

OVERVIEW

Purpose

- Characterization of the ionization process involve in LDTD-APCI where no liquid is present ;
- Proton transfer reactions characterization of a LDTD-APCI source in terms of Proton affinity and enthalpy of reaction ;
- Real-time determination of the major reactants present in the ionization region.

Method

A LDTD ion source was modified to control the amount of water vapor in the carrier gas during the desorption experiments. Different concentrations of water in air and nitrogen were used to monitor the reactive species present in the corona discharge. Exothermic reactions from proton transfer are evaluated in function of the theoretical values of proton affinities.

Results

The gas composition in the APCI region of a LDTD source differs from the one currently used in LC-APCI-MS. Since there is no solvent involved, water remains the main reactant. Hydronium ions (H₃O⁺) and their clusters ions (H₃O)⁺(H₂O)_n are monitored in the corona discharge as the gas flow (1-10 l/min), current (1-10 uA) and water concentration (1 ppm to saturation) are changed separately for air and nitrogen respectively. Modifications in the proportion of the reactant follow the behavior observed in PTR (proton transfer reaction) experiments and air contaminant analysis. Moreover, the low water cluster forms seems to be the most reactive species in the APCI. Acetaminophen shows fragmentation due to the excess of exothermic energy in the protonation reaction. Increasing the concentration of larger water clusters (higher proton affinity) shows less fragmentation.

INTRODUCTION

The LDTD ionization source is used in mass spectrometry as an alternative way to introduce samples in a mass spectrometer equipped with an atmospheric inlet. The LDTD uses a Laser Diode to produce and control heat on the sample support (Figure 1). The energy is then transferred through the sample holder to the **dry sample** which vaporizes prior to be **carried by a gas in an APCI region for ionization**. The purpose of this work is to present the mechanisms of protonation related to the reactive species in presence. Measurements of these reactants and typical protonation reactions are presented.

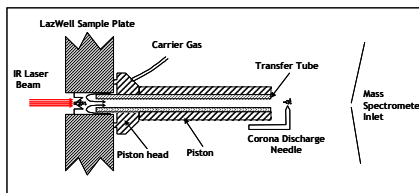


Figure 1 Schematic of the LDTD ionization source.

METHOD

Instrumentation (Figure 2)

- LDTD model T-360, Phytronix Technologies
- Thermo Fisher Scientific TSQ³ VantageTM



Figure 2 LDTD-MS/MS analytical system.

MS Parameters

- APCI (+)
- Optimized for low mass range
- Scan time : 0.1 s
- Q1 width : 0.70 amu
- DCV : 0 V
- Corona Current : 3 uA

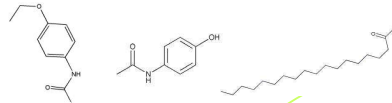
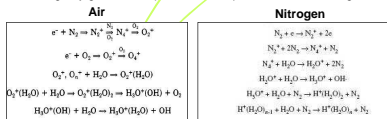


Figure 3 Chemical structure and molecular weight of tested compounds.

Sequence of Ion/Molecule Reactions

The APCI in LDTD is operated in "dry" condition as no liquid phase is introduced into the APCI region. Here are the species present in an APCI environment containing only gaseous components when operated with air or nitrogen.



Reactive Species

- Mainly hydronium ion-water clusters.
- At 25 °C with 21% relative humidity (6600 ppm) the majority of the water clusters contain from 5 to 8 water molecules¹.
- The proton affinities are as follows : H₃O⁺ (691 kJ/mol) < H₃O⁺(H₂O) (820 kJ/mol) < H₃O⁺(H₂O)₂ (835 kJ/mol).
- Note** : A higher proton affinity means that in the gas phase the base is stronger and that the conjugate acid is weaker.

LDTD-APCI Ionization Hypothesis

• The water traces in the carrier gas used in LDTD should produce enough water clusters in the APCI to produce the ionization.

• In "dry" environment (water traces) the main source of protons should be the water cluster forms.

• The more reactive water cluster species should be responsible for the high ionization efficiency of the LDTD-APCI process.

RESULTS

LDTD-APCI Characterization : Reactive Species

Water effect

The water clusters profile in the APCI, when air and nitrogen (with 3 ppm of water) are used as carrier gas, showed H₃O⁺(H₂O)_n with 0<n≤10 as reported in PTR experiments. Adding water into the carrier gas to reach the saturation lowers significantly the low water cluster (n < 3) content while the higher water clusters remain almost constant (Figure 4). The same behavior is observed when nitrogen is used as carrier gas.

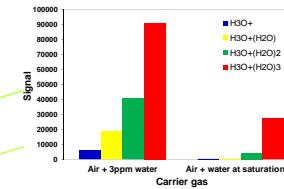


Figure 4 LDTD-MS/MS analytical system.

