Soft X-ray Atmospheric Pressure Photoionization in Liquid Chromatography—Mass Spectrometry

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he most common atmospheric pressure ion sources in liquid chromatography−mass spectrometry (LC-MS) are electrospray ionization (ESI), atmospheric pressure chemical ionization (APCI), and atmospheric pressure photoionization (APPI). APPI can efficiently ionize a wide range of compounds with different polarities and plays a special role in the analysis of nonpolar neutral compounds, such as steroids, polyaromatic hydrocarbons, and terpenes, which may be poorly ionized with ESI or APCI.1,2 In APPI, the ionization is initiated with 10 eV photons emitted by a vacuum ultraviolet (VUV) lamp. Since the ionization energies (IE) of most used LC eluents (e.g., methanol and acetonitrile) are higher than 10 eV, they are not efficiently ionized by VUV APPI. Therefore, a dopant having an IE below 10 eV, such as toluene, chlorobenzene, or anisole, is commonly added to the eluent to enhance the ionization efficiency. In the ionization process,3 the dopant is first photoionized by 10 eV photons, and a dopant radical cation is formed. If the IE of the analyte is smaller than that of the dopant, charge exchange reaction may occur, and a radical cation of the analyte is formed. In the other ionization pathway, the dopant donates a proton to the eluent molecule, which may react with the analyte by a proton transfer reaction if the proton affinity of the analyte is higher than that of the solvent molecule or its cluster. In the negative ion mode, the photoionization of the dopant forms thermal electrons that initiate the reactions leading to the ionization of analytes. The compounds with high electron affinity (EA) are ionized by electron capture or by charge exchange, and the compounds with high gas-phase acidity are ionized by proton transfer.4

The drawback of VUV APPI is that the use of dopant complicates the method, and commonly used dopants such as toluene and chlorobenzene are harmful for environment and health.

Higher-energy photons, such as soft X-ray photons, can efficiently ionize atoms and molecules without using a dopant. In the soft X-ray regime, photons have an energy of about 0.1−10 keV, which is about 10−1000 times higher than the energy of VUV photons. The energy of the soft X-ray photons is high enough to displace not only a valence electron but often also inner shell electrons, producing single and multiple charged compounds.5−7 The soft X-ray photons generated by a synchrotron light source or by an X-ray tube have been used to study the ionization and fragmentation of different types of small molecules, such as vanillin,6 alcohols,8,9 hydrocarbons,5,7,10 and amino acids.11 In all these studies, the compounds were ionized in the vacuum of a mass spectrometer, while few MS studies are available, in which soft X-ray photons were used for the ionization of compounds...
at atmospheric pressure. Riebe et al. examined the formation of reactant ions at atmospheric pressure from different gases and gas mixtures and ionization of alkyl nitrates in the negative ion mode, using 2.8 keV photons produced by a miniaturized soft X-ray APPI source.12 The same soft X-ray source was applied in the positive ion atmospheric pressure chemical ionization (APCI) of volatile organic compounds produced by various fungi, using gas chromatography-MS.13 Soft X-ray ion source has also been applied in different APCI methods aiming for selective ionization of atmospheric vapors, such as sulfuric acid, low-volatility organic compounds, and amines, in the negative ion mode. Thus far, soft X-ray ionization has not been presented as an ionization method in LC-MS.

Here, we present soft X-ray APPI as an ionization method in LC-MS for the first time. The ionization mechanism is examined with selected test compounds having different gas-phase energetics in the negative ion mode, using soft X-ray APPI source emitting 4.9 keV photons. The ionization efficiency of soft X-ray APPI is compared with that of VUV APPI in negative ion mode, and the feasibility of soft X-ray APPI is demonstrated in the LC-MS analysis of the selected test compounds.

