Co-funded by the Horizon 2020 programme of the European Union

Universität Rostock | HelmholtzZentrum münchen
JOINT MASS SPECTROMETRY CENTRE

Short course

Atmospheric pressure ionization techniques for high resolution mass spectrometry of complex samples

Rostock, 5.3.2018 – 7.3.2018

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JOINT MASS SPECTROMETRY CENTRE
Universität Rostock

Schedule

Atmospheric pressure ionization techniques for high resolution mass spectrometry of complex samples

Monday 5.3. 18:00 –	Arrival and casual get-together in a Rostock pub ("Trotzenburg") + Dinner	
Tuesday 6.3. 9:00 – 11:00	Welcome and start with tutorial lecture:	1. Atmospheric pressure ionization 2. Ion transport into vacuum 3. Bacis of FT-ICR MS
11:00 – 12:00	Lab tour (2 groups)	
12:00 – 13:00	Lunch	
13:00 – 15:00	Hands on – Electrospray ionization (direct infusion)	
15:30 – 17:00	Hands on – Atmospheric pressure chemical ionization	
18:30 – 22:00	Social event (guided tour downtown Rostock) and dinner	
Wednesday 7.3. 9:00 – 12:00	Data analysis of ESI, APCI and GC- APCI/APPI samples – comparison of ionization features, in parallel: running GC – APCI/APPI measurements	
12:00 – 13:00	Lunch	
13:00 – 14:00	Closing meeting and wrap up	

Safety

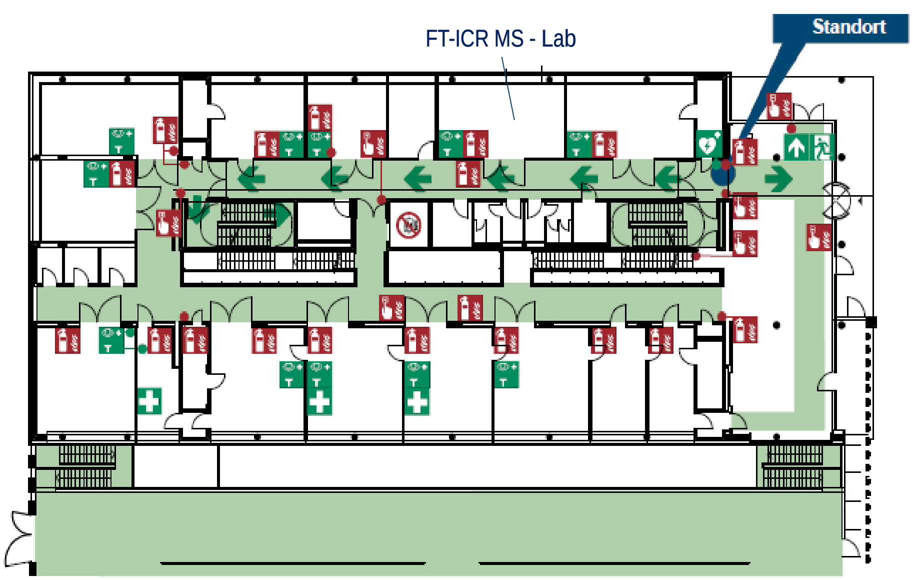
High magnetic fields – watch the marked 5 Gauss line

Laser safety lamp on – do not enter!

No eating, drinking, smoking in the lab

In case of emergency: see escape paths

Safety



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JOINT MASS SPECTROMETRY CENTRE

Short course lecture

Atmospheric pressure ionization techniques

for high resolution mass spectrometry of complex samples

Martin Sklorz
Analytical Chemistry University of Rostock

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Universität Rostock

Motivation

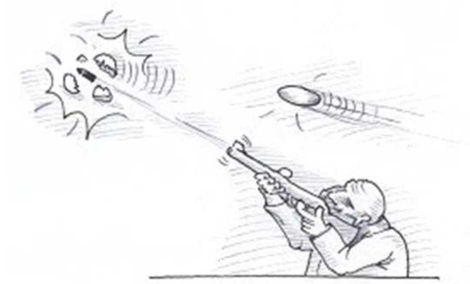
FT-ICR mass spectrometry

- High accuracy in mass determination
- Sum formula and isotopic fine structure accessible
- Universal, as every molecule has a mass, but mass spectrometry detects **ions** (mass per charge)

