Anal. Chem. **2010,** *82,* 2178–2181

Letters to *Analytical Chemistry*

Small Molecule Ambient Mass Spectrometry Imaging by Infrared Laser Ablation Metastable-Induced Chemical Ionization

Asiri S. Galhena,[†] Glenn A. Harris,[†] Leonard Nyadong,[†] Kermit K. Murray,[‡] and Facundo M. Fernández*,[†]

School of Chemistry and Biochemistry, Georgia Institute of Technology, Atlanta, Georgia 30332, and Department of Chemistry, Louisiana State University, Baton Rouge, Louisiana 70803

Presented here is a novel ambient ion source termed infrared laser ablation metastable-induced chemical ionization (IR-LAMICI). IR-LAMICI integrates IR laser ablation and direct analysis in real time (DART)-type metastable-induced chemical ionization for open air mass spectrometry (MS) ionization. The ion generation in the IR-LAMICI source is a two step process. First, IR laser pulses impinge the sample surface ablating surface material. Second, a portion of ablated material reacts with the metastable reactive plume facilitating gas-phase chemical ionization of analyte molecules generating protonated or deprotonated species in positive and negative ion modes, respectively. The successful coupling of IR-laser ablation with metastable-induced chemical ionization resulted in an ambient plasma-based spatially resolved small molecule imaging platform for mass spectrometry (MS). The analytical capabilities of IR-LAMICI are explored by imaging pharmaceutical tablets, screening counterfeit drugs, and probing algal tissue surfaces for natural products. The resolution of a chemical image is determined by the crater size produced with each laser pulse but not by the size of the metastable gas jet. The detection limits for an active pharmaceutical ingredient (acetaminophen) using the IR-LAMICI source is calculated to be low picograms. Furthermore, three-dimensional computational fluid dynamic simulations showed improvements in the IR-LAMICI ion source are possible.

The rapid development of ambient ionization techniques, the two most popular being desorption electrospray ionization (DESI)[1] and direct analysis in real time (DART),[2] has provided powerful analytical tools to substantially expand the scope of contemporary analytical mass spectrometry (MS). With more than two dozen different types of ambient ionization methods reported within the past few years,[3−5] the gap between the ambient environment and the vacuum system where analysis takes place has been effectively bridged. A variety of samples can now be analyzed in the open air in their native state without sample pretreatment. A particularly powerful mode of ambient ionization that has drawn significant attention is ambient mass spectrometry imaging (MSI).[6] The most compelling benefit of this mode is the capability of constructing chemical images of intact samples, sometimes in vivo, with remarkably high throughput and flexibility.[7] All ambient MSI experiments reported so far have been based on techniques involving a liquid spray for ionization and/or desorption purposes,[6,7] with plasma-based techniques remaining relatively unexplored.

Plasma-based ambient ion generation techniques such as DART, flowing atmospheric pressure afterglow (FAPA),[8] and desorption electrospray/metastable-induced ionization (DEMI)[9] make use of a gaseous flux of electronic and/or vibronicly excited metastable helium or nitrogen formed in a distal electrical discharge. These metastable species are directed through the ionization source where they can be heated to enhance desorption and/or isolated by a grid electrode to limit the exposure of the sample to ions and electrons. Under standard conditions, these metastables react with atmospheric water, oxygen, or other air components to produce reactive protonated water clusters. With the exception of microhollow cathode discharges, most plasma-based ambient ionization sources produce a reactive stream of at least several millimeters in diameter, preventing their use for

* Corresponding author. Phone: 404 385 4432. Fax: 404 385 6447. E-mail: facundo.fernandez@chemistry.gatech.edu.

[†] Georgia Institute of Technology.

[‡] Louisiana State University.

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10.1021/ac902905v
Published on Web 02/15/2010

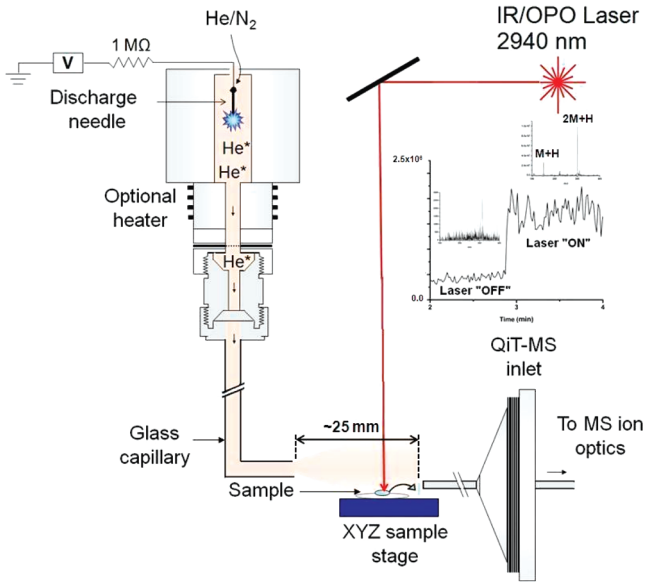

Figure 1. Schematic of the IR-LAMICI ion source coupled to a quadrupole ion trap mass spectrometer. The inset shows the total ion current trace observed for the analysis of a Tylenol tablet with the laser turned off and on.