**EXPERIMENTAL SECTION**

The selected test compounds, 2-naphthoic acid, 2-naphthol, 1,4-naphthoquinone, 1,4-dinitrobenzene, and hexachlorobenzene (Figure 1), were purchased from Sigma-Aldrich (Steinheim, Germany and Schweiz, Switzerland). Acetonitrile, methanol, and toluene were purchased from Honeywell International (Seelze, Germany). All chemicals were of analytical or chromatographic grade. The water was purified in a Milli-Q water purification system (Millipore, Molsheim, France). All test compounds were dissolved in a mixture of acetonitrile/water (90/10) with 0.1% formic acid for the LC-MS measurements. Measurements, the integrated fluids system of the MS was used to deliver the sample directly to the ion source at a flow rate of 90 μL min⁻¹. In the LC-MS experiments, an Acquity ultraperformance liquid chromatographic ethylene-bridged hybrid (UPLC BEH) C-18 column (100 mm × 2.1 mm i.d., 1.7 μm particle size) was used for chromatographic separation of the test compounds. Eluent A was 5% acetonitrile in Milli-Q water, and eluent B was 100% acetonitrile. The gradient was as follows: 10% B for 0–2 min, 50% B for 2–4 min, 100% B for 4–5.1 min, and 10% B for 5.1–8.0 min. The flow rate of the mobile phase was 400 μL min⁻¹, the injection volume was 3 μL with partial loop with needle-overfill injection mode, and the column temperature was 40 °C. In all dopant-assisted measurements, toluene was infused with a syringe pump (Pump 11 Elite, Harvard apparatus; Harvard Bioscience Inc., Holliston, MA, USA) to the solvent line before the ion source to create a 10% dopant solution.

The soft X-ray and VUV APPI ion sources were built in-house to the frame of the commercial Waters Zspray Nanoflow ion source. The plastic enclosure of the Nanoflow metal frame was removed, and the nano-ESI probe was replaced with an APCI nebulizer from the Zspray APPI/APCI ion source and positioned to the same distance from the MS inlet as in the APPI/APCI source. The Nanoflow frame was more open than the APPI/APCI source and had built-in XY stages, allowing a separate soft X-ray source (PhotoIonBar L12536; Hamamatsu Photonics K.K., Hamamatsu, Japan) or a krypton discharge VUV lamp (PKR 100; Heraeus Noblelight Ltd., Cambridge, UK) to be attached and placed near the MS inlet region. The VUV lamp emitted 10.0 and 10.6 eV photons. The photon energy in the soft X-ray source was 4.9 keV, and the source was powered with a separate controller (C12537; Hamamatsu). Only one of the emitters was directed toward the MS inlet at a time. The VUV APPI and soft X-ray emitters were positioned at the same distance from the MS inlet. Aluminum foil was wrapped around the ion source frame to block the harmful soft X-ray photons, and the minimum working distance from the soft X-ray source was 2 m when the source was on to ensure safe working conditions.

The mass spectra were acquired in the mass range of mass-to-charge ratio (m/z) 30–500 with a data acquisition frequency of 2 Hz. The cone and desolvation gas flow rates
were 0 and 800 L h\(^{-1}\), and the source and probe temperatures were 100 and 200 °C, respectively. In the LC-MS experiments, the probe temperature was set to 600 °C, due to the increased solution flow rate. The extraction and sampling cone values were set to 4.0 and 30.0, respectively.

## RESULTS AND DISCUSSION

The ionization process in negative ion soft X-ray APPI-MS was examined with the selected test compounds (Figure 1), including acidic groups (2-naphthol and 2-naphthoic acid) and those having positive EAs (1,4-naphthoquinone, 1,4-dinitrobenzene, and hexachlorobenzene). The same test compounds were used earlier to study the VUV APPI process. The spectra of 1,4-naphthoquinone and 2-naphthol showed [M\(^{-}\)H\(^{+}\)] ions at m/z 159, respectively, whereas the spectra of hexachlorobenzene showed abundant [M + Cl\(^{-}\)] ions at m/z 263 with a characteristic chlorine isotope pattern. Some fragment ions were also detected. The spectra of 2-naphthoic acid showed ion [M – H – CO\(_2\)]\(^{-}\) at m/z 127, and the spectra of 1,4-dinitrobenzene showed ion [M – 30\(^{-}\)] at m/z 138, which is formed either by the loss of NO or by a substitution reaction producing ion [M – NO\(_2\) + O\(^{-}\)].

