular ion
MALDI	matrix assisted laser desorption
$[M-H]^{-}$	deprotonated molecule
$[M+H]^{+}$	protonated molecule
MRM	multiple reaction monitoring
MS	mass spectrometry / mass spectrometer
MS/MS	tandem mass spectrometry
m/z	mass-to-charge ratio
NH_4OAc	ammonium acetate
NP-LC	normal phase-liquid chromatography
PA	proton affinity
PAESI	photon-assisted electrospray ionization
PAH	polyaromatic hydrocarbon
PI	photoionization
PID	photoionization detector
RP-LC	reversed phase-liquid chromatography
S	solvent
S_n	solvent cluster
S/N	signal-to-noise ratio
SFC	supercritical fluid chromatography
TFA	trifluoroacetic acid
TFC	turbulent flow chromatography
VUV	vacuum ultra-violet
UV	ultra-violet

1 INTRODUCTION

The development of liquid chromatography-mass spectrometric (LC-MS) techniques in the last few decades has made possible the analysis of trace amounts of analytes from complicated matrices. With LC, the analytes of interest can be separated from each other as well as from the interfering matrix, after which they can be reliably identified thanks to the sensitivity and specificity of MS. LC-MS has become an irreplaceable tool for many applications, ranging from the analysis of proteins or pharmaceuticals in biological fluids to the analysis of toxic substances in environmental samples.

Electrospray ionization (ESI) and atmospheric pressure chemical ionization (APCI) have thus far been the most popular interfaces for the coupling of LC with MS. In ESI, gas-phase ions of the analytes are formed by using a high electric field. Best ionization is achieved when the analytes are already charged in solution, and therefore ESI is best suited for the analysis of polar and ionic compounds. In APCI, the liquid sample is first evaporated, after which a charged plasma is formed by using corona discharge, and the ionization of analytes takes place by gas-phase reactions. The polarity of the analytes can be somewhat lower with APCI than with ESI, but completely non-polar analytes cannot be ionized by either of the two ionization methods. This leaves out a significant number of analytes, that are important for instance in environmental, pharmaceutical or biological applications. In addition, especially ESI is susceptible to signal suppression due to competition for charge between analytes, additives and impurities.

Atmospheric pressure photoionization (APPI) is a novel, alternative ionization method for LC-MS, which was originally developed in order to broaden the group of analytes that can be analyzed by LC-MS towards less polar compounds. The ionization process in APPI is initiated by 10 eV photons, emitted by a krypton discharge lamp. The photons can ionize compounds that possess ionization energies (IEs) below their energy (10 eV), which includes most larger molecules, i.e. possible analytes, but leaves out most of the typically used gases and solvents. Thus, the analytes can be ionized selectively, with minimum background interference. Furthermore, as the ionization of the analytes is dependent on the IE of the analyte, rather than its proton affinity (PA) like with ESI and APCI, the ionization of molecules of low polarity is also possible.

The main aims of this study were to clarify the ionization mechanism in APPI as well as to develop the method further. The ionization process in APPI and the effect of the solvent on it were thoroughly studied in positive (I) and negative (I, II) ion modes. For both studies, analytes and solvents with different properties were chosen, in order to admit ionization through different ionization mechanisms. The ionization process was explained in view of the different reactant ions formed depending on the solvent composition. Next, a new dopant for APPI, anisole, was introduced (III). The usefulness of anisole as the dopant was demonstrated in the analysis of low polarity analytes in high PA solvent, where the use of anisole could increase the ionization efficiency 100-fold.

Because it had been previously reported, that the ionization efficiency in APPI decreases as the solvent flow rate is increased, reasons for this ion loss were studied (IV). Two analytes in two different solvent environments were chosen in order to study the effect of the solvent flow rate on two different routes of ionization. The flow rate was varied and its effect on the analyte signal and on the total ion current was monitored. The measurements were performed with two different APPI sources to study the effect of the ion source construction on the ion

loss. In addition, the effects of temperature, gas flow and lamp current on the phenomenon were examined.

Recently, miniaturization of conventional analysis techniques has become popular due to short analysis times, low solvent consumption and the small amounts of sample that are required for sensitive analysis. Because it had been shown, that the ionization efficiency of APPI is better at low flow rates (IV), the miniaturization of APPI was found worth experimenting (V). The miniaturization was realized by combining a heated nebulizer microchip with the krypton discharge lamp of the conventional APPI. The use of the micro-APPI was demonstrated in the analysis of four compounds with different properties. The spectra were compared to spectra obtained with conventional APPI.

2 REVIEW OF THE LITERATURE

2.1 Liquid chromatography-mass spectrometry

The development of atmospheric pressure ionization (API) techniques such as atmospheric pressure chemical ionization (APCI) and electrospray ionization (ESI) has turned the coupling of mass spectrometry (MS) with liquid chromatography (LC) into a routine method. Both ionization techniques have found widespread applications as they allow the direct MS analysis of polar analytes in solution. However, both techniques are best suited for the analysis of polar compounds and other approaches are required when the analyte in question is non-polar.

2.1.1 Electrospray

Formation of gas-phase ions by electrospray was first achieved by Dole et al. who succeeded in forming negative macro-ions from a molecule beam [1]. Later, the group of Fenn interfaced electrospray with mass spectrometry [2, 3].

In ESI, the liquid sample is led to the ion source through a stainless steel needle, on which a high voltage (approx. ±5 kV) is conducted. The high voltage divides the charges in the solvent, so that if the voltage on the needle wall is positive, negative ions from the solvent are driven to the walls and the rest of the solution is left with a positive charge. The positive charges in the solution are repelled by each other and the positively charged needle walls, so that a cone-shaped flow is formed on the tip of the needle (Figure 1). Gradually the tip of the cone-shaped flow starts to spit out droplets that bear several positive charges on their outer surface. As the solvent evaporates from the droplets, they gradually become smaller, which increases the charge density on the droplet surface. According to Dole, the droplets slowly reach a state called “Rayleigh’s instability limit”, which causes them to undergo a series of coulombic fissions to smaller droplets until finally gas-phase ions are left [1]. Another theory, by Iribarne and Thomson, assumes that a competitive process called “ion evaporation” takes place, in which the droplets start emitting ions directly to the gas-phase after the repulsion forces on the droplet surface become high enough to break the surface tension [4]. Similarly, negatively charged ions can be formed by setting a negative voltage on the needle wall.

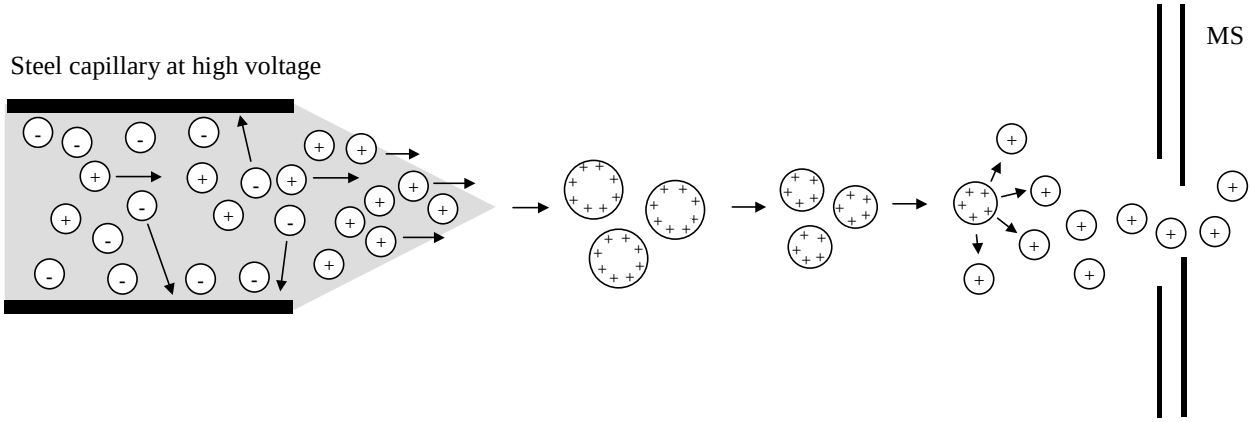

Figure 1. Schematic drawing of an electrospray ion source.

The ions formed in ESI are typically protonated molecules ($[M+H]^+$) or adducts (i.e. $[M+NH_4]^+$, $[M+Na]^+$) in positive ion mode and deprotonated molecules ($[M-H]^-$) in negative ion mode. The sensitivity that can be achieved by using ESI is excellent and, as the ionization takes place without heating, also large thermolabile molecules can be analyzed. However, because of the nature of ESI, it is best suited to ionization of polar compounds that can already be charged in the solution. Another disadvantage of ESI is signal suppression, which is caused by competition of the charge between the analytes and different solvent species. This limits the use of buffers and demands careful sample preparation in case of complicated sample matrices [5].

2.1.2 Atmospheric pressure chemical ionization

The ion source for atmospheric pressure chemical ionization (APCI) was first developed by Horning et al. [6]. They introduced the sample either as gas in a solvent-free fashion or with solvent, which was vaporized together with the sample. The ionization took place by chemical ionization (CI) reactions in an atmospheric pressure reaction chamber external to the low-pressure region of the mass spectrometer. In the first design, the ionization reactions were initiated by electrons emitted by a ^{63}Ni-foil [6-8]. The ^{63}Ni-source was soon replaced by a corona discharge needle, which created a similar spectrum, but had a much higher reactant ion intensity and thus a larger dynamic response. A schematic diagram of the heated nebulizer interface used as the APCI source is shown in Figure 2.

In APCI, the electrons emitted by the ^{63}Ni-foil or the corona discharge needle initially ionize the gas and solvent molecules present in the ion source. After this the ionization takes place via gas-phase ion-molecule reactions, such as proton transfer and charge exchange [7-9]. Proton transfer takes typically place when the analyte in question has high proton affinity (PA), whereas for charge exchange the analyte should possess low ionization energy (IE). Ionization through charge exchange can therefore also be utilized in the analysis of low PA and neutral compounds. However, in APCI, the formation of analyte molecular ions by charge exchange has mainly been achieved in aprotic solvents, such as benzene, whereas in more typical LC solvents the signal of the molecular ions is suppressed and protonated molecules dominate the spectra [7]. Molecular ions are also very reactive and are therefore easily neutralized in ion-molecule reactions with other gas-phase components. Consequently, the ionization efficiency for non-polar analytes is usually poor in APCI. In addition, APCI often suffers from high background, because of efficient ionization of high PA gases, solvents and impurities. Because of the heat required in the vaporization process, only relatively small and stable compounds up to about 1000-1500 Da can be analyzed.

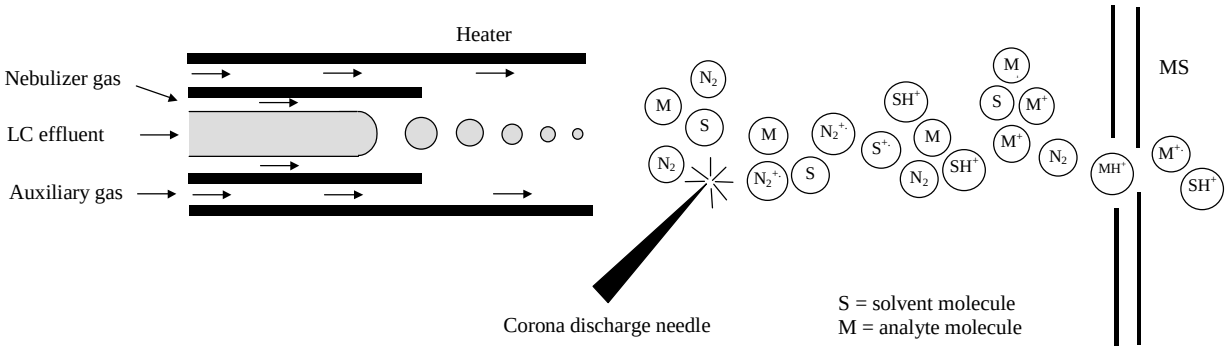

Figure 2. Schematic drawing of an atmospheric pressure chemical ionization source.

2.1.3 Atmospheric pressure photoionization

Photoionization has a long history as the detection method for gas chromatography (GC) [10-13]. It utilizes a discharge lamp that generates vacuum-ultraviolet (VUV) photons. If the photons are absorbed by a species that has ionization energy (IE) below the energy of the photons, then single-photon ionization may occur:

$$M + h\nu \rightarrow M^{+\cdot} + e^{-} \quad (1)$$

An alternative reaction route may take place, if a carrier gas, such as nitrogen, is used, that strongly absorbs the VUV radiation:

$$N_2 + h\nu \rightarrow N_2^* \quad (2)$$
$$N_2^* + M \rightarrow N_2 + M^{+\cdot} + e^{-} \quad (3)$$

The photoions formed by the two mechanisms generate a current, which flows through a collection electrode and forms the signal in the chromatogram [12]. The lamp is usually selected so that the energy of the photons is below the IE of the carrier gas and above the IE of the analytes.

The IE of a compound is dependent on the size and the structure of the compound. Large compounds and compounds that possess a high degree of conjugation typically have lower IEs than small and aliphatic molecules. Thus, typical analytes usually possess IEs in the range of 7-10 eV, whereas the common GC carrier gases have higher IE values (Table 1). Therefore, the analytes can be selectively ionized without interference from ionized carrier gas molecules. Often, a krypton discharge lamp that emits 10 eV and 10.6 eV (a minor fraction) photons is used.

Table 1. Selected ionization energies (IEs) [14].

Compound	IE (eV)
Toluene	8.83
Benzene	9.24
Acetone	9.70
n-Hexane	10.13
Methanol	10.84
Ammonia	10.07
Oxygen	12.07
Acetonitrile	12.20
Water	12.62
Carbon dioxide	13.78
Nitrogen	15.58

Photoionization has also been used as a detection method for LC [15-17]. Also with LC, selective ionization can be achieved because of the relatively high IEs of the LC solvents (Table 1). In the early reports on PI coupled to LC, the solvent eluting from the LC column had to be evaporated before the photoionization detector (PID), because the rate of

recombination of ions is much higher in liquid phase than in gas-phase. After the evaporation, the analytes were ionized in a manner similar to GC-PID. The selective ionization achieved by photoionization has also been utilized in the analysis of gaseous samples with ion mobility spectrometry (IMS) [18-20].

Revel'skii et al. were the first to connect APPI with mass spectrometry as they replaced the ^{63}Ni source of a heated nebulizer with a photoionization lamp [21, 22]. This resulted in extended dynamic range and much lower detection limits for their analytes. The gaseous mixture of various analytes was introduced to the ion source with the carrier gas and mass analyzed without separation. Years later, the potentiality of the APPI source as an interface for LC-MS was recognized by Bruins et al., who constructed their own APPI design and applied it in the analysis of liquid samples eluting from LC [23].

The APPI source presented by Bruins et al. was designed for coupling with a PE Sciex triple quadrupole mass spectrometer. The system includes a heated nebulizer and a housing, which are identical to the ones used in the Sciex APCI source. Nitrogen is used as nebulizing and lamp gases and oxygen as auxiliary gas. The source is dependent on addition of a dopant, which is added to the auxiliary gas line and vaporized together with the solvent in the heated nebulizer. A usual flow rate of the dopant is about 1/10 of the solvent flow rate, which in turn is typically 100-300 µl/min. The vapor is swept by nitrogen gas flow into the photoionization region mounted directly to the end of the heated nebulizer probe. The photoionization lamp used is a commercially available krypton discharge lamp emitting 10 eV (a minor fraction of 10.6 eV) photons. An electric potential of 1.2-1.5 kV is applied to the mounting bracket of the discharge lamp. The APPI source, described in more detail by Bruins et al. [23], is shown in Figure 3.

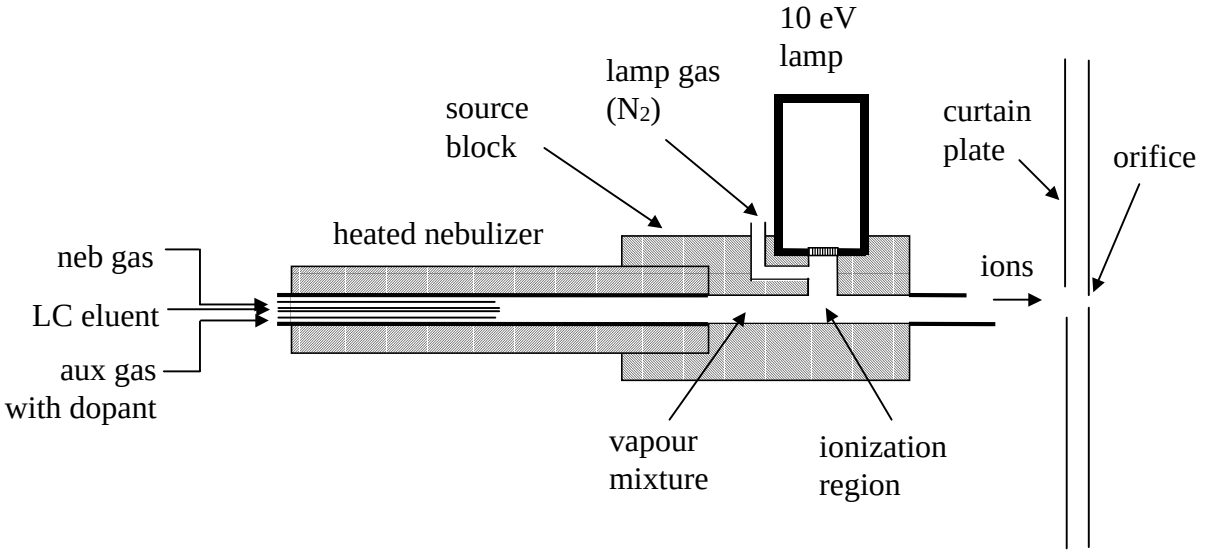

Figure 3. A schematic diagram of the APPI source designed by Bruins et al [23].

