Measurements of REMPI Spectra of Selected Ionization Labels at Reduced and Atmospheric Pressure

APLI

René Mönnikes; Hendrik Kersten; Ralf Schiewek; Matthias Lorenz; Oliver J. Schmitz; Siegmar Gäb; Klaus J. Brockmann; Thorsten Benter

BERGISCHE UNIVERSITÄT WUPPERTAL
Department of Chemistry
Wuppertal, Germany

Overview

The influence of different pressure stages for the REMPI laser ionization is shown. Study of different molecules with regard to the possibility of application for ionization labeling were performed. The use of ionization labels leads to identical mass spectroscopic response factors.

Introduction

The atmospheric pressure (AP) and medium pressure (MP) laser ionization (LI) techniques are two powerful ionization methods for mass spectrometry. Both are based on resonance-enhanced multiphoton ionization (REMPI) and are selective as well as sensitive towards aromatic compounds. Polar functional groups reduce the sensitivity of APLI strongly and aliphatic compounds are generally not ionized by two-photon-REMPI processes. To extend the ionization range of both, APLI and MPLI, to aliphatic and polar compounds, several aromatic ionization labels were investigated with respect to their spectroscopic properties and mass spectrometric response factors. One aim was to identify labels which are favorably ionizing at fixed frequency laser wavelengths in combination with GC- and LC-separation methods.

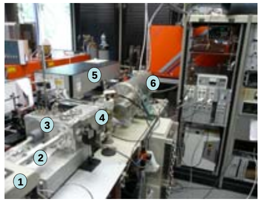
Methods

Laser system:	OPO (optical parametric oscillator) with SHG unit [GWU]. λ = 200 - 2550 nm 0,1- 1 mJ/pulse (200 -300 nm) 4 … 8 ns pulse duration 10 Hz.
Mass analyzers:	APLI: Bruker micrOTOF with Apollo API source. MPLI: Bruker I with home-build ion source
Parameters:	APLI: Direct injection via HPLC pump with 300 µL/min liquid flow. MPLI: Continuous inlet with a modified GC-injection stage; sample flow 1 mL/h; other parameters are given in Results section.

Results

Regarding the search of potential labels, we distinguish between application for GC and LC separation. For GC separations, benzene derivatives with low mass and high vapor pressures are used. The most feasible candidates were found to be 4-fluorotoluene and p-xylene. Both compounds exhibit their absorption maximum around 266 nm, which coincides with the fourth harmonic of Nd:YAG lasers. In contrast, LC labels are not restricted to small aromatic compounds. The utilization of labels with condensed aromatic rings leads to enhanced detection limits because of the higher absorption cross sections and intermediate state lifetimes. Anthracene shows good excitation characteristics at the KrF* excimer laser wavelength of 248 nm.
At atmospheric pressure, the recorded mass selected REMPI spectra show broadening in comparison with classically jet-cooled REMPI experiments, as expected. Only minor variations of the position of the absorption maximum were observed.
To examine the impact of the chemical properties of the target compounds on the spectroscopic features (e.g. shift in absorption maximum or overall absorption cross section) of the label-analyte complex, series of REMPI spectra were recorded. In all cases nearly identical spectroscopic behaviour of the bare label and the complex was observed. Assuming the same derivatization yield for the presently investigated analytes, these results lead to the conclusion that the mass spectrometric response factors for analytes tagged with the same REMPI label are virtually identical. This is of great benefit for quantitative analysis.

MPLI-System



1) GC
2) Transfer line
3) Ion source
4) Ion optics
5) Opo (optical parametric oscillator)
6) Flight tube

Figure 1: MS with a MPLI ion source (medium pressure laser ionization)

Ion sources

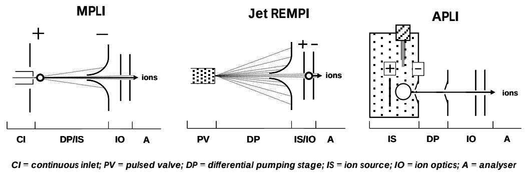


Figure 2: Schemes of the different ion sources for the laser ionization

APLI-System

1) Laser optic
2) APLI ion source
3) Bruker MicrOTOF
4) APCI sprayer

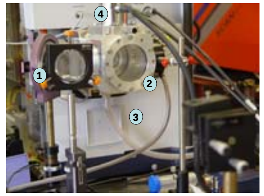


Figure 3: MS with an APLI ion source (atmospheric pressure laser ionization)

Toluene under various pressure conditions

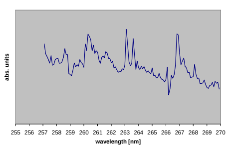


Figure 4:
Toluene at atmospheric pressure

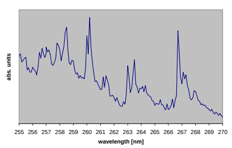


Figure 5:
Toluene at medium pressure
(~ 10 mbar)

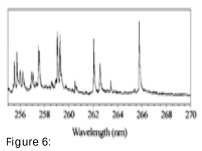


Figure 6:
Jet REMP TOFMS spectra of toluene
modified by *Anal. Chem.* 2004, 76, 2517-2524

Labeling based on Anthracene for HPLC applications

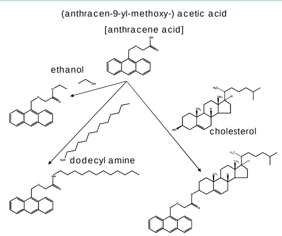


Figure 7: Label reactions of anthracene acid
for derivatization strategy please see poster nr.XX

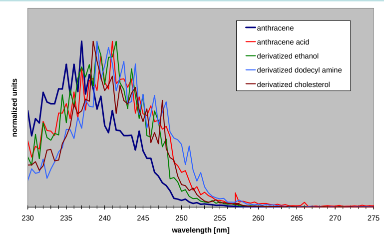


Figure 8: AP-REMPI spectra of anthracene, anthracene acid and anthracene-labeled analytes

Spectroscopic investigations of small labels

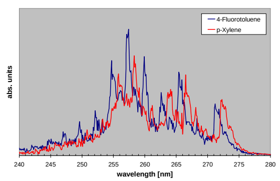


Figure 9:
REMPI spectra of 4-Fluorotoluene and p-xylene.