All the spectra of the test compounds measured by dopant-assisted VUV APPI with 10 eV photons are very similar to the spectra measured with soft X-ray APPI with 4.9 keV photons. This suggests that the reactant ion composition is similar in negative ion soft X-ray and VUV APPI. However, the advantage of soft X-ray APPI is that no dopant is needed to achieve high ionization efficiency in the negative ion mode, as shown in Figure 2, which presents a comparison of the ionization efficiencies between soft X-ray and VUV APPI with and without use of a dopant (toluene). The results show that the ionization efficiency is about 10–50 times better with soft X-ray APPI than with VUV APPI without the dopant. However, the use of dopant in VUV APPI significantly increased ionization efficiency achieving a level similar to that of soft X-ray APPI without dopant. In contrast, addition of the dopant did not significantly affect the ionization efficiency in the soft X-ray APPI. These results indicate that the formation of electrons directly from commonly used LC eluents, such as acetonitrile, methanol, and water, or atmospheric gases, is not sufficient to achieve maximum sensitivity with 10 eV VUV photoionization. This is because the IE of the LC eluents or atmospheric gases is higher than 10 eV. However, the addition of a dopant having IE below 10 eV can efficiently produce thermal electrons in VUV photoionization, which explains the significant improvement in ionization efficiency with dopant-assisted VUV APPI in comparison to VUV APPI without the

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**Figure 2.** Comparison of the ionization efficiencies of soft X-ray and VUV APPI with and without dopant (toluene) in the negative ion mode. The comparison measurements were done using infusion of the mixture of compounds in acetonitrile/water (50/50). The bars represent absolute abundances of the total ion currents and proportions of [M – H\(^{-}\)], M\(^{+}\), substitution products, and fragments. A = 2-naphthoic acid, B = 2-naphthol, C = 1,4-naphthoquinone, D = 1,4-dinitrobenzene, and E = hexachlorobenzene. 1 = soft X-ray APPI without dopant, 2 = VUV APPI without dopant, 3 = soft X-ray APPI with a dopant, 4 = VUV APPI with a dopant.
use of a dopant. The energy of the soft X-ray photons was 4.9 keV, high enough to efficiently release valence electrons and often also inner shell electrons without the use of a dopant from all kinds of molecules, including LC eluents and atmospheric gases, resulting in high numbers of thermal electrons and efficient ionization in the negative ion mode. For the same reason, the addition of dopant did not improve the ionization efficiency in soft X-ray APPI. The high ionization efficiency in negative ion soft X-ray APPI is clearly an advantage in comparison to dopant-assisted VUV APPI, because the use of a dopant complicates the analytical system and commonly used dopants (such as toluene and chlorobenzene) are harmful to the environment and health.

We also demonstrated the use of soft X-ray APPI in analysis of the test compounds by LC-MS. The flow rate was 0.4 mL min⁻¹, the gradient consisted of acetonitrile and water, and no dopant was used. The soft X-ray APPI LC-MS chromatograms presented in Figure 3 are the sum of the ion currents of the selected ions were the following: m/z 158 and 173 for 1,4-naphthoquinone, m/z 168 and 138 for 1,4-dinitrobenzene, m/z 171 and 127 for 2-naphthoic acid, m/z 159 and 143 for 2-naphthol, and m/z 263, 265, 267, and 269 for hexachlorobenzene. The limits of detection currently furnish no meaningful information because the soft X-ray setup was an early prototype and the ionization conditions as well as the LC method were not fully optimized. However, comparison between soft X-ray APPI without a dopant and dopant-assisted VUV APPI suggests that soft X-ray APPI can reach at least similar sensitivity as VUV APPI in the negative ion mode.

![Figure 3. Selected ion chromatograms of the test compounds measured by LC-MS using soft X-ray APPI without a dopant. The selected ions were the following: m/z 158 and 173 for 1,4-naphthoquinone, m/z 168 and 138 for 1,4-dinitrobenzene, m/z 171 and 127 for 2-naphthoic acid, m/z 159 and 143 for 2-naphthol, and m/z 263, 265, 267, and 269 for hexachlorobenzene.](image)

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All authors contributed to the writing of the manuscript, and all authors gave their approval to the final version of the manuscript.

#### Notes

The authors declare no competing financial interest.

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