The APPI source designed by Bruins et al. was developed with the aim to broaden the range of compounds that can be analyzed by API-techniques towards less polar molecules, that cannot be analyzed by ESI or APCI [23]. They tested the source for a group of compounds with different polarities and compared the results with those obtained with APCI. APPI turned out to be more sensitive towards all the test compounds, although it gave a higher signal for

the high PA compounds that formed protonated molecules, than for the non-polar low PA compounds that formed molecular ions. In negative ion mode, deprotonated molecules were observed.

Bruins et al. used a dopant with their APPI source in order to increase the ionization efficiency of the analytes. In atmospheric pressure, the photons lose easily their energy in collisions with instrument surfaces and gas-phase particles, which decreases the ionization efficiency. By using a large amount of a readily ionizable substance that has IE below the energy of the photons, the charge can be efficiently transferred to the analytes and the ionization efficiency can be enhanced [20, 24, 25]. The idea to use a dopant to increase the ionization efficiency has first been introduced in connection with PI-IMS [20, 24], where acetone [20], benzene, toluene and xylene [24] have successfully been applied as dopants. The use of benzene has also been reported as a dopant for APCI, where it enhanced the signal of molecular ions formed from low PA analytes by charge exchange [25].

Another APPI apparatus has been described by Syage et al. [26], which uses the same operational principle as the Bruins' source (Figure 4). The source described by Syage et al. is orthogonal, with the commercial name of PhotoMate [26, 27]. The source is similar to the Agilent Technologies APCI source, except that the corona discharge needle is replaced by a krypton discharge lamp emitting 10 eV photons. Unlike the Bruins' APPI source, the Syagen source can achieve significant ionization also without the presence of dopant, which is thought to be due to greater lamp radiance output [28]. Because of a brighter lamp, direct photoionization of the analytes can be obtained, although the sensitivity is mostly improved when dopant is used.

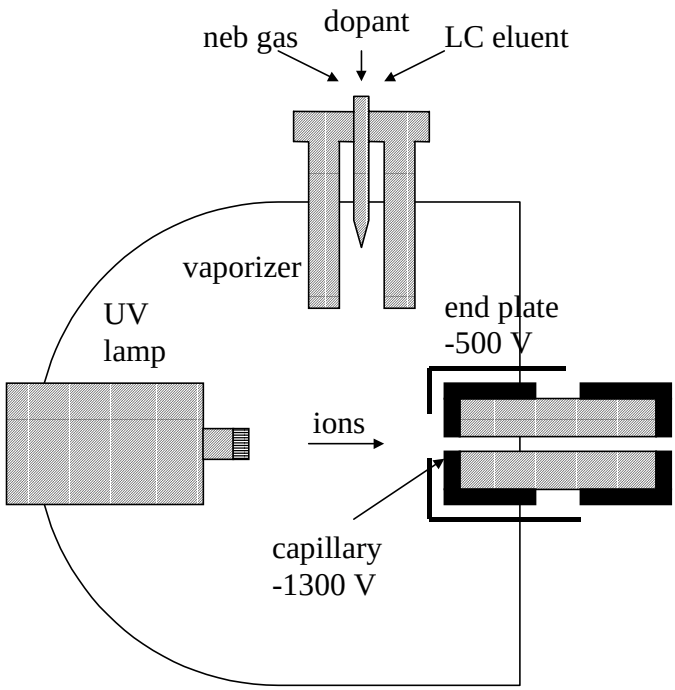

Figure 4. Schematic diagram of the APPI source designed by Syage et al. [27].

Applications

The introduction of APPI has aroused much interest amongst the analytical chemists of the world and the number of applications using APPI has grown rapidly (Table 2). Therefore, the applications are reviewed here briefly and only a few examples are discussed in greater depth.

The suitability of APPI in drug discovery and drug analysis has been examined by several research groups [29-37]. Keski-Hynnilä et al. compared ESI, APCI and APPI in the analysis of phase II metabolites of apomorfine, dobutamine and entacapone (catechols) in rat urine, rat liver cell cultures and human liver microsomes. ESI was found to be the best ionization method for the polar metabolites. Only part of the metabolites detected with ESI could be detected with APCI and APPI, probably because of poor evaporation and degradation of the ionic, thermolabile analytes [30]. Henion et al. in turn compared APPI and APCI in the analysis of idoxifene and its two metabolites in human plasma. They observed that chemical noise in APPI was a lot lower than in APCI, which resulted in improved selectivity for the analytes. Especially for the neutral metabolite SB20, APPI proved to give better sensitivity than APCI. Henion et al. also found that the flow rate of the solvent had a significant effect on the ionization efficiency of the analytes [32]. Hakala et al. compared the suitabilities of APPI and ESI in the analysis of pharmaceuticals used in permeability studies of Caco-2 cell lines. Both methods gave sufficiently low limits of detection, but APPI provided a broader linear range than ESI [36].

APPI has also gained interest in the analysis of steroids, as the ionization efficiencies for less polar steroids by using ESI or APCI can be poor [29, 38-43]. Leinonen et al. [38] compared ESI, APCI and APPI in the detection of free anabolic steroid fraction in human urine. Eluent composition, ion source parameters and fragmentation were optimized for ESI, APCI and APPI, after which the methods were compared with respect to specificity and detection limit. The use of dopant was essential for ionization in APPI, but its flow rate within a range of 5-25 µl/min did not affect the results. Toluene and acetone were compared as dopants and with toluene approximately 20-50 % higher sensitivity was achieved. All the APPI-MS spectra showed protonated analyte molecules and more extensive fragmentation than with APCI and ESI. The detection limits with LC-MS/MS using APPI and APCI were at the same level (0.08-0.9 nmol/ml) being somewhat higher than with ESI (0.06-0.5 nmol/ml). APPI has also been applied to the analysis of corticosteroids [39-41] and neurosteroids [42].

APPI has turned out to be a good alternative technique in the analysis of polycyclic aromatic hydrocarbons (PAHs), thanks to its capability to ionize non-polar molecules [44-47]. PAHs are a large group of chemicals, which are typically formed in incomplete combustion and are present in various environmental matrices. Many of the PAHs are known to be carsinogenic, which makes their analysis important in view of both health and environment. Because the PAHs lack polar groups, they are difficult to ionize using ESI or APCI. Both reversed phase- (RP) as well as normal phase-liquid chromatographic (NP-LC) conditions have been tested for the analysis of PAHs by APPI and better ionization efficiency has usually been achieved by using NP solvents. Takino et al. have studied compounds of environmental and toxicological interest (pentafluorooctane sulfonates, patulin and chloramphenicol) in challenging matrices, such as citrus fruits, apple juice and fish meat by using APPI-MS. In their comparisons with APCI-MS, APPI was found to be less susceptible to matrix effects and therefore very suitable to this kind of analyses [48-50]. In addition, APPI has been used in the analysis of pesticides [51-53], explosives [54, 55] and quinones [56-58].

Flavonoids are polyphenolic natural products that are distributed in higher plants and known to have various health effects. They have low IEs and can therefore in theory form molecular ions by charge exchange; in addition, they possess reasonable proton affinities and gas-phase acidities and can therefore also be ionized via proton transfer. Rauha et al. have done a thorough comparison between the positive and negative ion modes of ESI, APCI and APPI and their suitability in the analysis of flavonoids. They also studied the effect of the solvent on the different ionization techniques and found that APPI was more dependent on the solvent composition than the other techniques. In APPI, mainly protonated molecules of the analytes were observed in positive ion mode and deprotonated molecules in negative ion mode. The differences between the ionization efficiencies of the three techniques were not significant, although negative ion ESI provided the best conditions for the detection of the flavonoids [59]. APPI-MS has also been successfully used in the analysis of simple sugars, which can be very problematic. By using small amounts of acetic acid, formic acid and trifluoroacetic acid (TFA) in the mobile phase, Perkins et al. were able to ionize mono- and disaccharides as their acylate anions [60]. Delobel et al. have studied the suitability of APPI in the analysis of hydrophobic peptides, and APPI was found to ionize them with similar ionization efficiency as ESI. In addition, highly specific in-source B- and C-type fragmentation pathways were observed to take place in APPI, which could give valuable information about the peptide sequences. According to Delobel et al., APPI would work best as a complementary technique to ESI; ESI giving stable multiply protonated ions and APPI sequence information [61]. APPI has also been used in the analysis of food components [41] and non-polar vitamins [62].

In most comparisons between ESI, APCI and APPI, APPI has given equal or better sensitivity than APCI and been less susceptible to matrix effects than ESI or APCI [32, 33, 35, 40]. It has even been found to be a suitable interface for capillary electrophoresis-mass spectrometry (CE-MS), because it tolerates phosphate buffers better than ESI [34]. Especially in the analysis of non-polar analytes, the sensitivity of APPI has been a lot higher than the sensitivity obtained with ESI or APCI, whereas ESI still seems to work best for polar analytes [41, 63]. The newest trend in the development of APPI seems to be combining it with other atmospheric pressure ion sources, ESI [46, 64] and APCI [65]. Hanold et al. have successfully combined APPI with ESI and have been able to analyze simultaneously polyaromatic hydrocarbons, proteins, peptides and steroids, which have very different characteristics and thus could not be ionized simultaneously by using ESI or APPI alone [46]. Dorcier et al. observed a large increase in the signal-to-noise ratio of coordination and organometallic compounds when a photon source was added to an ESI source [64]. Also the combination of APPI with APCI has extended the range of compounds that can be analyzed in a single run and increased the analyte signal levels [65].

Table 2. APPI applications

Analytes	Separation method	Other ion sources	Reference
Agrochemicals	-	LPPI, APCI	[51]
Steroids and pharmaceuticals	RP-LC	APCI	[66]
Polyaromatic hydrocarbons	RP- and NP-LC	-	[44]
Lead optimization, steroids	Preparative LC	ESI, APCI	[67]
Ubi- and menaquinones	-	APCI	[56]
Steroids	RP- and NP-LC	APCI	[43]
Fat-soluble vitamins	RP-LC	-	[62]
Polyaromatic hydrocarbons	RP- and NP-LC	APCI	[45]
Pharmaceuticals	RP- and NP-LC	ESI, APCI	[31]
Flavonoids	RP-LC	ESI, APCI	[59]
Catechol glucuronides	RP-LC	ESI, APCI	[30]
Anabolic steroids	RP-LC	ESI, APCI	[38]
Steroids and neurosteroids	RP- and NP-LC	APCI	[42]
Drug discovery compounds	RP-LC	APCI	[29]
Sugars	LC	APCI	[60]
Phenolic antioxidants and glucorticosteroids	LC	ESI, APCI	[41]
Idoxifene and its metabolites	RP-LC	APCI	[32]
Aromatic imines and amines	-	-	[58]
Explosives	RP-LC	APCI	[54]
Tricothecene mycotoxins	-	-	[68]
Steroids and metabolites	NP-LC	-	[69]
Steroids and pharmaceuticals	SFC, HPLC	ESI, APCI	[40]
Menaquinones and ubiquinones	RP-LC	APCI	[57]
Explosives	LC	APCI	[55]
PAHs	LC	EC-APCI, ESI	[47]
Phenyl ureas and carbamate pesticides	LC	ESI, APCI	[52]
Proteins, steroids, peptides and PAHs	-	ESI	[46]
Drug discovery compounds	RP-LC	ESI, APCI	[37]
Caco-2 samples dosed with a cocktail of pharmaceuticals	RP-LC	ESI, APCI	[36]
Polar and non-polar PAHs	RP-LC	ESI	[63]
Hydrophobic peptides	RP-LC	ESI, MALDI	[61]
Pharmaceutical bases	CE	ESI	[34]
Bromine-based fire retardants	-	-	[70]
Organic fluorochemicals	TFC, RP-LC	-	[50]
Mycotoxins	RP-LC	APCI	[48]
Chloramphenicol	RP-LC	APCI	[49]
Corticosteroids	-	ESI, APCI	[39]
Furocumarine	-	APCI	[71]
Coordination and organometallic compounds	-	APCI, PAESI, MALDI	[64]
Clozapine, lonafarnib and drug discovery compounds in rat plasma	RP-LC	APCI	[33]
Drug discovery compounds in rat plasma	RP-LC	APCI	[72]
Post-harvest fungicides	RP-LC	-	[53]
PAHs in sediment	RP-LC	-	[73]

3 AIMS OF THE STUDY

The primary aim of the study was to clarify the ionization mechanism of atmospheric pressure photoionization-mass spectrometry and to develop the technique further.

Specifically, the aims of the research were

- to study the ionization process of APPI and the effect of the solvent thoroughly in positive (I) and negative (I, II) ion modes
- to test the suitability of APPI to compounds with different functional groups in positive (I) and negative (I, II) ion modes
- to introduce anisole as a new dopant for APPI and to test its suitability for a group of molecules with different ionization energies and proton affinities (III)
- to study the effect of the solvent flow rate on the ionization efficiency in APPI and to clarify the reasons for the signal loss at high solvent flow rates (IV)
- to develop a new, microfabricated ion source for mass spectrometry, which is based on the principle of APPI and to demonstrate its suitability for the analysis of different compounds (V)

4 MATERIALS AND METHODS

The chemicals, instruments and analytical methods used in the study are briefly described in this section. The chemicals and instruments are listed in tables, whereas the analytical procedures of each publication are shortly described. More detailed descriptions can be found in the original publications I-V.

4.1 Chemicals

The chemicals used in the study are listed in Table 3. All the chemicals were of analytical or chromatographic grade. Structures of the studied compounds are shown in Figure 5.

4.2 Instrumentation

The instrumentation used in the study is listed in Table 4.

4.3 Atmospheric Pressure Photoionization Mass Spectrometry (APPI-MS) - Ionization Mechanism and the Effect of Solvent on the Ionization of Naphthalenes (I)

Seven compounds were analyzed in 13 solvent systems in order to clarify the ionization mechanism in positive and negative ion APPI. 10^{-5} M samples were prepared in each solvent, after which the samples were injected into continuous solvent flow by using loop injection. Dopant and solvent flow rates were 0.02 ml/min and 0.2 ml/min respectively. HPLC-grade toluene was used as the dopant. The instrument was operated in positive and negative ion modes. Same mass spectrometric parameters were used for all analytes. Gas-phase reactions between the dopant and the different solvent system species were studied by tandem mass spectrometric experiments. First a toluene molecular ion was formed by 70 eV electron ionization (EI) from neutral toluene, after which it was selected for further reactions with a neutral solvent. Spectra of the formed product ions were collected.

4.4 Negative Ion-Atmospheric Pressure Photoionization-Mass Spectrometry (II)

Seven compounds were analyzed in 17 solvent systems in order to clarify the ionization mechanism in negative ion APPI. 10^{-5} M samples were prepared in each solvent, after which the samples were injected into continuous solvent flow using an autosampler. Dopant and solvent flow rates were 0.02 ml/min and 0.2 ml/min respectively. HPLC-grade toluene was used as the dopant. The instrument was operated in negative ion mode. Same mass spectrometric parameters were used for all analytes. The performances of APPI and APCI were compared.

4.5 Anisole, a New Dopant for Atmospheric Pressure Photoionization-Mass Spectrometry of Low Proton Affinity, Low Ionization Energy Compounds (III)

The performances of toluene and anisole as dopants were compared in the analysis of 14 compounds in acetonitrile. The 10^{-5} M samples were injected by loop injection into a continuous solvent flow and analyzed by APPI-MS. Dopant and solvent flow rates were 0.02 ml/min and 0.2 ml/min respectively. The instrument was operated in positive ion mode. Same mass spectrometric parameters were used for all analytes.

Table 3. Chemicals used in the study.