Ionization requests for complex mixtures:

- Universal
  …mass spectrometric signals reflects composition of neutrals
- Preserve molecular ion information
  …No fragmentation or adduct formation
- Low “matrix” effects and linear response

Excurse EI: electron impact ionization



In high vacuum, no collision and therefore no chemical reaction:

simply: M + e- → M+· + 2e-

70 eV electron energy

Ionization potential for most organic compounds: 8-15 eV

→ **Universal**, but needs transfer of neutrals into high vacuum

- Often loss of molecular ion information
- Gain of structural information

Motivation

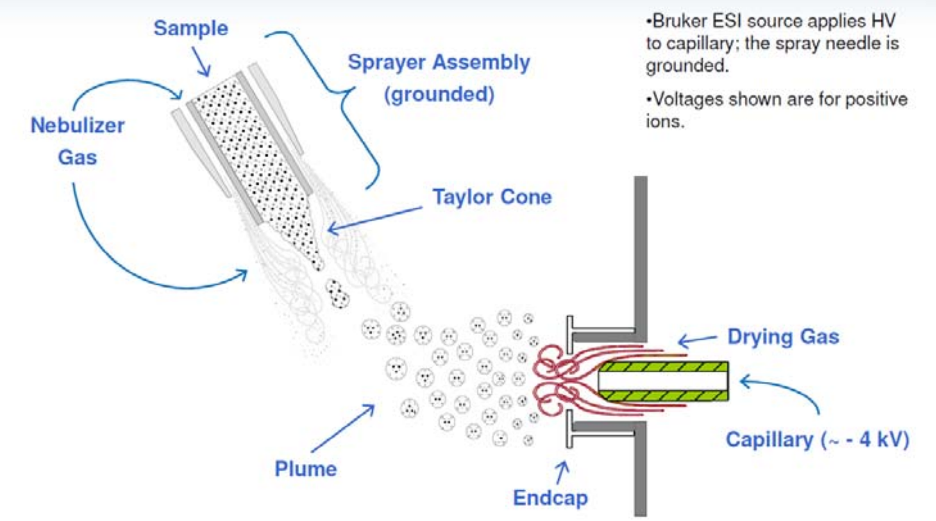
Common to all atmospheric pressure ionization techniques

→ Mean free path (N_2 @ 1013mbar, 273K): ~75nm
Time between collision: ~ 200ps
Mean residence time in ion source ~10ms!

→ Millions of collisions
Reactions can/will occur
Collisional cooling leads to less fragmentation

→ High sensitivity obtainable
High pressure leads to density of analye molecules

ESI APCI APPI APLI



- Bruker ESI source applies HV to capillary; the spray needle is grounded.
- Voltages shown are for positive ions.

John B. Fenn: Science 1989, Mass Spec Rev 1990, JAMS 1993, Noble prize 2002

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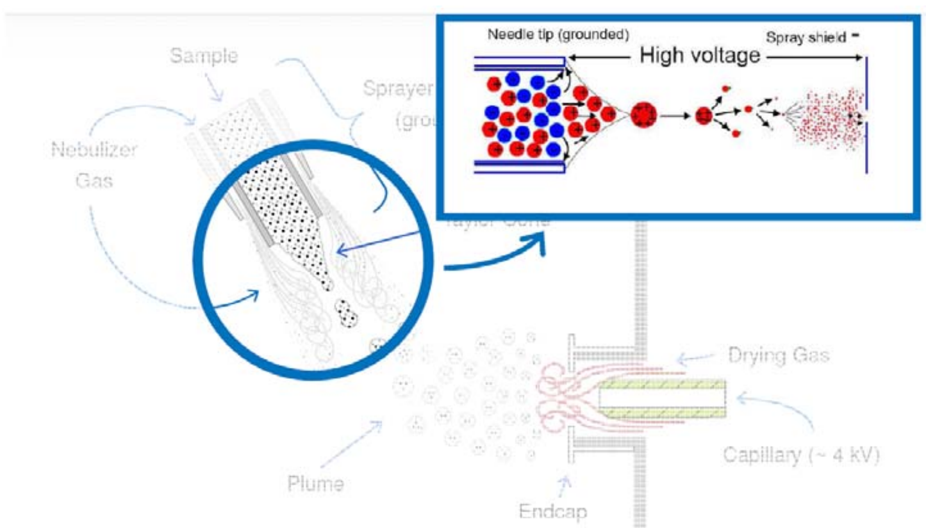
ESI APCI APPI APLI