Carrier Gas Flow Effect

The LDTD carrier gas flow was varied from 0 to 10 L/min and the water clusters, H₃O⁺(H₂O)_n (1≤n≤4), were monitored. The low water cluster species (H₃O⁺ and H₃O⁺(H₂O)) reach a maxima at a gas flow of 3-4 L/min (Figure 5). The optimal carrier gas flow in LDTD is reported to be between 3 and 4 L/min and seems to be associated to the presence of the lower water clusters.

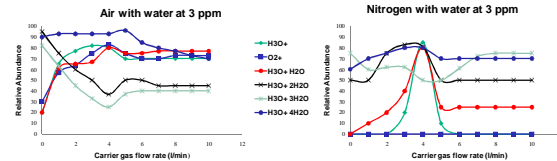


Figure 5 Carrier gas flow effect on the water cluster profile.

Corona Current Effect

• The corona current has been varied from 1 to 6 uA and the water clusters profile does not change.

Protonation Reaction in LDTD-APCI

- The proton transfer is energetically favored if the proton affinity of the respective analyte is greater than the reactant one¹.
- Molecules of proton affinity greater than the hydronium clusters produce an exothermic reaction.
- Smaller clusters of lower proton affinity can react more efficiently than larger ones.
- Exothermic reactions involving the lower water clusters may produce an excess of energy. Thus, fragmentation of a protonated molecule² may occur if its internal energy reaches the dissociation limit.
- Small hydronium clusters have low proton affinities. As observed in the water effect experiment (Figure 4), increasing humidity in the APCI region reduces the number of small clusters³. Therefore, the sensitivity for detecting low proton affinity molecules will decrease accordingly and the fragmentation due to an excess of energy in protonation reaction will also be reduced.

Behavior of Low Proton Affinity Compound

Fatty acids are known in mass spectrometry to have low proton affinity². Stearic acid (285 → 153) has been analyzed under LDTD-(+)APCI using different types of carrier gas (different APCI gaseous environments). The highest signal was recorded when dry air with water at 3 ppm was used (Table 1). Increasing the water vapor until saturation is obtained lowers the stearic acid signal as predicted (Figure 4). The introduction of methanol or acetonitrile decreases even more the signal because they not only reduce the number of small clusters but also the larger ones. In fact they undergo a direct protonation reaction with the water clusters ((M+H)⁺ observed). The ionization efficiency of low proton affinity molecules illustrates the important role of small hydronium clusters species in LDTD. The use of methanol and acetonitrile in LC-APCI generates unfavorable ionization conditions as compared to the "dry" environment observed in LDTD ion source.

Table 1 Stearic Acid signal at different carrier gas composition.

	Dry air (Water at 3 ppm)	Air saturated in water	Dry air with Methanol vapor	Dry air with acetonitrile vapor
Stearic acid area count (average, n=3)	5256	196	53	3

Fragmentation from Exothermic Reaction

Thermal energy in the LDTD source used to desorb molecules, as well as the ionization process, might cause fragmentation. Phenacetin is used to show the protonation fragmentation phenomena. In Figure 6, the molecular ions of phenacetin and acetaminophen are observed in both APCI mode (+ and -). In the phenacetin spectra in positive mode (Figure 6C), there is 2% of fragmentation visible at the mass 152 which corresponds to acetaminophen. If this fragmentation was due to a thermal process, we should observe a corresponding peak at mass 150 in the negative ion spectra of phenacetin (Figure 6D), which is not the case. Thus we conclude that the fragmentation occurs during the protonation reaction.

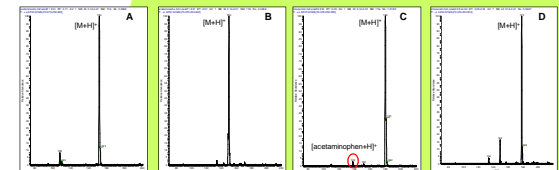


Figure 6 Full mass spectra of A) acetaminophen in (+)APCI, B) acetaminophen in (-)APCI, C) phenacetin in (+)APCI, and D) phenacetin in (-)APCI obtained from LDTD experiments.

Conclusion : High Efficiency Ionisation in LDTD-APCI

- Water traces in air or nitrogen produce low proton affinity reactant leading to high efficiency protonation APCI.
- Increasing the amount of water decreases small hydronium clusters, which reduces the protonation efficiency.
- Molecular fragmentation from an excess of energy might occur during the protonation reaction.
- The use of solvents in the ppm range lower the efficiency of protonation a lot more than what water may cause.

References

1. Sumner J, Nicol G and Kiebarle P. 1988. Analytical Chemistry, 60 : 1300-1307.
2. Tari A. et al. 2004. International Journal of Mass Spectrometry, 239, 161-169.
3. Sinha V. et al. 2009. International Journal of Mass Spectrometry, 282, 108-111.