Chemical	Producer	Note	Publication
2-Acetonaphthone	Sigma-Aldrich, Steinheim, Germany	Standard	I
Acetic acid	Rathburn, Walkerburn, Scotland	Solvent	I, II
Acetone	Sigma-Aldrich, Steinheim, Germany	Dopant	II, III
Acetonitrile	Rathburn, Walkerburn, Scotland	Solvent	I, II
	Merck, Darmstadt, Germany		III, IV
Acridine hydrochloride	Sigma-Aldrich, Steinheim, Germany	Standard	III, IV, V
Ammonium hydroxide	J. T. Baker, Deventer, Holland	Solvent	I, II
Ammonium acetate	Merck, Darmstadt, Germany	Solvent	I, II
Anisole (99.9 %)	Fluka Chemie AG, Neu-Ulm, Switzerland	Dopant	III, V
Anisole-d_3	Sigma-Aldrich, Steinheim, Germany	Dopant	III
Anisole-d_8	Sigma-Aldrich, Steinheim, Germany	Dopant	III
Anthracene	Sigma-Aldrich, Steinheim, Germany	Standard	III
Carbamazepine	Sigma-Aldrich, Steinheim, Germany	Standard	III
Catechin	Sigma Chemical Co., St Louis, MO, USA	Standard	III
Chloroform	Sigma-Aldrich, Steinheim, Germany	Solvent	I
	Rathburn, Walkerburn, Scotland		II, IV
1,4-dinitrobenzene	Sigma-Aldrich, Steinheim, Germany	Standard	II
Diphenyl sulfide	Sigma-Aldrich, Steinheim, Germany	Standard	III
2-Ethylnaphthalene	Sigma-Aldrich, Steinheim, Germany	Standard	I, III, IV
Formic acid	Riedel-de Haën, Seelze, Germany	Solvent	II
2,5-Furandione	Sigma-Aldrich, Steinheim, Germany	Standard	II
Hexachlorobenzene	Sigma-Aldrich, Steinheim, Germany	Standard	II
Hexane	Sigma-Aldrich, Steinheim, Germany	Solvent	I
	Rathburn, Walkerburn, Scotland		II
Luteolin	Extrasynthése, Genay, France	Standard	III
Methanol	J. T. Baker, Deventer, Holland	Solvent	I, II, V
	Merck, Darmstadt, Germany		III, IV
3-Methyl-2,5-furandione	Sigma-Aldrich, Steinheim, Germany	Standard	II
Midazolam	Hoffman-La Roche, Basel, Switzerland	Standard	III
Naphthalene	J. T. Baker, Phillipsburg, NJ, USA	Standard	III
2-Naphthaleneethanol	Sigma-Aldrich, Steinheim, Germany	Standard	I
1-Naphthalenemethylamine	Sigma-Aldrich, Steinheim, Germany	Standard	I, III
2-Naphthoic acid	Sigma-Aldrich, Steinheim, Germany	Standard	II, V
2-Naphthol	Sigma-Aldrich, Steinheim, Germany	Standard	I, II, III, V
1,4-Naphthoquinone	Sigma-Aldrich, Steinheim, Germany	Standard	I, II, V
2-Naphthylacetic acid	Sigma-Aldrich, Steinheim, Germany	Standard	I
Propranolol hydrochloride	ICN Biomedicals Inc., Aurora, OH, USA	Standard	III
Testosterone	Fluka Chemie AG, Neu-Ulm, Switzerland	Standard	III
Toluene	Sigma-Aldrich, Steinheim, Germany	Dopant	I, II
	J. T. Baker, Deventer, Holland		II, V
	J. T. Baker, Phillipsburg, NJ, USA		III, IV
Trifluoroacetic acid	Fluka Chemie AG, Neu-Ulm, Switzerland	Solvent	II
Verapamil hydrochloride	ICN Biomedicals Inc., Aurora, OH, USA	Standard	III
Water (Milli-Q Plus)	Millipore, Molsheim, France	Solvent	I, II

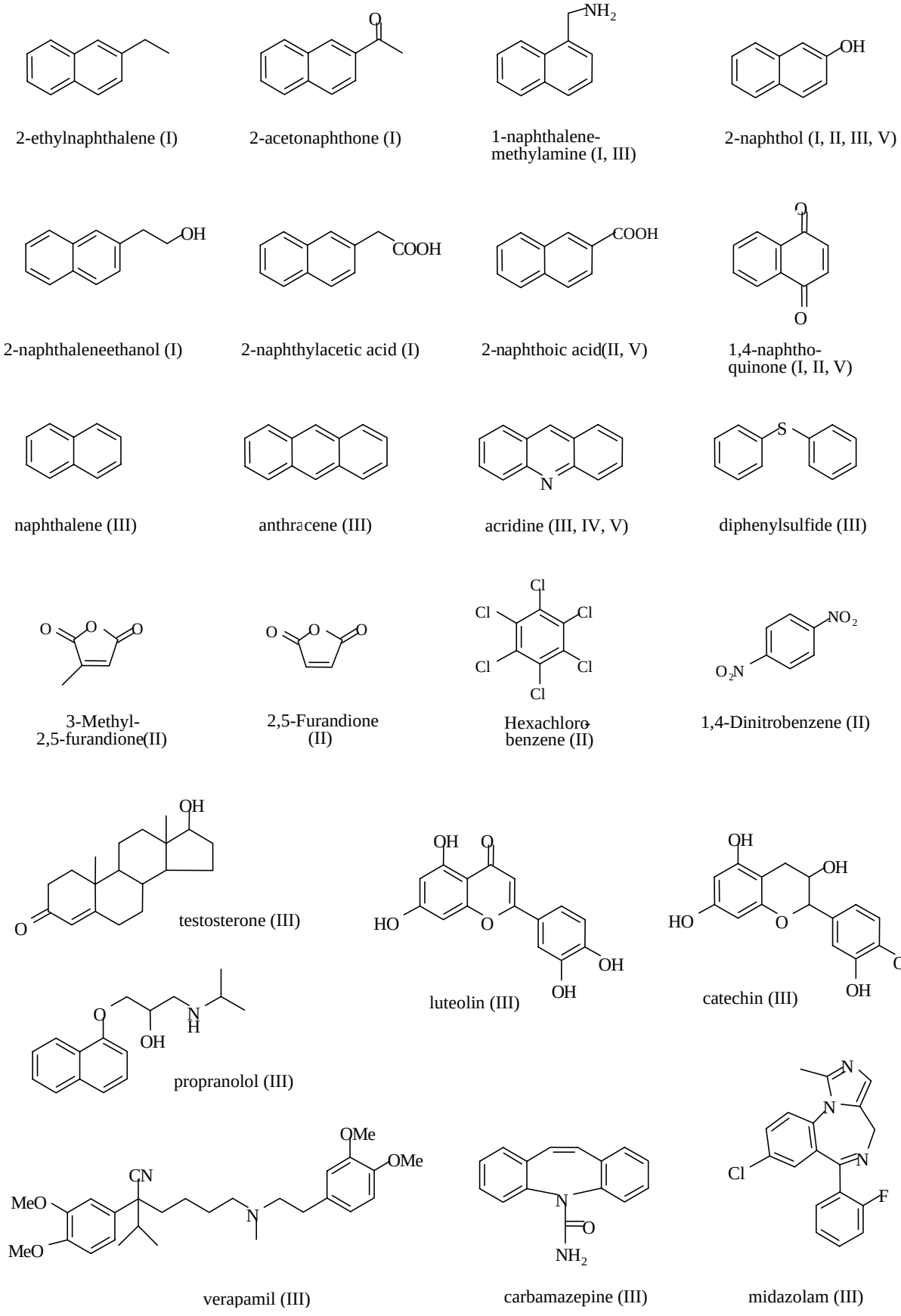

Figure 5. Structures of the studied compounds (publication given in parentheses).

Table 4. Instrumentation used in the study.

Publication	Solvent delivery system	Sample introduction	Mass spectrometer	Ionization method
I	Harvard Apparatus Inc. micro-syringe pump, Holliston, MA, USA	50 µl loop injection	PE Sciex API 3000 triple quadrupole mass spectrometer, Sciex, Concord, Canada	Atmospheric pressure photoionization prototype, Machine Shop, University of Groningen, the Netherlands
			Extrel pentaquadrupole mass spectrometer, Pittsburgh, PA, USA	Electron ionization (EI) by 70 eV electrons
II	HP 1100 series binary pump, Hewlett Packard, Waldbronn, Germany	HP 1100 autosampler, Agilent Technologies, Waldbronn, Germany	PE Sciex API 3000 triple quadrupole mass spectrometer, Sciex, Concord, Canada	Atmospheric pressure photoionization prototype, Machine Shop, University of Groningen, the Netherlands
				Atmospheric pressure chemical ionization heated nebulizer interface, Sciex, Toronto, Canada
III	Applied Biosystems 140 B solvent delivery system, Foster City, CA, USA	50 µl loop injection	PE Sciex API 365 triple quadrupole mass spectrometer, Sciex, Concord, Canada	Atmospheric pressure photoionization prototype, Machine Shop, University of Groningen, the Netherlands
IV	Applied Biosystems 140 B solvent delivery system, Foster City, CA, USA	Continuous flow	PE Sciex API 365 triple quadrupole mass spectrometer, Sciex, Concord, Canada	Atmospheric pressure photoionization prototype, Machine Shop, University of Groningen, the Netherlands
			Agilent 1100 Series LC/MSD Trap, Agilent Technologies, Germany	Agilent G1971A APPI Source, Agilent Technologies, Palo Alto, CA, USA
V	Harvard Apparatus Inc. micro-syringe pump, Holliston, MA, USA	Continuous flow	PE Sciex API 300 triple quadrupole mass spectrometer, Sciex, Concord, Canada	Miniaturized heated nebulizer chip and a krypton discharge lamp
		50 µl loop injection		Atmospheric pressure photoionization prototype, Machine Shop, University of Groningen, the Netherlands

4.6 Effect of the Solvent Flow Rate on the Ionization Efficiency in Atmospheric Pressure Photoionization-Mass Spectrometry (IV)

Effect of the solvent flow rate on the ionization efficiency in positive ion APPI was studied by following the signal of two analytes at different solvent flow rates (50-1000 µl/min). Two different APPI sources – Sciex prototype source and Agilent/Syagen source - were used in the measurements (Table 4). With the Sciex prototype source the proportions of dopant, solvent and analyte were kept constant at all flow rates, whereas with the Agilent/Syagen source the measurements were also performed without the dopant. In addition to the MS measurements also the ion currents on the curtain/end plate were measured, which gave the total amount of ions in the ion source. The mass spectrometric parameters were optimized for both analytes when the Sciex prototype was used, whereas with the Agilent/Syagen source the same mass spectrometric parameters were used for both analytes. The instrument was operated in positive ion mode.

4.7 Atmospheric Pressure Photoionization-Mass Spectrometry with a Microchip Heated Nebulizer (V)

A heated nebulizer microchip (Microelectronics Centre, Helsinki University of Technology, Finland) and a 10 eV Model PKS 100 krypton discharge lamp (Cathodeon Ltd., Cambridge, England) were combined for the miniaturization of APPI. The heated nebulizer microchip consisted of fluidic and gas inlets, a mixer and a nozzle etched onto silicon wafer, which was anodically bonded to a Pyrex glass wafer, on which an aluminum heater was sputtered (Figures 6 and 7). The mass spectrometer was fitted with an x,y,z-stage (Proxeon, Odense, Denmark) equipped with a teflon holder (The Finnish School of Watchmaking, Espoo, Finland), on which the chip was placed. A more detailed description of the instrumentation and the microchip fabrication process is given in publication V.

The performance of the micro-APPI was demonstrated in the analysis of 4 compounds. The spectra of the analytes obtained using micro-APPI and the conventional APPI were compared. The analytes were introduced by continuous flow (micro-APPI) or loop injection (conventional APPI). With micro-APPI, sample solution flow rates of 0.05-5 µl/min were tried. The dopant (toluene or anisole) was delivered as mixed to the solvent. With conventional APPI, the dopant and solvent flow rates were 0.02 ml/min and 0.2 ml/min, respectively. The mass spectrometric parameters were optimized for each analyte.

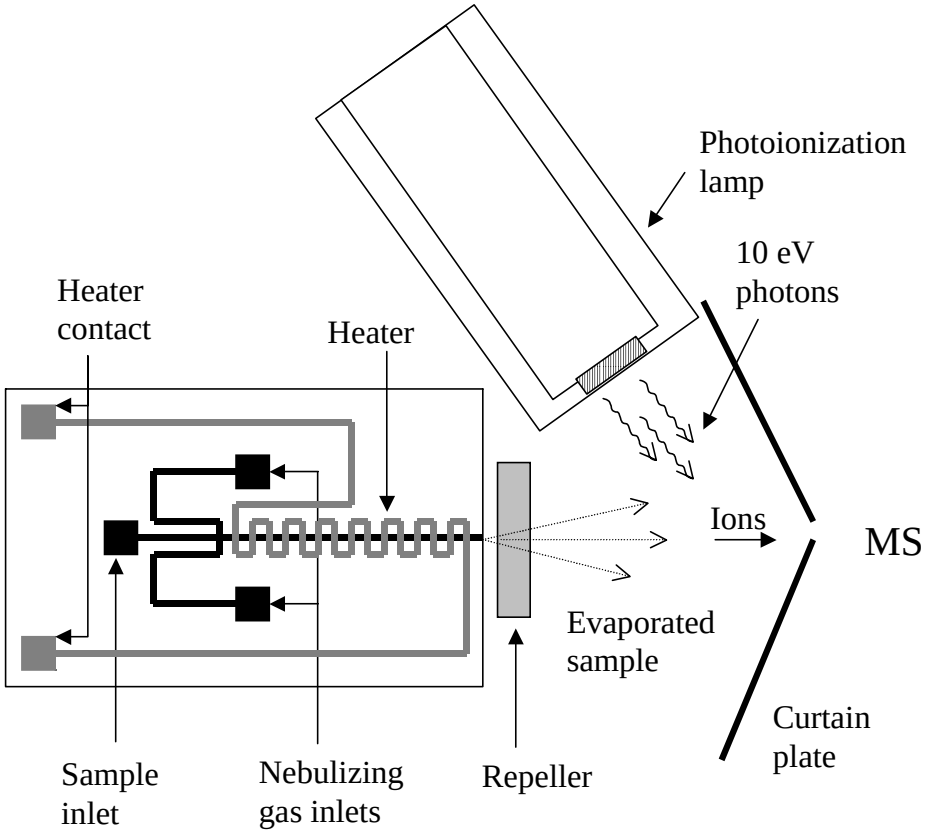

Figure 6. A schematic diagram of the microfabricated APPI

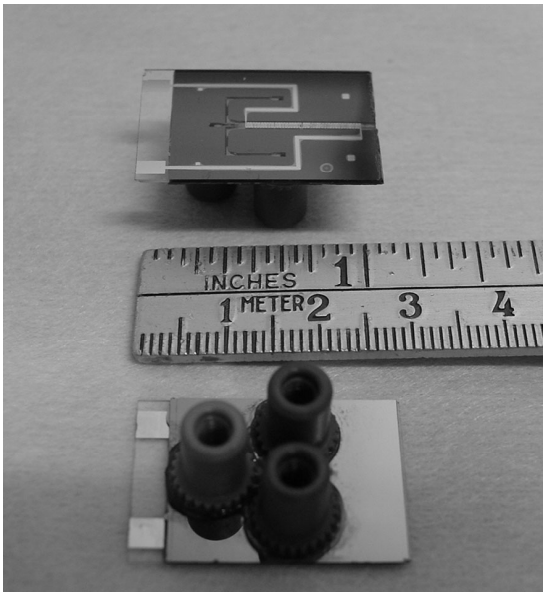

Figure 7. A photograph of the heated nebulizer microchip.

5 RESULTS AND DISCUSSION

The results obtained in this work are described shortly in this section. More details can be found in the original publications (I-V).

5.1 Ionization process in positive ion APPI

5.1.1 Formation of reactant ions

The ionization process in APPI is directly dependent on the reactant ion composition, which in turn depends on solvent, dopant, gases and impurities as well as components of the surrounding air. The formation of reactant ions in positive ion APPI was studied by analyzing 13 solvent systems (Table 5) with toluene as the dopant. All the solvent systems had IEs above 10 eV but different PAs (Appendix 1). In addition, the solvents are typically used in LC-MS, and therefore their effect in the ionization efficiency was found important in view of applications. The spectra of the different solvents as well as the spectrum of toluene alone were recorded by using APPI (Table 6). In addition, the formation of reactant ions was further studied by selected ion-molecule reactions between the toluene molecular ion ($C_7H_8^{+\cdot}$) and the solvents by tandem mass spectrometry (Table 7).

Table 5. The solvents used in the experiment.