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ESI APCI APPI APLI

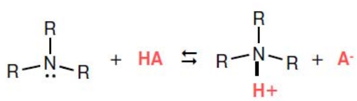
ESI = solution chemistry

Positive Ion Mode

Formation of **protonated** molecular ions

$M + HA \rightleftarrows [M+H]^+ + A^-$

Example:

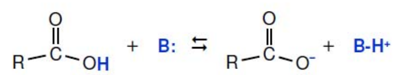


Negative Ion Mode

Formation of **deprotonated** species

$M + B \rightleftarrows [M\text{-}H]^- + BH^+$

Example:



HA: Modifiers for basic components

0.1 - 1 %	formic acid
0.1 - 1%	acetic acid
<0.05%	trifluor acetic acid (toxic!)

B: Modifiers for acidic components:
< 10mM ammonium hydroxide
or buffers as ammonium formate
or ammonium acetate

Most often only „acid/base - charged“ molecules are detectable!

ESI APCI APPI APLI

ESI = solution chemistry

Pros:

- No evaporation necessary → even nonvolatile compounds are ionized
- Collisional cooling leads to low fragmentation
- Multiple charged ions available → high molecular weight compounds accessible

works well for polar compounds
most suitable for peptides and proteins

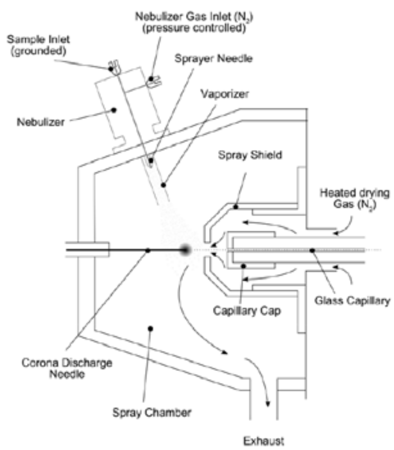
Problems:

- Molecule-ion-interaction in liquid
- Adduct formation (Na^+, K^+) e.g. from sample or glassware
- Cluster formation ($[M+Na+CH_3CN]^+$, $[M+H+MeOH]^+$, $[M+H+M]^+$…
- High matrix suppression → all analytes compete to limited (surface-)charge
- Quantitative results difficult (use internal standards!)

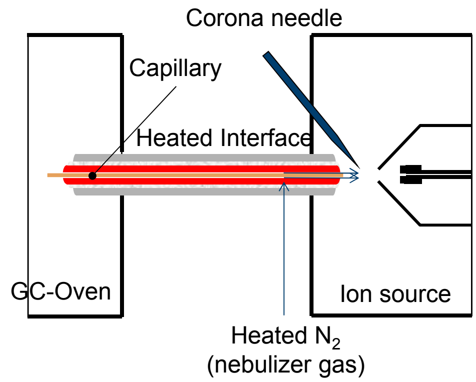
Interferences and contaminants compare: Keller et al. Anal Chim Acta 2008