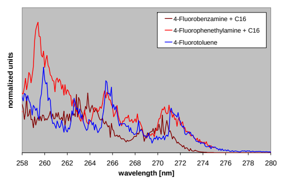
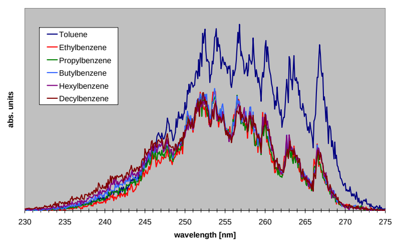


Figure 10:
REMPI spectra of 4-fluorotoluene in comparison with 4-fluorobenzamine-labeled palmitic acid and 4-fluorophenethylamine-labeled palmitic acid.

REMPI-Spectra of Alkylbenzene under MP conditions



Method:

continuous varporization with a modified GC-injection stage

concentration of each compound: 10 µmol/L

Figure 11: Alkylbenzenes in a wavelength range between 230 nm and 275 nm

Conclusions

The use of ionization labeling increases the method of laser ionization in view of aliphatic and polar compounds. Otherwise, these analytes would not be accessible for the laser ionization. Additionally, it offers the advantage of response factor free measurements. Though, the derivatization yield for different analytes has to be the same.

The search for label molecules has brought a few potential candidates so far. It seems to be obvious that the first functional group in a substituent directly at the ring system has the largest influence on the spectroscopical characteristics.

Kann mann das an Beispielen erläutern??? Anmerkung von Klaus.

Outlook

The influence of different substituents will be investigated in more detail. Furthermore, the role of the molecules temperature and the impact on the absorption during the ionization process has to be understand and analysed.

Literature

1) Constapel, M.; Schellenträger, M.; Schmitz, O. J.; Gäb, S.; Brockmann, K. J.; Giese, R.; Benter, Th. Atmospheric-pressure laser ionization: a novel ionization method for liquid chromatography/mass spectrometry. *Rapid Communications in Mass Spectrometry* **2005**, *19*, 326-336.
2) Droste, S.; Schellenträger, M.; Constapel, M.; Gäb, S.; Lorenz, M.; Brockmann, K. J.; Benter, T.; Lubda, D.; Schmitz, O. J. A silica-based monolithic column in capillary HPLC and CEC coupled with ESI-MS or electrospray-atmospheric pressure laser ionization-MS. *Electrophoresis* **2005**, *26*, 4098-4103.
3) Schiewek, R.; Schellenträger, M.; Mönnikes, R.; Lorenz, M.; Giese, R.; Brockmann, K. J.; Gäb, S.; Benter, Th.; Schmitz, O. J. Ultra-sensitive Determination of Polycyclic Aromatic Compounds with Atmospheric-Pressure Laser Ionization as an Interface for GC-MS. *Analytical Chemistry* **2007**, 79, 4135-4140.
4) Lorenz, M.; Schiewek, R.; Brockmann K. J.; Schmitz, O. J.; Gaeb, S.; Benter, Th. The Distribution of Ion acceptance in Atmospheric Pressure Ion sources: Spatially Resolved APLI Measurements. *JASMS* **2008**, *1*, 400-410.
5) Benter, Th.; Schmitz, O. J. Atmospheric Pressure Laser Ionization. In *Advances in LC-MS instrumentation*, Journal of Chromatography Library 72, Cappiello, A. Ed.; 2007; p. 89.
6) Benter, Th. Atmospheric Pressure Laser Ionization. In *The Encyclopedia of Mass Spectrometry*, Gross, M. L., Caprioli, R. N., Eds., 1st ed.; Elsevier: Oxford, U.K., 2007.
7) Schmitz, O. J.; Benter, Th. Atmospheric Pressure Laser Ionization. In *Lasers in Chemistry*, Lackner, M., ED., VCH-Whiley, *in press*.
8) Schmitt-Kopplin, P.; Englmann, M.; Rossello-Mora, R.; Schiewek, R.; Lorenz, M.; Brockmann, K.; Benter, Th.; Schmitz, O. J. Combining Chip.ESI with APLI (cESILI) as a multimode source for the analysis of complex mixtures with ultrahigh resolution mass spectrometry. *Submitted to Analytical and Bioanalytical Chemistry.*
9) Schiewek, R.; Lorenz, M.; Giese, R.; Brockmann, K.; Benter, Th.; Gäb, S.; Schmitz O. J. Development of a multi-purpose ion source for LC- and GC-API MS. *Submitted to Analytical and Bioanalytical Chemistry*.

Acknowledgement

Financial support is gratefully acknowledged:

Cognis GmbH, Ford Motor Company, State of NRW, Studienstiftung des deutschen Volkes.