1.	Hexane
2.	Chloroform
3.	Water
4.	Methanol
5.	Acetonitrile
6.	Water/Methanol (50:50 %)
7.	Water/Acetonitrile (50:50 %)
8.	Water/Methanol/Acetic acid (50:50:0.1 %)
9.	Water/Methanol/Ammonium acetate (50:50:0.1 %)
10.	Water/Methanol/Ammonium hydroxide (50:50:0.1 %)
11.	Water/Acetonitrile/Acetic acid (50:50:0.1 %)
12.	Water/Acetonitrile/Ammonium Acetate (50:50:0.1 %)
13.	Water/Acetonitrile/Ammonium hydroxide (50:50:0.1 %)

The main ion in the spectra of the low PA solvents (water, hexane, chloroform, solvents 1-3, Table 5) was the toluene molecular ion, $C_7H_8^{+\cdot}$, formed in the photoionization of toluene (Scheme 1, Reaction 1). In the solvents containing methanol or acetonitrile (Solvents 4-13, Table 5), protonated solvent molecules or their dimers, trimers or solvent-water clusters were observed instead of $C_7H_8^{+\cdot}$, indicating that $C_7H_8^{+\cdot}$ had donated a proton to the solvent cluster (Scheme 1, Reaction 2). In addition to the recognized ions, the APPI-MS spectra of the solvents also contained many unknown ions that could originate from impurities or be the products of gas-phase ion-molecule reactions. These ions can take part in the ionization process and thus complicate it.

The proton transfer from the $C_7H_8^{+\cdot}$ to the solvent can only take place, if the PA of the solvent is above that of the benzyl radical (Scheme 1, Reaction 2). However, according to literature, this is not the case with acetonitrile and methanol, which should make the proton transfer

from $C_7H_8^{+\cdot}$ to them thermodynamically impossible (Appendix 1)[14]. The proton transfer between $C_7H_8^{+\cdot}$ and the solvent was therefore explained by the formation of solvent and solvent water clusters, which have been reported to have higher PAs than the individual solvent monomers [74, 75] and were also observed in the spectra of these solvents (Table 6). The same conclusion has also been made by Bruins and his co-workers [76].

Table 6. The main ions in the positive ion APPI spectra of solvents 1-5. The measurements were done using an orifice voltage of 0 V.

Solvent	m/z 92[a] (rel. abund.)	S_nH^+ m/z (rel. abund.)	Observed ions m/z (rel. abund.)
Toluene[b]	92 (100)	-	95 (74), 96 (39), 101 (34), 107 (30), 108 (47), 109 (98), 111 (97), 112 (34)
Hexane	92 (52)		84 (88), 94 (62), 95 (31), 99 (100), 101 (34), 108 (17), 109 (40), 111 (35), 115 (23)
Chloroform[c]	92 (87)	-	108 (100), 91 (72), 107 (57), 93 (56), 109 (38), 105 (34), 74 (29), 106 (18), 111 (14)
Water	92 (100)	-	83 (15), 95 (21), 96 (21), 101 (22), 107 (23), 108 (42), 109 (26), 111 (59), 112 (18)
Methanol	-	51 (7.9) $[CH_3OH+H_2O+H]^+$, 65 (100) $[2xCH_3OH+H]^+$, 97 (6.5) $[3xCH_3OH+H]^+$	59 (9.5), 60 (9.4), 73 (14), 74 (60), 87 (36), 101 (10), 115 (7.7)
Acetonitrile	-	42 (5.1) $[CH_3CN+H]^+$, 83 (100) $[2xCH_3CN+H]^+$	59 (21), 60 (8.3), 84 (17), 100 (5.9), 101 (22), 115 (4.8), 142 (3.8), 148 (10)

[a] m/z 92 = $[C_7H_8^{+\cdot}]$, toluene radical cation

[b] The toluene (used as a dopant) spectrum was overloaded and therefore the relative abundances of the ions should be taken as suggestive

[c] Chloroform spectrum was recorded by using a declustering potential of 20 V.

Scheme 1. The reactions in positive ion APPI.

D (dopant) + 10 eV photons	$\rightarrow$	$D^{+\cdot} + e^-$,	if IE (D) < 10 eV	(1)
$D^{+\cdot}$ + n S	$\rightarrow$	$[D\text{-}H]^{\cdot} + S_nH^+$,	if PA (S_n) > PA ($[D\text{-}H]^{\cdot}$)	(2)
$D^{+\cdot}$ + n S	$\rightarrow$	no reaction,	if PA (S_n) < PA ($[D\text{-}H]^{\cdot}$)	(3)
$S_nH^+ + M$	$\rightarrow$	$[M+H]^+ + n S$,	if PA (M) > PA (S_n)	(4)
$D^{+\cdot} + M$	$\rightarrow$	$M^{+\cdot} + D$,	if IE (M) < IE (D)	(5)
M (analyte) + 10 eV photons	$\rightarrow$	$M^{+\cdot} + e^-$,	if IE (M) < 10 eV	(6)
$D^{+\cdot} + M$	$\rightarrow$	$[D\text{-}H]^{\cdot} + [M+H]^+$,	if PA (M) > PA ($[D\text{-}H]^{\cdot}$)	(7)

The reactions between $C_7H_8^{+\cdot}$ and the solvents were studied further by selected ion-molecule reactions in the collision chamber of a tandem mass spectrometer (Table 7). Reactions of $C_7H_8^{+\cdot}$ with methanol produced ion m/z 65, which can be $[(MeOH)_2+H]^+$ or a fragment of

$C_7H_8^{+\cdot}$ or a mixture of these two entities, whereas reactions of $C_7H_8^{+\cdot}$ with acetonitrile mainly produced m/z 42 $[ACN+H]^+$. These results prove that $C_7H_8^{+\cdot}$ upon low energy collisions can donate a proton to dimers and higher clusters of methanol and acetonitrile, although direct proton transfer from $C_7H_8^{+\cdot}$ is thermodynamically unfavorable. This situation is also possible in the APPI process, which explains the disappearance of $C_7H_8^{+\cdot}$ in the APPI-MS spectra with solvents containing methanol or acetonitrile. For methanol, the PA of methanol clusters was calculated to be at least 77 kJ/mol ($PA(C_7H_7^{\cdot})$ – PA(MeOH), Appendix 1) higher than that of the methanol monomer and in the case of acetonitrile the difference in PAs would be at least 52 kJ/mol ($PA(C_7H_7^{\cdot})$ – PA(ACN), Appendix 1). The selected ion-molecule reactions between $C_7H_8^{+\cdot}$ and water, hexane and chloroform showed no proton transfer (Table 7), because of the low PAs of these solvents and their possible clusters (Scheme 1, Reaction 3). This is in agreement with the observation made in the APPI experiments that the ionized toluene fails to transfer a proton to these solvents and instead remains in the APPI system.

The formed reactant ions depend not only on the solvent but also on the dopant used and are expected to be somewhat different when acetone or anisole are used as dopants than with toluene. However, the principle for the proton transfer between the dopant molecular ion and the solvent is the same and depends on the PAs of the solvent and the dopant. Some observed differences in the situations between toluene and anisole are discussed in chapter 5.1.3.

Table 7. The ions formed in the ion-molecule reactions between $C_7H_8^{+\cdot}$ (m/z 92) and solvent molecules

Solvent	Observed ions m/z (rel. abund.)
Hexane	83 (2.8), 85 (57) $[hexane-H]^+$, 91 (49), 92 (100), 93 (9.0)
Chloroform	83 (28), 85 (16), 91 (100), 92 (29), 139 (15) $[C_7H_7+CHCl]^+$, 141 (2.5)
Water	65 (37), 66 (3.9), 91 (100), 92 (50)
Methanol	59 (57), 65 (14), 67 (6.7), 68 (3.1), 73 (41), 80 (44), 91 (100), 92 (52), 93 (7.7), 117 (51)
Acetonitrile	39 (13), 42 (25) $[CH_3CN+H]^+$, 65 (38), 66 (3.1), 91 (100), 92 (20), 106 (5.5), 132 (3.4)
Ammonia	65 (45), 66 (6.9), 91 (100), 92 (46)

$91 = [C_7H_8^{\cdot}-H]^+$
$92 = C_7H_8^{+\cdot}$

5.1.2 Effect of the solvent on the ionization of naphthalenes (I)

Next, the effect of the reactant ion composition on the APPI ionization process in positive ion mode was studied by analyzing 6 naphthalenes with different functional groups (1-naphthalenemethylamine, 2-acetonaphthone, 2-naphthol, 2-naphthaleneethanol, 2-ethylnaphthalene and 2-naphthylacetic acid, Figure 5) in the solvent compositions of Table 5. All the naphthalenes had low IEs, but the PAs of the attached functional groups were different (Appendix 1). The low IEs made possible the ionization through charge exchange, whereas the ability of the compounds to be ionized by proton transfer was dependent on their PAs.

Figure 8 shows the absolute abundances (peak areas) of the total ion currents of the studied naphthalenes in the different solvent systems. The relative abundances of the protonated molecule ($[M+H]^+$) and the molecular ion ($M^{+\cdot}$) indicate the mechanisms by which the

naphthalenes were ionized. It was assumed that the protonated molecules were formed by proton transfer from the protonated solvent species to the analyte (Scheme 1, Reaction 4), whereas the $M^{+\cdot}$ ions were assumed to have formed by charge exchange between $C_7H_8^{+\cdot}$ and the analyte (Scheme 1, Reaction 5). The formation of $M^{+\cdot}$ directly by photoionization (Scheme 1, Reaction 6) was not the main mechanism in our experiments, since the ionization efficiency decreased approximately two orders of magnitude when toluene was removed.

Formation of abundant $M^{+\cdot}$ of the naphthalenes could only be obtained with water, hexane and chloroform (Solvents 1-3, Figure 8). The poor signal of $M^{+\cdot}$ in rest of the solvents was thought to be because of the neutralization of $C_7H_8^{+\cdot}$ by proton transfer with the solvent (Scheme 1, Reaction 2). Efficient charge exchange can only be expected if $C_7H_8^{+\cdot}$ remains in the APPI source (Scheme 1, Reaction 3) and if the compound to be ionized has an ionization energy below that of the dopant. The results showed, that non-polar, low IE compounds that cannot be ionized by electrospray or APCI, can indeed be ionized by charge exchange in APPI and the ionization efficiency for these compounds can be enhanced by using low PA solvent systems.

Protonated molecules produced by proton transfer were mainly formed from 1-naphthalenemethylamine and 2-acetonaphthone, which were also estimated to have the highest PAs (Figure 8). Obviously, the PAs of the rest of the naphthalenes were too low for efficient protonation (Appendix 1). The signal of the protonated molecules was highest with solvent systems capable of producing protonated reactant ions as a result of proton transfer from $C_7H_8^{+\cdot}$ to the solvent (Solvents 4-13). In low PA solvents (Solvents 1-3) that do not produce protonated solvent molecules, 1-naphthalenemethylamine that has lower IE than 2-acetonaphthone, formed $M^{+\cdot}$, whereas 2-acetonaphthone still formed an $[M+H]^+$ (Figure 8). The proton was assumed to have originated from background ions, protonated reaction products or water clusters as it could not have originated from the solvent.

The addition of ammonium acetate or ammonium hydroxide (Solvents 9, 10, 12 and 13) significantly deteriorated the ionization efficiency for all the naphthalenes except 1-naphthalenemethylamine (Figure 8). This was thought to be due to the high PA of ammonia, which prevents the proton transfer to analytes of lower PA. Only 1-naphthalenemethylamine, which was estimated to have the highest PA (Appendix 1), was not affected by the presence of ammonia. Note that the PA of ammonia as reported in reference [14] is lower than the estimated proton affinities of 2-acetonaphthone and 2-naphthol. However, the PAs of ammonia clusters are higher than those of monomeric ammonia and may also exceed the PA of 2-acetonaphthone.

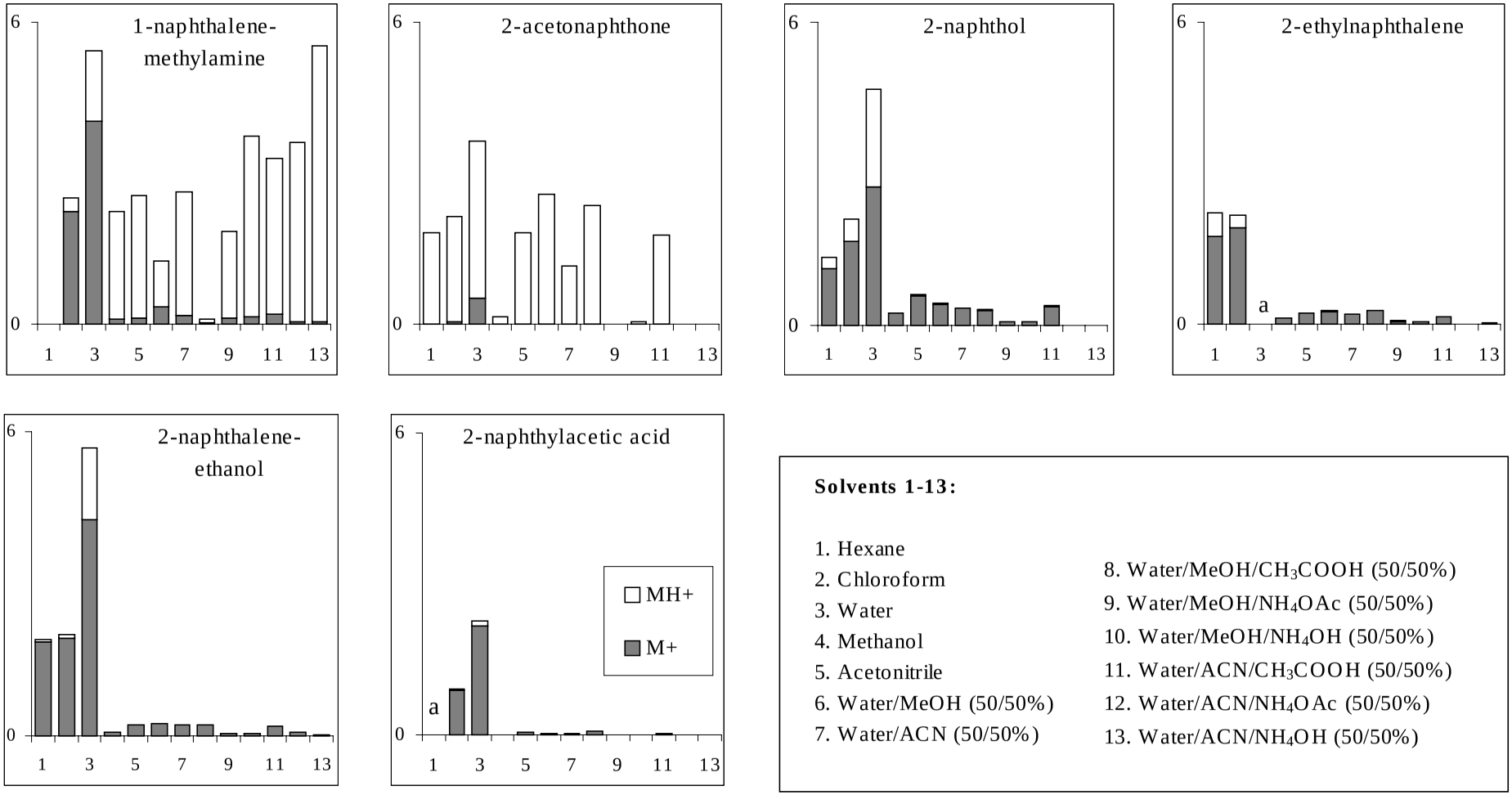

Figure 8. The absolute abundance of the total ion currents (peak areas) and the relative proportions of $M^{+\cdot}$ and $[M+H]^+$ for the studied naphthalenes in 13 different solvent compositions. X: Solvents 1-13, Y: absolute abundance in arbitrary units. A: the analyte could not be dissolved in the solvent in question and was therefore left out of the study.

5.1.3 Effect of the dopant (I, III)

In dopant assisted APPI, the 10 eV photons emitted by the photoionization lamp first ionize the dopant producing a dopant molecular ion ($D^{+\cdot}$). The formed $D^{+\cdot}$ can either remain in the system or react further with solvent molecules, analytes or impurities. Two different dopants have been studied in this work: toluene (I-V) and anisole (III, V). Both have IEs below 10 eV as requested, but their PAs are different enough to cause significant differences in the ionization process (Appendix 1).

As mentioned above, the $D^{+\cdot}$ of toluene reacted with solvents such as methanol and acetonitrile by proton transfer, but stayed in the system when low PA solvents, such as hexane and chloroform were used. Figure 9a shows the spectrum of acetonitrile with toluene as the dopant: the main ions observed were protonated solvent and solvent-water clusters, formed in the proton transfer between the $D^{+\cdot}$ of toluene and the solvent. The situation with anisole as the dopant was quite different (Figure 9b). The only ion observed in the spectrum was the $D^{+\cdot}$ of anisole, indicating that the proton transfer from $D^{+\cdot}$ to the solvent had not taken place. This was thought to be due to the higher PA of the deprotonated anisole molecular ion, which prevented the proton transfer between the $D^{+\cdot}$ of anisole and the solvent (Appendix 1).