ESI **APCI** APPI APLI

APCI - evaporated liquid



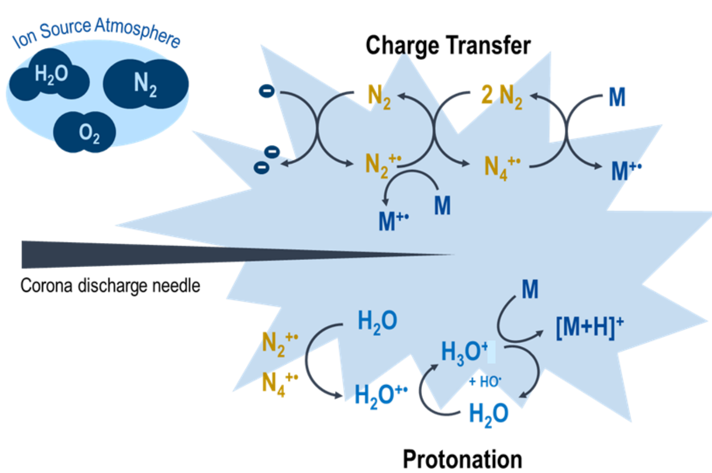
APCI - gas



Horning et al., Anal Chem 1973, , McEven et al.JASMS 2005, Benter et al. ABC 2008

ESI **APCI** APPI APLI

ACPI = gas-phase chemistry



Ionization potential

Molecule	IE (eV)
Nitrogen	15.58
Carbon dioxide	13.78
Water	12.62
Acetonitrile	12.20
Oxygen	12.07
Methanol	10.84
Isopropanol	10.17
n-Hexane	10.13
Heptane	9.93
Isooctane	9.80
Benzene	9.24
Furan	8.88
Toluene	8.83
Anisole	8.20
Naphthalene	8.14
Triethylamine	7.53

Proton affinity

Molecule	PA kJ/mol
Oxygen	422
Nitrogen	495
Carbon dioxide	541
n-Hexane	672
n-Heptane	677
n-Octane	684
Water	691
Benzene	750
Methanol	754
Toluene	784
Isopropanol	793
Acetonitrile	794
Naphthalene	803
Furan	804
Anisole	840
Triethylamine	982

webbook.nist.gov, Hunter&Lias J Phys Chem Rev Data 1998, Hunter&East J Phys Chem 2002

APCI = gas-phase chemistry

Pros:

- Collisional cooling reduces fragmentation (compared to EI)
- No formation of Na and K adducts
- Higher linear dynamic range and less matrix effects (compared to ESI)
- Ideal for hyphenation to gas and liquid chromatography

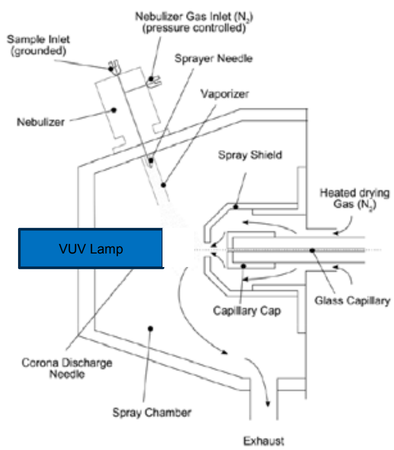
works well for intermediate polar compounds
most suitable for “small molecules” (metabolomic, petroleomic)

Problems:

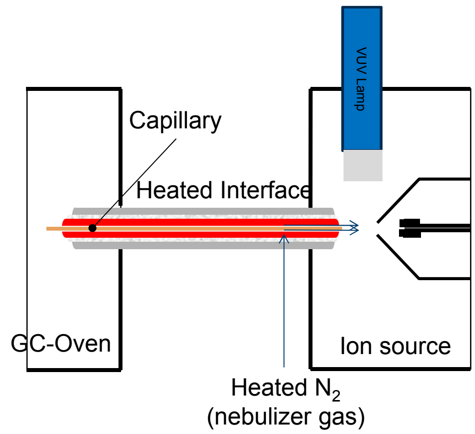
- Need evaporation → not applicable for thermo labile components
- Molecule-ion-interaction in gas-phase
- Complex gas-phase chemistry (reactants, solvent, concentration, positioning….)
- Adduct formation with oxygen
- Corona current needs to be tuned, position of sprayer and needle is critical