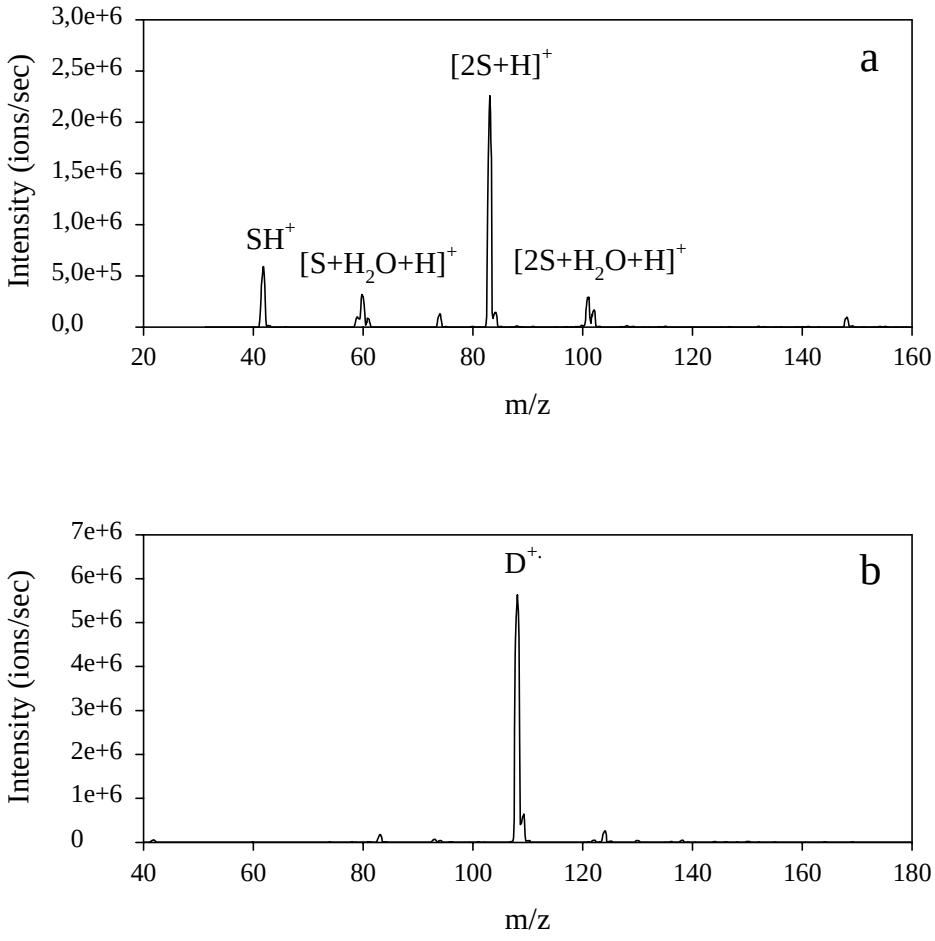

Figure 9. Background mass spectra using acetonitrile as the solvent and toluene (a) and anisole (b) as dopants. S: acetonitrile, D: dopant.

The use of anisole as the dopant was demonstrated for the analysis of a miscellaneous group of compounds (Figure 5). As an example, Figure 10 shows the absolute abundance of 2-naphthol in acetonitrile, with toluene, acetone and anisole as dopants. 2-Naphthol has low IE and low PA and is therefore usually ionized by charge exchange (Scheme 1, Reaction 5). With toluene and acetone as dopants, the ionization efficiency for 2-naphthol was poor as the $D^{+\cdot}$ was neutralized by the solvent and the ionization of 2-naphthol by charge exchange was thus prevented. However, because of the higher PA of the $[D\text{-}H]^{\cdot}$ of anisole, the anisole $D^{+\cdot}$ stayed in the system even with acetonitrile and 2-naphthol could efficiently be ionized by charge exchange. This resulted in a 100-fold signal increase, compared to the situations with the other two dopants. Therefore, for the analysis of non-polar, low PA and low IE analytes in high PA solvents, the ionization efficiency can be significantly improved if anisole is used as the dopant.

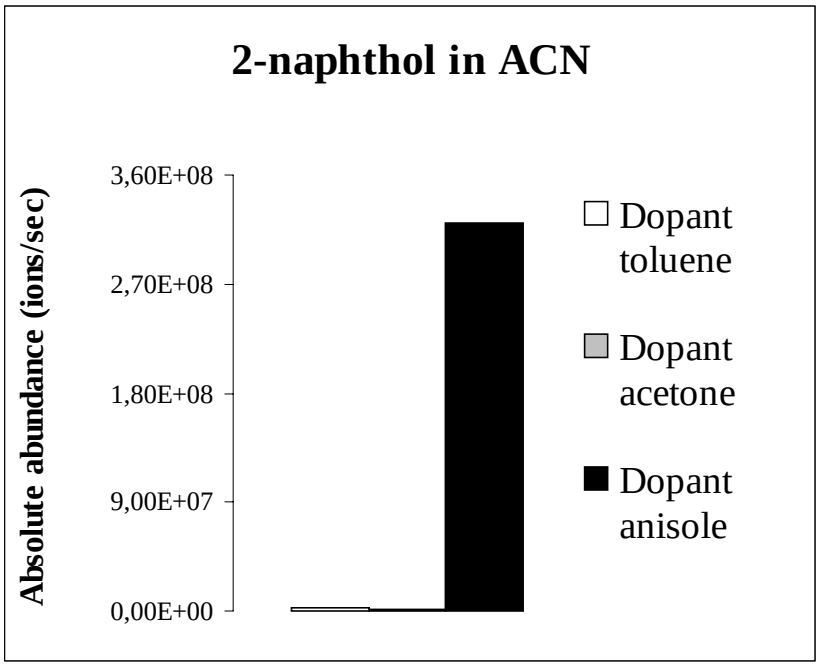

Figure 10. The absolute abundances of the total ion current for 2-naphthol in acetonitrile by using toluene, acetone and anisole as dopants.

For compounds that could be ionized both through charge exchange and proton transfer, the use of anisole as the dopant promoted the formation of molecular ions through charge exchange. An example is given in Figure 11 for carbamazepine in acetonitrile, which showed $[M+H]^+$ when toluene was used as the dopant (Figure 11a), but $M^{+\cdot}$ when anisole was used (Figure 11b). In some cases the degree of fragmentation was more intensive with anisole than with toluene, probably due to formation of $M^{+\cdot}$, which fragments more readily than $[M+H]^+$. For analytes that are mainly ionized through proton transfer (Scheme 1, Reaction 4), a somewhat weaker signal was observed with anisole as the dopant. This was thought to be because of the lack of protonated solvent molecules, which decreased the efficiency of proton transfer.

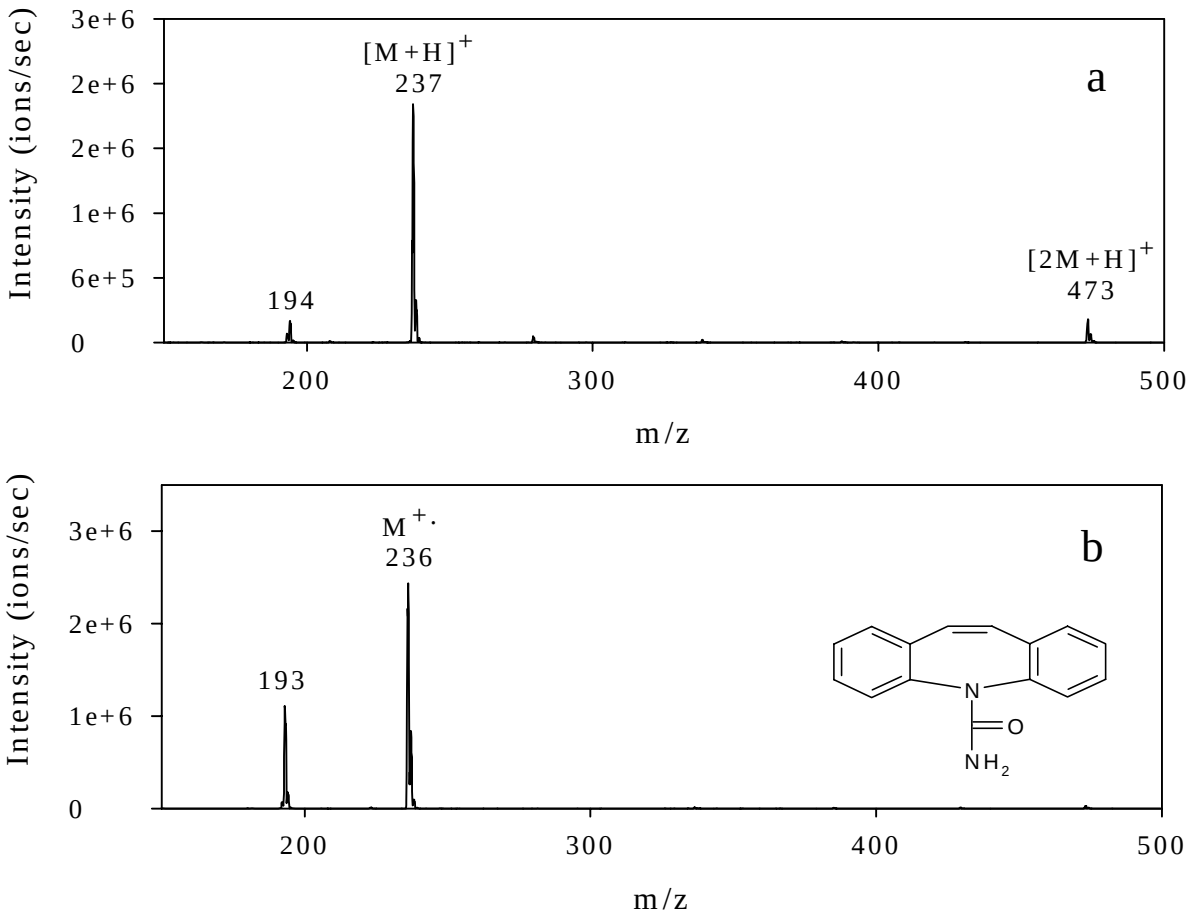

Figure 11. Mass spectra of carbamazepine in acetonitrile by using toluene (a) and anisole (b) as dopants. The ions at m/z 193 and 194 are due to loss of HNCO.

5.1.4 Effect of the solvent flow rate (IV)

To investigate the effect of the solvent flow rate on the ionization efficiency, acridine and 2-ethylnaphthalene were chosen as model compounds. High PA acridine was analyzed in acetonitrile, where it formed an intense protonated molecule ($[M+H]^+$) by proton transfer (Scheme 1, Reaction 4), whereas low PA 2-ethylnaphthalene was analyzed in chloroform, where it formed a molecular ion ($M^{+\cdot}$) by charge exchange (Scheme 1, Reaction 5). The measurements were done using two APPI sources – Sciex prototype source and Agilent/Syagen source – to investigate the effect of the differences in their construction (Figures 3 and 4). Dopant (toluene) was used in all the experiments done with the Sciex prototype source, whereas the measurements done with the Agilent/Syagen APPI could also be performed without dopant. The ionization efficiencies for the different analytes at different flow rates were recorded by keeping the relative proportions of the dopant, analyte and solvent constant (dopant flow rate was 10 % of the solvent flow rate). The ion currents at the curtain plate and orifice of the Sciex MS (Figure 3) and at the end plate and capillary of the Agilent MS (Figure 4) were also recorded in order to obtain a more truthful representation of the amount of ions in the ion source than is given by the reconstructed total ion current measured with the mass analyzer, which can be affected by poor transportation of ions into the mass analyzer and by discrimination against low mass ions.

The results from the flow rate experiments are presented in Figures 12 and 13. The curtain plate and orifice currents (Sciex prototype source) and the end plate and capillary currents (Agilent/Syagen source) were observed to decrease as the flow rate was increased in all the experiments where dopant was present, indicating a loss of reactant ions at higher flow rates (Figures 12a, 12c, 13a and 13c; orifice and capillary currents not shown). The currents seemed to be especially dependent on the signal of the dopant molecular ion as its decrease was observed to take place simultaneously and, as in the measurements done without the dopant, the currents were in minimum (Figures 12c and 13c). The severe loss of reactant ions in the system was assumed to have a strong effect also on the ionization efficiency of the analytes.

The signal of $M^{+\cdot}$ of 2-ethylnaphthalene measured in chloroform was observed to be highly dependent on the flow rate (Figure 12). The decrease of the 2-ethylnaphthalene signal at higher flow rates was thought to be due to the gradual depletion of the toluene molecular ion, which was also observed to take place (Figures 12b and 12d). The dependence of the 2-ethylnaphthalene signal on the presence of the dopant molecular ion was further confirmed by the observation that, with the Agilent/Syagen source, ionization of 2-ethylnaphthalene could not at all be achieved without the dopant (Figure 12d). This indicates, that the ionization of 2-ethylnaphthalene takes place by charge exchange rather than direct photoionization (Scheme 1, Reactions 5 and 6, respectively).

The possible neutralization reactions for the dopant molecular ions are presented in Scheme 2. The strong decrease of curtain and end plate currents suggests that the neutralization of the dopant molecular ion takes place by reactions that produce neutral species; i.e. by recombination with a negative ion, electron capture, charge exchange with negative ion or discharge against ion source walls (Scheme 2, Reactions 3-6). Recombination reactions are possible with APPI, since positive and negative ions can be present simultaneously. The role of charge exchange and proton transfer with neutrals (Scheme 2, Reactions 7 and 8) in the neutralization of the dopant molecular ion cannot be ruled out either. With chloroform as the solvent, proton transfer with the solvent is unlikely, but solvent impurities may be involved in the loss of dopant and analyte ions.

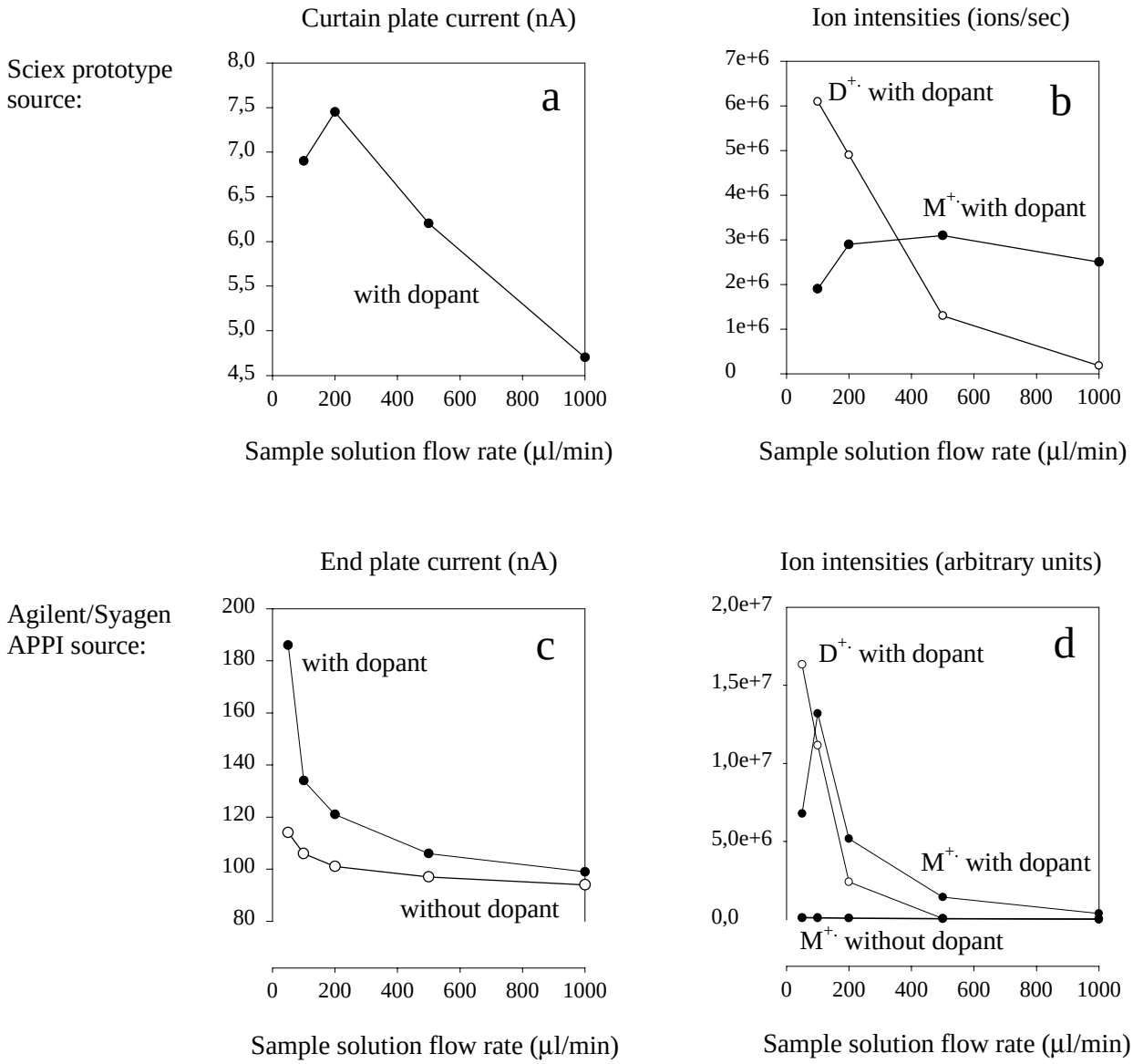

Figure 12. Effect of the solvent flow rate on the ionization of 2-ethylnaphthalene. a-b: 2-ethylnaphthalene in chloroform by using the Sciex prototype source. a: curtain plate current, b: intensities of $M^{+\cdot}$ of 2-ethylnaphthalene and $D^{+\cdot}$ of toluene. Chloroform flow rate 100-1000 µl/min, dopant (toluene) flow rate 10-100 µl/min and 2-ethylnaphthalene concentration 200 nM. c-d: 2-ethylnaphthalene in chloroform by using the Agilent/Syagen APPI source. c: end plate current with and without the dopant; d: intensities of $M^{+\cdot}$ of 2-ethylnaphthalene and $D^{+\cdot}$ of toluene with and without the dopant. Chloroform flow rate 50-1000 µl/min, dopant flow rate 0 or 5-100 µl/min and 2-ethylnaphthalene concentration 1.4 µM.

Scheme 2. Possible loss mechanisms for photons and ions in APPI

Loss of photons:				
Collisions to surfaces				(1)
Absorption by dopant, solvent and sample molecules without ion formation				(2)
Loss of positive ions (including dopant), with reduction of the total ion current:				
Recombination with a negative ion:	$M^+ + X^-$	$\rightarrow$	MX	(3)
Electron capture:	$M^+ + e^-$	$\rightarrow$	M	(4)
Charge exchange with a negative ion:	$M^+ + X^-$	$\rightarrow$	M + X	(5)
Discharge against source walls:		$\rightarrow$	neutralization	(6)
Loss of positive ions (including dopant), without reduction of the total ion current:				
Charge exchange with a neutral:	$M^+ + X$	$\rightarrow$	$M + X^+$	(7)
Proton transfer with a neutral:	$[M+H]^+ + X$	$\rightarrow$	$M + [X+H]^+$	(8)

The signal of the protonated molecule of acridine in acetonitrile was observed to saturate when the flow rate was increased, but the effect was not as strong as it was on the signal of 2-ethylnaphthalene (Figures 13b and 13d). In fact, the signal-to-noise ratio (S/N) of acridine even increased, as the total ion current decreased more abruptly than the signal of $[M+H]^+$ of acridine. Acridine was thought to be less effected by the flow rate than 2-ethylnaphthalene because the formation of protonated molecules is not directly dependent on dopant molecular ions but instead on proton donors in the system. This was supported by the fact that efficient ionization with the Agilent/Syagen source was also achieved without the dopant (Figure 13d). Slight saturation of the $[M+H]^+$ signal of acridine was observed nevertheless, possibly due to decreased amount of proton donors in the system as the dopant was depleted or due to competing reactions by impurities. Another possible reason for the ion loss is the discharge of ions to ion source walls (Scheme 2, Reaction 6). This hypothesis was supported by the observation, that the $[M+H]^+$ signals saturated more with the Sciex source, than with the Agilent/Syagen APPI source that uses a more open ionization space (Figures 3, 4, 13b and 13d).

The effect of temperature and gas flows on the flow rate dependency was tested, and it turned out that more heat and more powerful nebulization was needed at higher solvent flow rates. However, even with the highest possible temperature (500 °C) and gas flows, the analyte signal was observed to level off at high flow rates. Therefore, the apparent ion loss could not be explained by insufficient nebulization and evaporation.

Different lamp currents were investigated in order to explain whether insufficient lamp output could be the cause of ion loss at high solvent flow rates. The overall ionization efficiency was somewhat improved at higher lamp current, but the effect of high solvent flow rate stayed the same, indicating that the output of photons emitted by the lamp has no influence on the flow rate dependence of the signal.

Whether the ion loss at high solvent flow rates is caused by absorption of photons by the solvent (Scheme 2, Reaction 2) was tested by delivering the dopant to the Sciex prototype source together with the lamp gas rather than with the auxiliary gas as usual (Figure 3). This

was hoped to lead to more efficient ionization of the dopant in front of the lamp window before the possible absorption of photons by the solvent. However, the signal was observed to saturate the same way as earlier and it was concluded that either the loss of photons in non-ionizing interaction with solvent molecules is not important or that the mixing of solvent vapor with lamp gas is such an efficient process that a clean sheath of dopant in lamp gas just in front of the lamp window cannot be established and absorption of the photons by solvent vapor cannot be avoided.

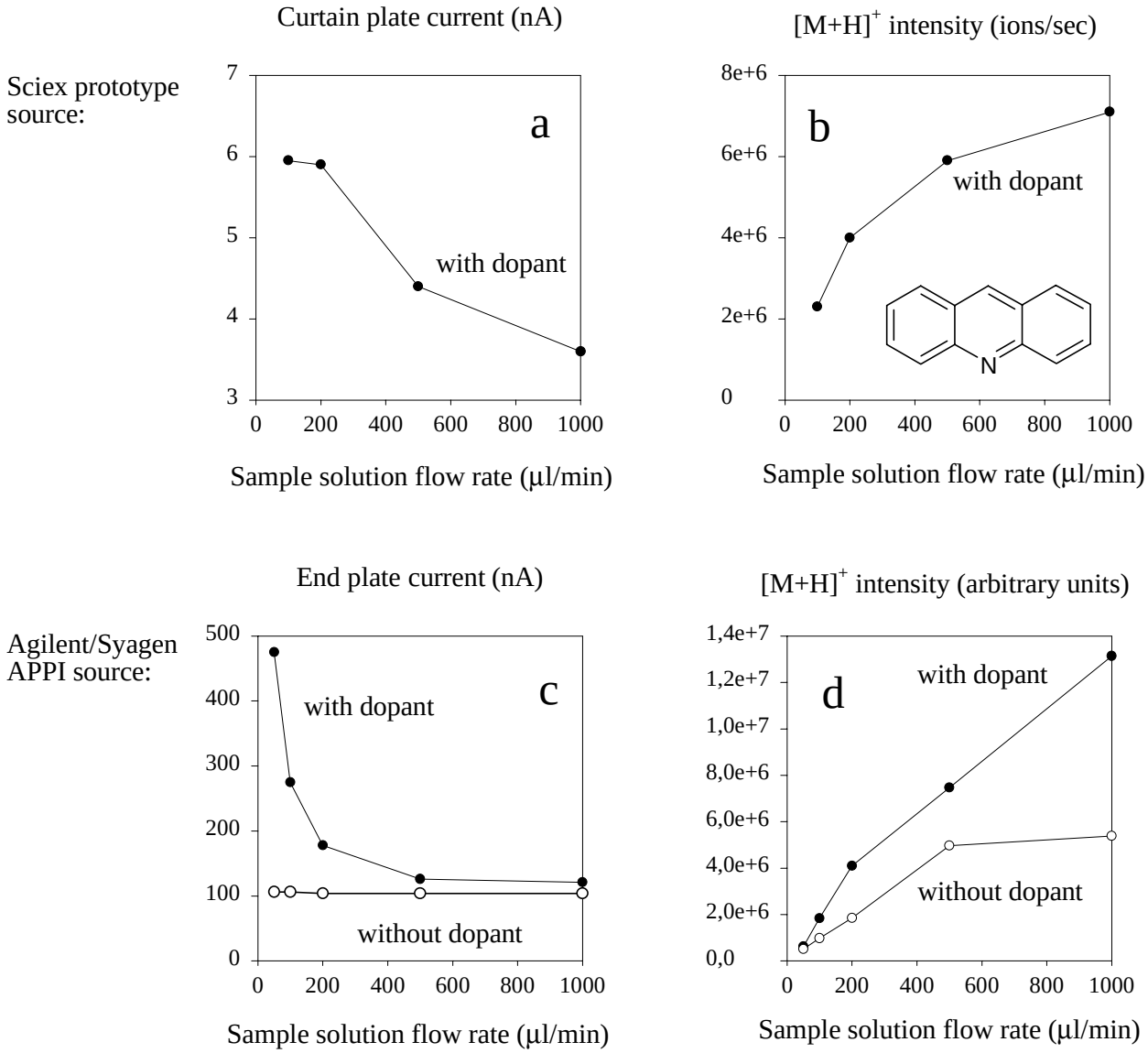

Figure 13. Effect of the solvent flow rate on the ionization of acridine. a-b: acridine in acetonitrile by using the Sciex prototype source. a: curtain plate current; b: $[M+H]^{+\cdot}$ intensity. Acetonitrile flow rate 100-1000 µl/min, dopant flow rate 10-100 µl/min and acridine concentration 200 nM. c-d: acridine in acetonitrile by using the Agilent/Syagen APPI source. c: end plate current; d: $[M+H]^{+\cdot}$ intensity. Acetonitrile flow rate 50-1000 µl/min, dopant flow rate 0 or 5-100 µl/min and acridine concentration 200 nM.

5.2 Ionization process in negative ion APPI (I, II)

The ionization mechanism in negative ion APPI was studied by analyzing a series of compounds (2-naphthoic acid, 2-naphthol, 3-methyl-2,5-furandione, 2,5-furandione, hexachlorobenzene, 1,4-dinitrobenzene and 1,4-naphthoquinone, Figure 5) in 17 different solvent systems (Table 8). The studied compounds possessed either high gas-phase acidities, positive electron affinities (EA) or both (Appendix 1) and could therefore be ionized in negative ion mode either by proton transfer to form a deprotonated molecule, or by electron capture or charge exchange to form a negative molecular ion (Scheme 3). On the other hand, the wide variety of solvent systems with different polarities and gas-phase acidities provided a possibility to study systematically the ionization process as well as the effect of the solvent in negative ion APPI. Furthermore, the solvent compositions studied are commonly used in liquid chromatography and therefore interesting in the view of applications.

5.2.1 Thermal electrons

The ionization process in negative ion APPI is thought to be initiated by low-energy electrons released in the photoionization of the dopant (Scheme 3, Reaction 1), although it has also been suggested that the electrons are released from the metal surfaces of the ion source when radiated by the photons [70]. In our study, the role of the dopant was supported by the observation that the signal intensity decreased abruptly when the dopant was not used. The low-energy electrons may be captured by species, such as analytes, solvents or gases, that possess positive electron affinities (Scheme 3, Reactions 2-5), and the resulting ions can react further with other species (Scheme 3, Reactions 6-13).

Table 8. The solvents used in the study.

1.	Hexane
2.	Chloroform
3.	Water
4.	Methanol
5.	Acetonitrile
6.	Water/ methanol (50:50 %)
7.	Water/ acetonitrile (50:50 %)
8.	Water/ methanol/ acetic acid (50:50:0.1 %)
9.	Water/ methanol/ ammonium acetate (50:50:0.1 %)
10.	Water/ methanol/ ammonium hydroxide (50:50:0.1 %)
11.	Water/ methanol/ formic acid (50:50:0.1 %)
12.	Water/ methanol/ trifluoroacetic acid (50:50:0.1 %)
13.	Water/ acetonitrile/ acetic acid (50:50:0.1 %)
14.	Water/ acetonitrile/ ammonium acetate (50:50:0.1 %)
15.	Water/ acetonitrile/ ammonium hydroxide (50:50:0.1 %)
16.	Water/ acetonitrile/ formic acid (50:50:0.1 %)
17.	Water/ acetonitrile/ trifluoroacetic acid (50:50:0.1 %)

Scheme 3. Possible reactions in negative ion APPI

$D + h\nu$	$\rightarrow$	$D^{+\cdot} + e^-$		(1)
$M + e^-$	$\rightarrow$	$M^{-\cdot}$,	if EA (M) > 0 eV	(2)
$S + e^-$	$\rightarrow$	$[S\text{-}F]^{\cdot} + F^-$,	if EA (M) > 0 eV, F = fragment	(3)
$S + e^-$	$\rightarrow$	$S^{-\cdot}$,	if EA (S) > 0 eV	(4)
$O_2 + e^-$	$\rightarrow$	$O_2^{-\cdot}$		(5)
$S + [X\text{-}H]^-$	$\rightarrow$	$[S\text{-}H]^- + X$,	if $\Delta_{acid}G$ (S) < $\Delta_{acid}G$ (X)	(6)
$M + [X\text{-}H]^-$	$\rightarrow$	$[M\text{-}H]^- + X$,	if $\Delta_{acid}G$ (M) < $\Delta_{acid}G$ (S)	(7)
$M + X^{-\cdot}$	$\rightarrow$	$M^{-\cdot} + X$,	if EA (M) > EA (X)	(8)
$M^{-\cdot} + O_2$	$\rightarrow$	$[M\text{-}X+O]^- + OX^{\cdot}$,	X = H, Cl, NO_2	(9)
$M + O_2^{-\cdot}$	$\rightarrow$	$[M\text{-}X+O]^- + OX^{\cdot}$,	X = H, Cl, NO_2	(10)
$S + O_2^{-\cdot}$	$\rightarrow$	$[S\text{-}H]^- + HO_2^{\cdot}$,	if $\Delta_{acid}G$ (S) < $\Delta_{acid}G$ ($HO_2^{\cdot}$)	(11)
$M + O_2^{-\cdot}$	$\rightarrow$	$[M\text{-}H]^- + HO_2^{\cdot}$,	if $\Delta_{acid}G$ (M) < $\Delta_{acid}G$ ($HO_2^{\cdot}$)	(12)
$M + O_2^{-\cdot}$	$\rightarrow$	$M^{-\cdot} + O_2$,	if EA (M) > EA (O_2) = 0.451 eV	(13)

5.2.2 Formation of reactant ions

The background spectra of the neutral (Solvents 1-7, except chloroform) and basic solvents (Solvents 10 and 15) showed ions that originated from ionization of toluene (used as the dopant) or atmospheric gases present in the ion source (m/z 46, 59, 60, 61 and 77, Table 9). Chloroform (Solvent 2) was an exception as it showed Cl^- and other ions with characteristic chlorine isotope patterns, probably formed by dissociative electron capture (Scheme 3, Reaction 3) and gas-phase reactions between fragments of chloroform and other gas-phase components. The background changed radically when acids or ammonium acetate (Solvents 8, 9, 11, 12, 13, 14, 16, 17) were introduced to the system, and very abundant deprotonated molecules of the acids ($[CH_3COO]^-$, $[HCOO]^-$ and $[CF_3COO]^-$) were observed instead of the ions m/z 46, 59, 60, 61 and 77. This was thought to be due to neutralization of the atmospheric anions ($[NO_2]^-$, $[CO_3]^-$, $[HCO_3]^-$ etc.) by proton transfer with the components of higher gas-phase acidity (Scheme 3, Reaction 6). In addition, all the background spectra contained several ions that could not be identified. Furthermore, the mass spectrometer used discriminates against low m/z values, leaving many important ions, such as $O_2^{-\cdot}$ and CH_3O^- undetectable.

5.2.3 Ionization of the analytes

Analytes of high or moderate gas-phase acidity (2-naphthoic acid, 2-naphthol, 3-methyl-2,5-furandione and 2,5-furandione, Figure 5) were ionized through proton transfer, forming deprotonated molecules ($[M\text{-}H]^-$) (Figure 14; Scheme 3, Reaction 7). In addition, fragments, solvent adducts, substitution products and other gas-phase reaction products were observed in their spectra. Deprotonation is possible for compounds that have higher gas-phase acidities than the reactant molecules in the ion source, i.e. their $\Delta_{acid}G$s are below those of the reactants (Appendix 1). Alternatively it can be presented that the deprotonation of the analyte takes place if the proton affinity (PA) of the deprotonated analyte is below that of the reactant anion.

The formation of negative molecular ion $M^{-\cdot}$ by electron capture or charge exchange (Scheme 3, Reactions 2 and 8, respectively) is possible for compounds of positive EA - in this study 2,5-furandione, hexachlorobenzene, 1,4-dinitrobenzene and 1,4-naphthoquinone (Figure 5,

Appendix 1). However, an intense $M^{-\cdot}$ was only observed in the 1,4-dinitrobenzene and 1,4-naphthoquinone spectra, in addition a weak $M^{-\cdot}$ was observed in the 2,5-furandione spectra in solvents 3, 4, 5 and 10 (Figure 14). Hexachlorobenzene spectra showed solely a phenoxide ion $[M\text{-}Cl+O]^-$; similar phenoxide ions were also observed in 1,4-naphthoquinone and 1,4-dinitrobenzene spectra ($[M\text{-}H+O]^-$ and $[M\text{-}NO+O]^-$, respectively). The phenoxide ions were thought to result from substitution reactions between the analyte $M^{-\cdot}$ and oxygen or the neutral analyte and superoxide ion $O_2^{-\cdot}$ (Scheme 3, Reactions 9 and 10). Especially for 1,4-naphthoquinone, the formation of phenoxide ions seemed to compete with the formation of negative molecular ions as the proportion of $[M\text{-}X+O]^-$ increased in solvents containing additives, whereas in pure solvents the formation of $M^{-\cdot}$ was more efficient (Figure 14). Also, in comparisons done with APCI, the proportion of phenoxide ions of 1,4-naphthoquinone was observed to be a lot lower than with APPI.