ESI APCI **APPI** APLI

APPI - liquid



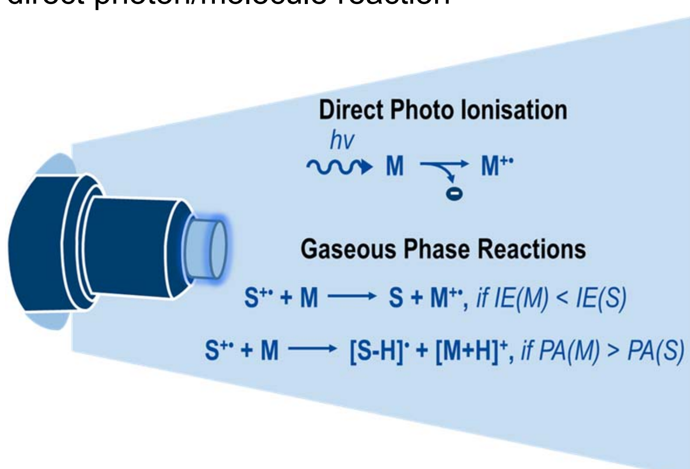
APPI - gas



Horning et al., Anal Chem 1973, , McEven et al.JASMS 2005, Benter et al. ABC 2008

ESI APCI **APPI** APLI

APPI = direct photon/molecule reaction



+ gas-phase chemistry (dopant or solvent or matrix)!!!

ESI APCI **APPI** APLI

Ionization potential

Molecule	IE (eV)
Nitrogen	15.58
Carbon dioxide	13.78
Water	12.62
Acetonitrile	12.20
Oxygen	12.07
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Furan	8.88
Toluene	8.83
Anisole	8.20
Naphthalene	8.14
Triethylamine	7.53

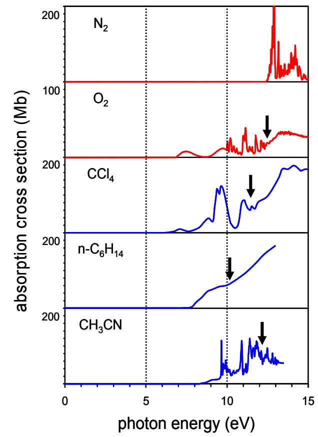
Direct PI

$M + h\nu \rightarrow M^{\bullet+} + e^-$

Krypton lamp
10eV …10.6eV

Xenon lamp
8.4eV

Solvent and impurities are not ionized, but may adsorb photons



webbook.nist.gov, Short et al. JASMS 2006

ESI APCI **APPI** APLI

APPI = direct photon/molecule reaction and gas-phase chemistry

Pros:

- Collisional cooling reduces fragmentation (compared to EI)
- Higher linear dynamic range and less matrix effects (compared to APCI)
- Low noise
- Ideal for hyphenation to gas chromatography

works well for compounds with comparable low IP
most suitable for "small and even less polar molecules"

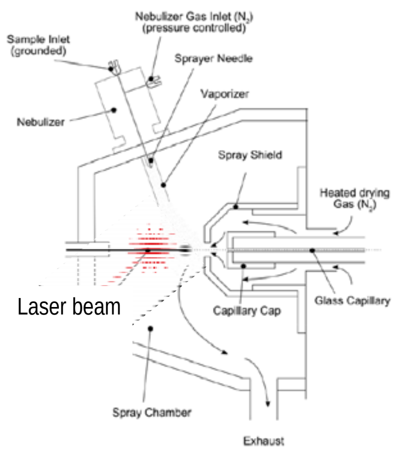
Problems:

- Need evaporation → not applicable for thermo labile components
- Still molecule-ion-interaction occurs (formation of radical cation vs. protonation)
- Dopants and solvents may lead to complex gas-phase chemistry
- Adduct formation with oxygen
- Lamp has a limited lifetime