5.2.4 Role of oxygen

The formation of oxidation products indicated the presence of oxygen in the APPI source, although only high purity nitrogen was used as nebulizer and auxiliary gases. The most probable origin for oxygen is the room air, as the ion source operates in atmospheric pressure and is not airtight. Oxygen has positive electron affinity (Appendix 1), and it can therefore capture electrons from its surroundings and form a superoxide ion $O_2^{-\cdot}$ (Scheme 3, Reaction 5). $O_2^{-\cdot}$ is a relatively strong gas-phase base ($\Delta_{acid}G$ of $HO_2^{\cdot}$ = 1451 kJ mol^{-1} [14]), which can deprotonate solvents and analytes that have higher gas-phase acidities (Scheme 3, Reactions 11 and 12). In addition, $O_2^{-\cdot}$ can react by charge exchange with solvents or analytes that possess electron affinities above that of O_2 (Scheme 3, Reaction 13). It is also not clear, whether the substitution reactions involve O_2 or $O_2^{-\cdot}$ (Scheme 3, Reactions 9 and 10) [77, 78].

5.2.5 Effect of the solvent

Negative ion APPI was also observed to be very dependent on the solvent composition. Solvents of high gas-phase acidities (Solvents 8, 9, 11-14, 16 and 17) as well as solvents of positive EA (Solvents 2, 12 and 17) were observed to deteriorate the ionization efficiency of all the analytes (Figure 14). For the solvents of high gas-phase acidities, this was thought to be due to formation of low PA anions such as $HCOO^-$, CH_3COO^-, and CF_3COO^- (Table 9), which can prevent the deprotonation of the analytes. The low PA anions can also protonate and neutralize the superoxide ion (Scheme 3, Reaction 11), which then cannot react with the high EA analytes through charge exchange (Scheme 3, Reaction 13). Analytes, that possessed higher gas-phase acidities than the solvents could, however, be deprotonated and at some cases, with enhanced selectivity (2-naphthoic acid, Figure 14). The solvents of positive EAs (chloroform and trifluoroacetic acid, Solvents 2, 12 and 17) were thought to deplete the low-energy electrons electrons from the system (Scheme 3, Reactions 3 and 4) and thus prevent direct electron capture by oxygen (Scheme 3, Reaction 5) or analytes of positive EA (Scheme 3, Reaction 2). Since superoxide ion is not formed, charge exchange (Scheme 3, Reaction 13) and proton transfer (Scheme 3, Reaction 12) between the superoxide ion and the analytes are also inhibited, which would further explain the sensitivity loss. Another possibility is, that TFA deteriorates the ionization efficiency because of its high acidity and chloroform forms HCl in dissociative electron capture, which also is a strong gas-phase acid and could therefore prevent the deprotonation of the analytes.

Table 9. The background ions of the solvents observed in negative ion APPI

Solvent	Ions observed
Hexane	42 (21) $[CNO]^-$, 45 (15), 46 (30) $[NO_2]^-$, 59 (27), 60 (100) $[(N_2)O_2]^-$; $[CO_3]^-$, 61 (83) $[HCO_3]^-$, 77 (25), 100 (18), 117 (77), 121 (16)
Chloroform	35 (15) $[Cl]^-$, 93 (87), 95 (58), 97 (13) , 109 (13), 115 (17), 117 (100), 118 (14), 135 (41), 137 (17)
Water	42 (14) $[CNO]^-$, 46 (56) $[NO_2]^-$, 59 (27), 60 (65) $[(N_2)O_2]^-$; $[CO_3]^-$, 61 (100) $[HCO_3]^-$, 77 (25), 93 (17), 107 (28), 117 (70), 121 (19)
Methanol	46 (67) $[NO_2]^-$, 59 (28) 60 (57) $[(N_2)O_2]^-$; $[CO_3]^-$, 61 (26) $[HCO_3]^-$, 75 (68), 77 (47), 100 (29), 117 (95), 123 (28), 137 (100)
Acetonitrile	42 (92) $[CNO]^-$, 45 (18), 46 (22) $[NO_2]^-$, 59 (16), 100 (17), 107 (13), 117 (100), 118 (17), 121 (12)
Water/methanol	45 (27), 46 (72) $[NO_2]^-$, 59 (44), 60 (64) $[(N_2)O_2]^-$; $[CO_3]^-$, 61 (86) $[HCO_3]^-$, 75 (39), 77 (49), 100 (24), 117 (100), 121 (21)
Water/acetonitrile	42 (85) $[CNO]^-$, 45 (26), 46 (37) $[NO_2]^-$, 59 (31), 60 (34) $[(N_2)O_2]^-$; $[CO_3]$-, 61 (16) $[HCO_3]^-$, 100 (16), 107 (24), 117 (100), 121 (22)
Water/methanol/CH_3COOH	59 (100) $[CH_3COO]^-$, 117 (34), 157 (22), 142 (11)
Water/methanol/NH_4OAc	59 (100) $[CH_3COO]^-$, 117 (50), 141 (93), 142 (9.0), 157 (20)
Water/methanol/NH_4OH	45 (38), 46 (45) $[NO_2]^-$, 59 (61), 60 (52) $[(N_2)O_2]^-$; $[CO_3]^-$, 61 (86) $[HCO_3]^-$, 73 (22), 75 (39), 77 (39), 95 (31), 117 (100)
Water/methanol/CHOOH	45 (100) $[HCOO]^-$, 113 (93), 117 (43), 129 (17)
Water/methanol/TFA	69 (23) $[CF_3]^-$, 113 (100) $[CF_3COO]^-$, 227 (100) $[2xCF_3COOH-H]^-$, 249 (52) $[CF_3COONa+CF_3COO]^-$, 265 (29) $[CF_3COOK+CF_3COO]^-$, 385 (12)
Water/acetonitrile/CH_3COOH	59 (100) $[CH_3COO]^-$, 117 (35), 119 (6.4), 139 (8.1), 141 (99), 157 (22), 212 (9.2)
Water/acetonitrile/NH_4OAc	59 (100) $[CH_3COO]^-$, 117 (53), 118 (8.1), 119 (7.5), 141 (92), 157 (19)
Water/acetonitrile/NH_4OH	42 (68) $[CNO]^-$, 45 (23), 46 (19) $[NO_2]^-$, 59 (32), 60 (24) $[(N_2)O_2]^-$; $[CO_3]^-$, 61 (26) $[HCO_3]^-$, 95 (35), 107 (18), 117 (100), 121 (18)
Water/acetonitrile/CHOOH	45 (91) $[HCOO]^-$, 113 (100), 117 (41), 129 (19), 181 (9.3)
Water/acetonitrile/TFA	69 (65), 113 (64) $[CF_3COO]^-$, 114 (32), 205 (48), 226 (17), 227 (76) $[2xCF_3COOH-H]^-$, 249 (100) $[CF_3COONa+CF_3COO]^-$, 265 (43) $[CF_3COOK+CF_3COO]^-$, 267 (17), 385 (57)

Only 10 most intensive ions were included in the table. Ions that had intensity < 6 % of the maximum were not included.

NH_4OAc = ammonium acetate, TFA = trifluoroacetic acid.

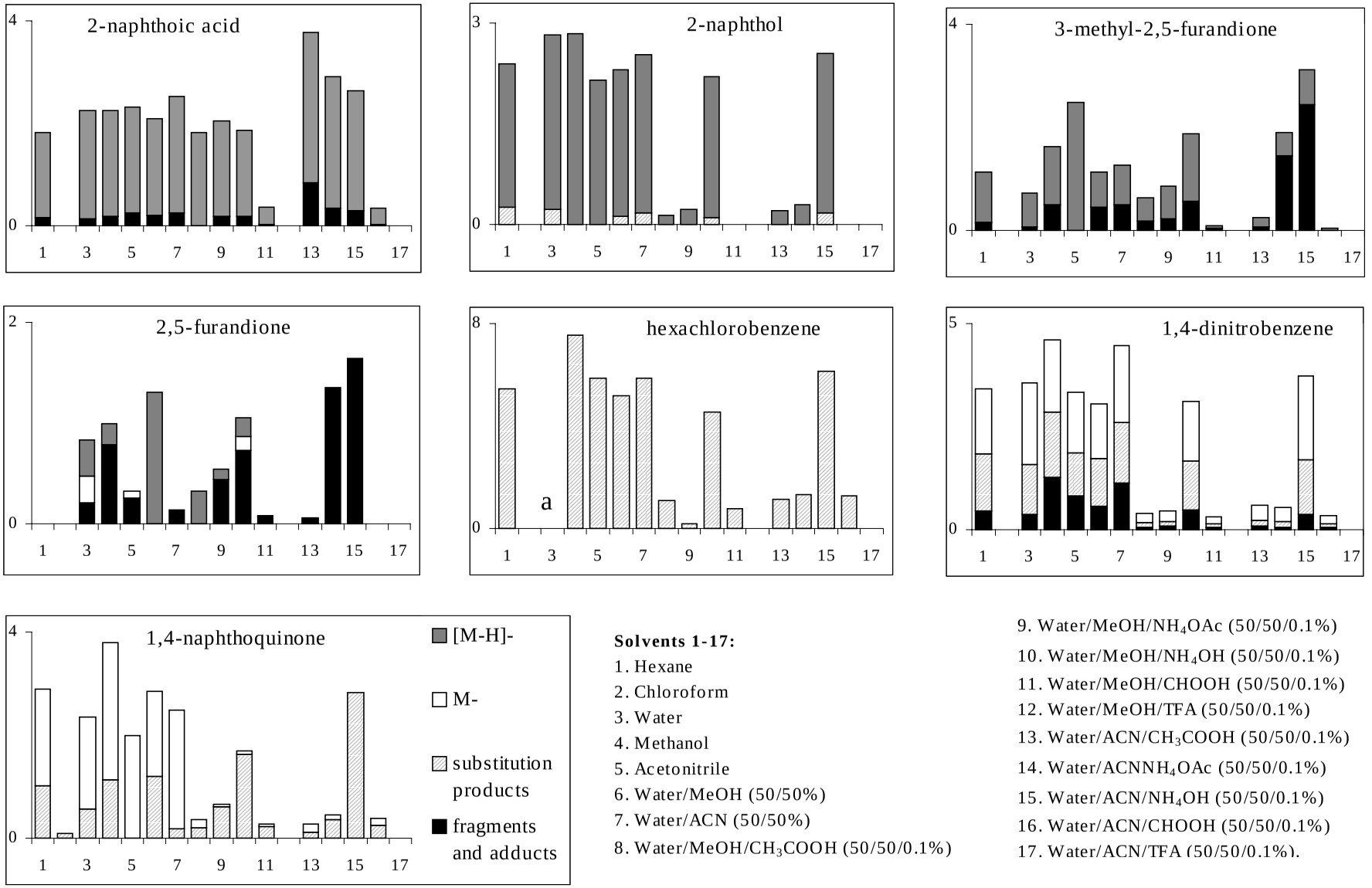

Figure 14. The absolute abundances of the total ion currents and the relative proportions of [M-H]$^-$, M$^-$, fragments and adducts and substitution products. X: solvents 1-17, Y: absolute abundance in arbitrary units. A: the compound could not be dissolved in the solvent in question and was therefore left out of the study.

5.3 Miniaturization of the APPI source (V)

Miniaturization of the APPI source was realized by combining a heated nebulizer microchip, designed for miniaturization of APCI [79], with the krypton discharge lamp, also used with conventional APPI. The heated nebulizer microchip consisted of fluidic and gas inlets, a mixer, a nozzle and a heater (Figures 6 and 7). The sample evaporating from the nozzle was ionized in the gas-phase by initiation of 10 eV photons, as with conventional APPI.

The positions of the nebulizer chip and the krypton discharge lamp were found to be very critical for efficient ionization and were optimized together by monitoring the intensity of the toluene molecular ion signal. The best signal was obtained when the lamp was positioned as close to the MS orifice as possible with the nebulizer microchip in its immediate proximity. Because of the large area demanded by the lamp, the place of the chip had to be compromised and the chip could not be fitted as close to the orifice as when the nebulizer chip was used with the corona discharge needle [79]. Therefore, a modified design with a smaller lamp could permit the position of the nebulizer chip closer to the mass spectrometer orifice, which could increase the sensitivity. A repeller was needed to create a suitable electric field to transfer the ions from the ion source into MS. This was achieved by attaching a metal plate, which was connected to a high voltage supply (1.3-2 kV), in front of the microchip (Figure 6).

The spectra obtained with the micro-APPI and the conventional APPI were compared in the analysis of four compounds. Sensitivities of the two ion sources were not compared, as the optimization of the micro-APPI had not been completed. Dopant (toluene or anisole), which was introduced as part of the solvent, was found necessary for efficient ionization in all measurements. Acridine and 2-naphthol were chosen as analytes in positive ion mode, because they both have low ionization energies, but different proton affinities (PAs). Acridine has high PA and it can readily accept protons from protonated solvent molecules or other species of lower PAs. A protonated molecule ($[M+H]^+$) of acridine, formed by proton transfer, was observed in the acridine spectra with the micro-APPI as well as with the conventional APPI source (Figures 15a and 15b). 2-Naphthol was chosen as an example of low PA compounds that cannot be ionized by proton transfer (I, III). Anisole was used as the dopant when 2-naphthol was analyzed, because of its charge exchange promoting properties (III). The 2-naphthol spectra showed an intense molecular ion ($M^{+\cdot}$) with both sources (Figure 15c and 15d), indicating that efficient ionization of non-polar analytes can also be achieved with the micro-APPI, as shown earlier for conventional APPI (I, III).

In negative ion mode, 1,4-naphthoquinone and 2-naphthoic acid were chosen as model compounds. 1,4-Naphthoquinone has positive electron affinity (EA) and therefore it can be ionized by electron capture or charge exchange [14]. Substitution reactions with atmospheric oxygen have also been reported to be possible (II). In this study, the 1,4-naphthoquinone spectra showed both a negative molecular ion ($M^{-\cdot}$), formed by electron capture or charge exchange as well as phenoxide ions formed by substitution reactions (Figures 15e and 15f). However, the proportion of the phenoxide ions was much higher with the conventional APPI than with the micro-APPI, which indicates that either the substitution reactions are catalyzed by the metal surfaces inside the conventional APPI ion source or they are dependent on the residence time, which may be shorter with the micro-APPI. 2-Naphthoic acid, which was the other analyte used in the negative ion mode, was chosen because of its high gas-phase acidity and thus the tendency to form a deprotonated molecule ($[M-H]^-$) by proton transfer. As assumed, the 2-naphthoic acid spectra showed a deprotonated molecule ($[M-H]^-$) with both ion sources (Figures 15 g and 15h).

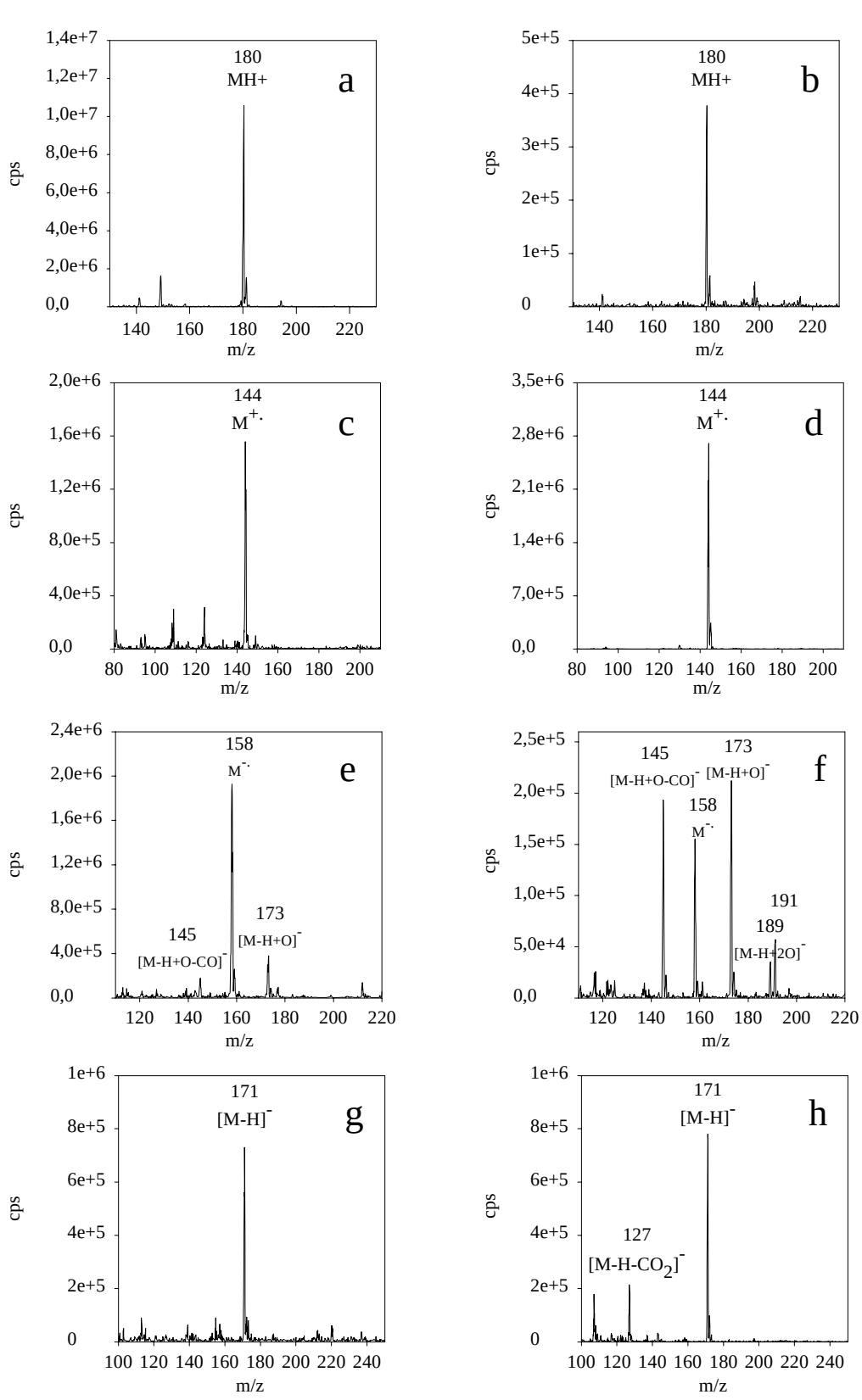

Figure 15. Spectra of the analytes using micro- and conventional APPI.
a: Acridine (100 µM), b: Acridine (100 µM), c: 2-Naphthol (100 µM), d: 2-Naphthol (10 µM), e: 1,4-Naphthoquinone (1 mM), f: 1,4-Naphthoquinone (100 µM), g: 2-Naphthoic acid (1 mM), h: 2-Naphthoic acid (100 µM). Micro-APPI (a, c, e and g): solvent methanol/toluene (9:1), except c: solvent methanol/anisole (9:1); flow rate 2.5 µl/min, except e: flow rate 5 µl/min. Conventional APPI (b, d, f and h): 50-µl loop injection, solvent methanol (200 µl/min), dopant toluene (20 µl/min), except d: dopant anisole (20 µl/min).

The suitability of different flow rates was tested by monitoring the signal of the three main fragments of the $[M+H]^+$ of acridine (measured in MRM mode) at flow rates of 0.05-5 µl/min (Figure 16). Ionization was achieved at all flow rates and the relative abundances of the three product ions were observed to stay the same throughout the measurement. However, the absolute abundance of the signal was observed to rise as the flow rate was increased, being most intense when the flow rate was >1 µl/min. This was thought to be due to the mass flow dependency of the system at these flow rates or due to insufficient amount of the dopant (10 % of solvent), as it has been shown earlier that the amount of the dopant is a crucial factor in APPI and may limit the sensitivity (IV).

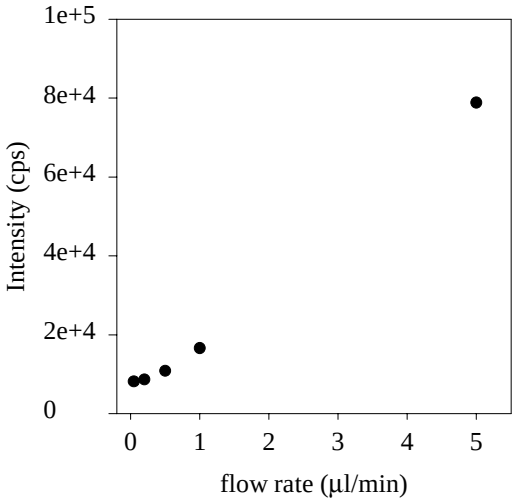

Figure 16. The signal of the three main product ions of the $[M+H]^+$ of acridine (100 µM) in methanol/toluene (9:1) at flow rates 0.05-5 µl/min with the micro-APPI (measured in MRM mode).

The performance of the micro-APPI was evaluated by introducing 100 µM acridine in methanol/toluene (9:1) with flow rate of 2.5 µl/min and by monitoring the signal of 3 main product ions of acridine $[M+H]^+$. The micro-APPI produced a highly stable ion current as demonstrated in a five-hour continuous measurement (Figure 17). The stable performance of the micro-APPI is an important step in the development of a robust quantitative interface for the connection of microfluidic devices to mass spectrometer.

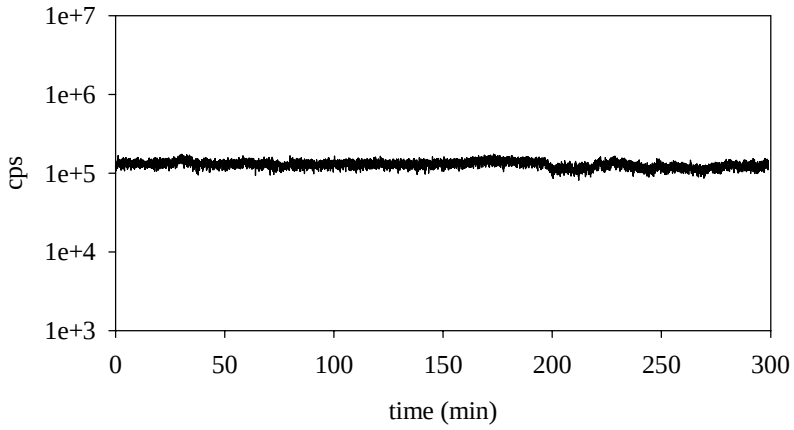

Figure 17. Stability of the micro-APPI; acridine (100 µM) in methanol/toluene (9:1), MRM of 3 main fragments of $[M+H]^+$ of acridine.

6 SUMMARY AND CONCLUSIONS

In positive ion APPI, the main ionization reactions were proton transfer and charge exchange: the reaction route depended on the proton affinities (PAs) of the analytes, the solvent and the dopant. The ionization efficiency was clearly dependent on the presence of dopant, which indicates that, at least in the conditions of our studies, the initiative reaction was the photoionization of the dopant and the formation of dopant molecular ion ($D^{+\cdot}$). $D^{+\cdot}$ was observed to react with the solvents and analytes through proton transfer and charge exchange. Proton transfer from $D^{+\cdot}$ to solvent took place when solvents or solvent clusters that possessed higher PA than the deprotonated $D^{+\cdot}$ ($[D\text{-}H]^{\cdot}$) were present. Thus formed protonated solvent molecules and solvent molecule clusters could in turn protonate the high PA analytes. When the formation of protonated solvent molecules was inhibited, for example when the $[D\text{-}H]^{\cdot}$ of the dopant possessed higher PA, the efficiency of proton transfer was observed to decrease slightly. Formation of protonated analytes by proton transfer was also suppressed when solvents and additives that have PAs above those of the analytes were present. However, for the analysis of high PA analytes, additional selectivity could perhaps be gained by the use of high PA additives, as possible impurities of lower PA would be left unionized.

Non-polar compounds that possess low ionization energies (IEs) and PAs were observed to be ionized by direct charge exchange with $D^{+\cdot}$, and were therefore very dependent on its presence. When high PA gas-phase species, such as solvent and impurities, were present, $D^{+\cdot}$ was readily neutralized by proton transfer, which prevented the possibility for charge exchange and decreased the ionization efficiency for non-polar analytes. However, the wish to analyze non-polar analytes is often the reason for choosing APPI as the ionization method, as their analysis cannot be achieved by APCI or ESI. Therefore, conditions that promote ionization of non-polar compounds and minimize the interference by high PA species are often wanted. By choosing the dopant and solvent so, that the PA of the deprotonated dopant molecular ion is higher than that of the solvent, neutralization of $D^{+\cdot}$ by proton transfer can be prevented. This can be done by choosing a low PA solvent, such as water, hexane or chloroform, that cannot accept protons or by choosing a dopant, such as anisole, whose $[D\text{-}H]^{\cdot}$ has higher PA than the typical LC solvents and, therefore, does not give up its protons as easily. Also, the IE of the analyte should be lower than the IE of the dopant to admit the charge exchange between the dopant and the analyte.

In negative ion APPI, the analytes were ionized by proton transfer, electron capture, charge exchange and substitution reactions. Analytes that possess high gas-phase acidities formed deprotonated molecules by proton transfer, whereas analytes of high electron affinity (EA) formed negative molecular ions by electron capture or charge exchange. In addition, phenoxide ions formed by substitution reactions with oxygen were observed. Also in negative ion APPI, the ionization efficiency was highly dependent on the solvent composition. Solvents of high gas-phase acidity suppressed the ionization of all analytes, as they prevented the deprotonation of the analytes and neutralized $O_2^{-\cdot}$, which otherwise could react with high EA analytes by charge exchange. Halogenated solvents had the same effect, but it is not certain whether this was because of their positive EA or formation of high gas-phase acidity species. Negative ion APPI cannot be considered as universal as positive ion APPI, but sensitive and selective analysis of analytes of high gas-phase acidities or positive EAs can surely be achieved, as long as the solvent composition is carefully optimized. The inhibition of proton transfer reactions may increase the selectivity for analytes that are ionized through electron capture. However, as deprotonated ions were observed in all the solvents of this research, this may be difficult to achieve. The effect of the substitution reactions on the

ionization efficiency is not clear, but at least they contribute to the complexity of the spectra. More substitution products were observed with conventional APPI than with APCI or micro-APPI, which indicates that either the substitution reactions are catalyzed by the APPI source surfaces or they are dependent on the resident times, which may be different in different sources. Altogether, there is still a lot to explore in negative ion APPI before all the factors affecting it are understood.

Significant improvement in the signal of low IE, low PA analytes in reversed phase-liquid chromatography (RP-LC) solvents was achieved when toluene, used earlier as the dopant, was replaced with anisole. Because of the higher PA of the anisole $[D\text{-}H]^{\cdot}$, the $D^{+\cdot}$ of anisole does not react as readily with the solvent by proton transfer and is therefore available for efficient charge exchange with the analytes. This was observed to cause a 100-fold improvement in the signal of some of the tested low polarity analytes. Thus, separation of low polarity compounds by RP-LC and their on-line detection by MS has finally become possible, thanks to the sensitivity achieved by APPI and anisole as the dopant. The benefit gained with anisole as the dopant should be taken into practice and applied in the analysis of real samples in real matrices as it is expected to improve the selectivity for compounds ionized by charge exchange and facilitate their analysis even in difficult matrices. Also, it would be worth testing whether other substances could give similar or even better sensitivity improvement or lead to selective ionization of specific compounds when used as dopants. Finally, the effect of different dopants in negative ion APPI has still not been tested. Dopants of very low IE could perhaps increase the amount of thermal electrons in the system and thus lead to increase in the overall ionization efficiency in negative ion mode. Different dopants might also affect the ionization routes, such as the formation of substitution products.

Solvent flow rate was found to be an important factor in APPI, which has to be taken into account when application methods are developed. In this study, it was shown that solvent flow rates that are too high may reduce the sensitivity due to neutralization of the dopant molecular ions and/or loss of photons. On the other hand, at low flow rates, a sufficient amount of the dopant must be present to ensure efficient ionization. Non-polar analytes, which are ionized through charge exchange, were shown to be more dependent on the amount of dopant molecular ions than polar, high PA analytes and therefore require more careful optimization of the analysis conditions. Although the overall signal was often found to decrease due to increasing flow rate, in some cases the signal-to-noise ratio (S/N) of the analytes increased. Therefore, experiments on the effect of the solvent flow rate on the signal of the analytes and especially on S/N should be carried out when developing application methods with APPI. It would also be interesting to study, whether replacing toluene with anisole as the dopant, removes the flow rate dependence, as anisole molecular ions are not as easily neutralized due to the higher PA of anisole $[D\text{-}H]^{\cdot}$.

Finally, a micro-APPI source was developed and its use in the analysis of four compounds was demonstrated. Efficient ionization of the analytes was achieved in positive and negative ion modes and mainly the same ions were observed as with conventional APPI. Development of the micro-APPI source, which can be used at very low flow rates makes possible the combination of micro/nano-LC or microfluidic devices to MS, which is not possible with commercial APPI. Furthermore, the manufacturing costs of micro-APPI are much lower than those of conventional APPI. The long term stability of the micro-APPI source, as well as the capability of analyzing polar and non-polar analytes from very small sample volumes are advantages over present ESI chips and make the micro-APPI a considerable alternative for interfacing low flow rate separation systems with MS. In future, the performance of the

micro-APPI can be improved by optimizing the voltage and the shape and position of the repeller. Moreover, a smaller lamp design would admit the nebulizer chip to be placed closer to the MS orifice, which may also increase the ionization efficiency. A new chip design, which has a special inlet for the dopant, is now being fabricated. It will make the source more practical, as it will allow the use of solvents that are not soluble to typical dopants.

Overall, APPI holds great potential as an ionization technique that overcomes the difficulties associated with APCI and especially with ESI in the analysis of non-polar, neutral compounds. In particular, the use of anisole is expected to give excellent efficiency and selectivity for low IE, low PA analytes. However, application of APPI to the analysis of non-polar analytes has begun surprisingly slowly, and there are still many unexplored areas that could benefit from the advantages of APPI. It is hoped that new applications that outline the potentials of APPI and further explain its complicated ionization process will emerge. Despite of the work done in this study, the ionization process in APPI is still far from clear and a lot of work is needed before it can be totally understood. Especially, the negative ion mode has obtained little attention and should be examined more deeply. In addition, the testing of new dopants in positive and negative ion modes could open new perspectives and lead to significant sensitivity and selectivity improvements. New information on the ionization process could probably also be obtained by systematic comparison of the two commercial APPI sources and by explaining the reasons for their different behavior. Other future challenges will be the combination of APPI and micro-APPI with other API sources as well as the combination of APPI with matrix-assisted laser desorption ionization (MALDI).

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APPENDICES

Appendix 1. Selected energetics.

Compound	IE (eV)	PA (kJ/mol)	EA (eV)	$\Delta_{acid}G$ (kJ/mol)
Acetic acid	10.65	783.7	-	1429
2-Acetonaphthone	8.31	909.0[a]	-	-
Acetone	9.703	812.0	0.0015	1514
Acetonitrile	12.2	779.2	0.01	1528
Ammonia	10.07	853.6	-	-
Anisole	8.20	839.6	-	1648
Benzene	9.243	750.4	-	1644
Benzoic acid	9.3	821.1	-	1393
Benzyl radical	7.2	831.4	-	-
Carbon dioxide	13.777	540.5	-	-
Chlorine	12.97	513.6	3.6	-
Chloroform	11.37	-	0.622	1464
1,4-Dinitrobenzene	10.3	-	2.003	-
2-Ethylnaphthalene	7.95	835.9[a]	<0.195	-
Formic acid	11.33	742.0	-	1415
2,5-Furandione	11.07		1.440	-
Hexachlorobenzene	9.0	-	0.915	-
n-Hexane	10.13	-	-	-
HNO_2	< 11.3	-	-	1396
$HO_2^{\cdot}$	11.35	660.0	-	1451
Hydrochloric acid	12.74	556.9	-	1373
Methanol	10.84	754.3	-	1565
3-Methyl-2,5-furandione	10.7	-	1.297	-
2-Naphthaleneethanol	-	826.3[a]	-	-
1-Naphthalenemethylamine	8.05	961.2[a]	-	-
2-Naphthoic acid	-	-	-	1370[c]
2-Naphthol	7.87	865.2[a]	-	1408
1,4-Naphthoquinone	9.5	-	1.813	-
2-Naphthylacetic acid	8.05	823.4[b]	-	-
Nitrogen	15.581	493.8	-	-
NO_2	9.586	591.0	2.273	-
Oxygen	12.1	421.0	0.451	-
Phenol	8.49	817.3	-	1432
Phenyl radical	-	884.0	1.096	-
Toluene	8.83	784.0	-	1567
Trifluoroacetic acid	11.5	711.7	-	1328
Trifluoroacetic acid	11.5	711.7	-	1328
Water	12.6	691.0	-	1607

IE, ionization energy; PA, proton affinity; EA, electron affinity; $\Delta_{acid}G$, gas-phase acidity

[a] The PAs of the naphthalenes were estimated from the PAs of the corresponding phenyl compounds [14]. The substituted naphthalene ring was estimated to have a 47.9 kJ/mol higher PA than a substituted benzene ring. This was calculated from the PAs of toluene (784 kJ/mol) and 2-methylnaphthalene (831.9 kJ/mol).

[b] The PA of naphthaleneacetic acid was estimated by comparing the PAs of acetic acid (783.7 kJ/mol), propanol (786.5 kJ/mol) and naphthaleneethanol (826.3 kJ/mol) as the PA of the phenylacetic acid could not be found in the literature [14].

[c] The gas-phase acidity of 2-naphthoic acid was estimated from the gas-phase acidities of 2-naphthol (1408 kJ mol^{-1}), phenol (1432 kJ mol^{-1}) and benzoic acid (1393 kJ mol^{-1}) [14].