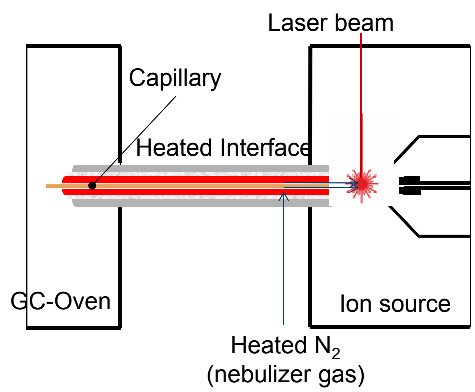
Syage_et al. Am Lab 2000, Kauppila et al. Anal Chem 2002, Hanold et al. Ana Chem 2004, Kauppila, Syage, Benter Mass Spec Rev. 2017

ESI APCI APPI **APLI**

APLI - evaporated liquid



APLI - gas



Schmidt et al. Anal Chem 1999, Kersten et al. JASMS 2011

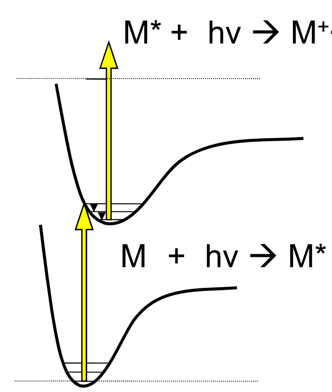
ESI APCI APPI **APLI**

APLI (REMPI) uses UV photons
248nm (5.00 eV)
266nm (4.66 eV)

Two photon process

High photon density (~10^7 W/cm^2)
Short pulses (6ns…10ns)

Selective for **aromatic and polyaromatic compounds,** having a relatively large lifetime of the intermediate S1 or S2 states



Boesl et al. Int J Mass Spec Ion Process 1994, Zimmermann et al RCM 1996

ESI APCI APPI **APLI**

APLI = direct photon/molecule reaction

Pros:

- Collisional cooling reduces fragmentation (compared to EI)
- Higher linear dynamic range and less matrix effects (compared to APPI)
- Compatible to common LC-solvents
- Extreme high sensitivity

works well for compounds with aromatic core (low IP)
most suitable for polycyclic aromatic hydrocarbons and derivatives

Problems:

- Need evaporation → not applicable for thermo labile components
- Ionization efficiency depends strongly on compound structure and laser fluence
- Laser safety

Constapel et al. RCM 2005, Schiewek etal Anal Chem 2007, Brinkhaus et al. ABC 2017

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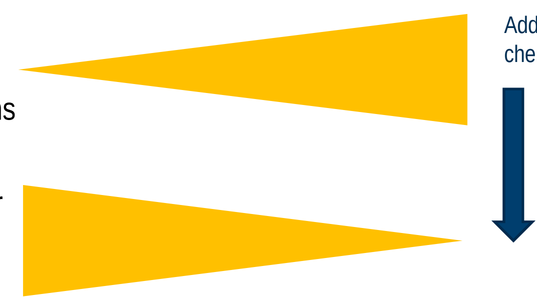
Summary

ESI* APCI APPI APLI

Direct formation of radical cations by electrons or photons

Protonation and other gas or liquid phase chemistry



Additional chemistry by dopant assisted Ionization

Reactions can/will take place and depend on:

- reactant concentration
- reaction enthalpies and rates
- fluid dynamics (flows and source design)

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Ionization requests for complex mixtures

- Universal
  …mass spectrometric signals reflects composition of neutrals

→ **NO**

Ionization efficiency varies extremely by:

- The applied technique
- Physical and chemical properties of the targeted compounds
- Background and matrix components

Ionization requests for complex mixtures

- Preserve molecular ion information
  …No fragmentation or adduct formation
- Low "matrix" effects and linear response

→ YES

Think about:

- Diluting the sample
- Using pure solvents and gases
- Optimizing solvent composition and instrumental settings
- Clean up and separation steps before mass spectrometric analysis
- Analyzing the same sample with different dilution ratios
- Spiking the sample or adding isotope labeled standards
- Checking the ratio of radical cation / protonated species
- ….

Rem: Additional fragmentation and adduct formation may happen
during ion transport from atmospheric pressure to high vacuum