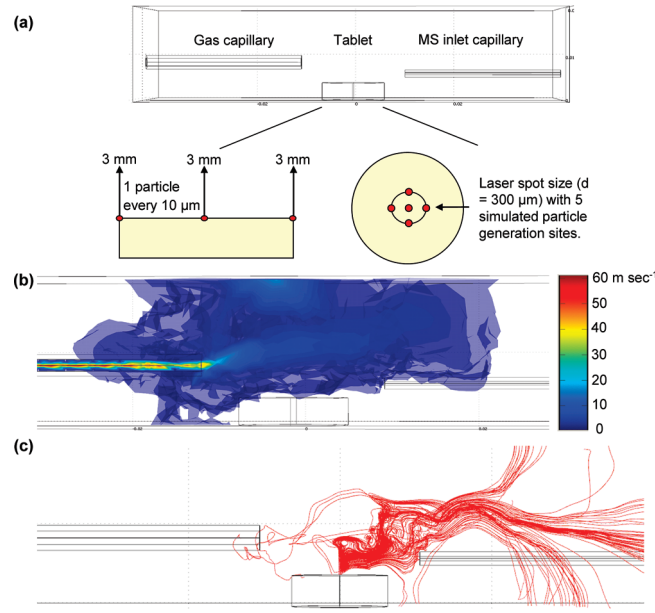

Figure 2. Three-dimensional computational fluid dynamic simulations of the IR-LAMICI setup: (a) 150 particles were simulated originating from a 300 μm-diameter laser spot; (b) gas velocity map and velocities; (c) particle trajectories originating from the positions outlined in part a. The laser spot diameter was measured by taking an optical image (Olympus BX41, optical microscope) of a single shot laser ablation spot.

direct MSI. Recognizing this limitation, Hieftje and co-workers[10] coupled flowing atmospheric-pressure afterglow (FAPA) to Nd:YAG laser ablation in an enclosed configuration. Although carried out in the enclosed environment, such two-step desorption/ionization experiment has the advantage of not being limited by the lateral resolution provided by a diverging liquid jet. Herein, we report the first results obtained using a new ambient air technique, named infrared laser ablation metastable-induced chemical ionization (IR-LAMICI). In comparison to the FAPA imaging experiment,[10] IR-LAMICI is performed in an open-air configuration, thus preventing deposition of ablated material in the ion source enclosure walls. Additionally, this technique should enable the examination of sizable samples and the use of large-area X−*Y* sample stages for imaging purposes. The capabilities of this approach are explored by imaging pharmaceutical tablets, screening counterfeit drugs, and probing the surface of algal tissues for natural product down-selection purposes.

The IR-LAMICI source (Figure 1) was interfaced to a LCQ Deca *XP*+ ion trap mass spectrometer (Thermo Finnigan, San Jose, CA). A custom-made metastable generation chamber[11] equipped with a 90° bent glass nozzle (o.d. 0.3 cm) was mounted in front of the mass spectrometer sampling inlet. Laser ablation was achieved with a Nd:YAG laser-driven optical parametric oscillator (OPO, Vibrant IR, Opotek Inc., Carlsbad, CA) operated at 2940 nm with a pulse length of 4 ns at 20 Hz frequency and 2 mJ per pulse. In the present configuration, the laser pulses impinged orthogonally from above the sample surface with quasi-circular focal spots of ~300 μm diameter. A fraction of the ablated sample material was directed toward the mass spectrometer capillary inlet by the flow of the metastable plume in which gas-phase chemical ionization of analyte molecules occurred, generating protonated or deprotonated species in positive and negative ion modes, respectively. Without laser illumination, no salient ionic species were observed in the mass spectrum as the metastable flux was not in direct contact with the sampled surface (insert, Figure 1). However, intense analyte peaks were observed when laser pulsing was started, suggesting that ions are produced by direct interaction of neutral aerosol particles with gas-phase reactive species. The base peak ion intensity was observed to be largely dependent on the energy of the laser beam and the position of the inlet capillary. Optimum signal intensity was observed for the mass spectrometer inlet capillary positioned ~1 mm above the sampling surface and ~25 mm away from the metastable gas nozzle (Figure 1).

To better understand the atmospheric transport of the ablated particles, a time-resolved (t = 200 ms) three-dimensional computational fluid dynamic simulation with particle tracing was created with COMSOL Multiphysics (COMSOL, Burlington, MA). The simulated setup consisted of the metastable gas glass nozzle, a typical pharmaceutical tablet, and the inlet capillary. Because of convergence limitations, the models were constructed without electric fields and the reduced pressure transport influence of the inlet capillary. Particle trajectories originating on the tablet surface and extending upward every 10 μm from five points on a laser spot size of 300 μm in diameter (total of 150 particles) were simulated (Figure 2a). The simulated gas velocity map depicts the high-velocity gas jet deflecting slightly upward above the tablet surface and the inlet capillary (Figure 2b). The upward gas flow affects particle flow sending a large part of the simulated particles above the inlet capillary (Figure 2c). Gas flow recirculation directing some particles backward toward the gas capillary was also observed, a phenomenon previously predicted for DART MS.[11] These simulations indicate that further

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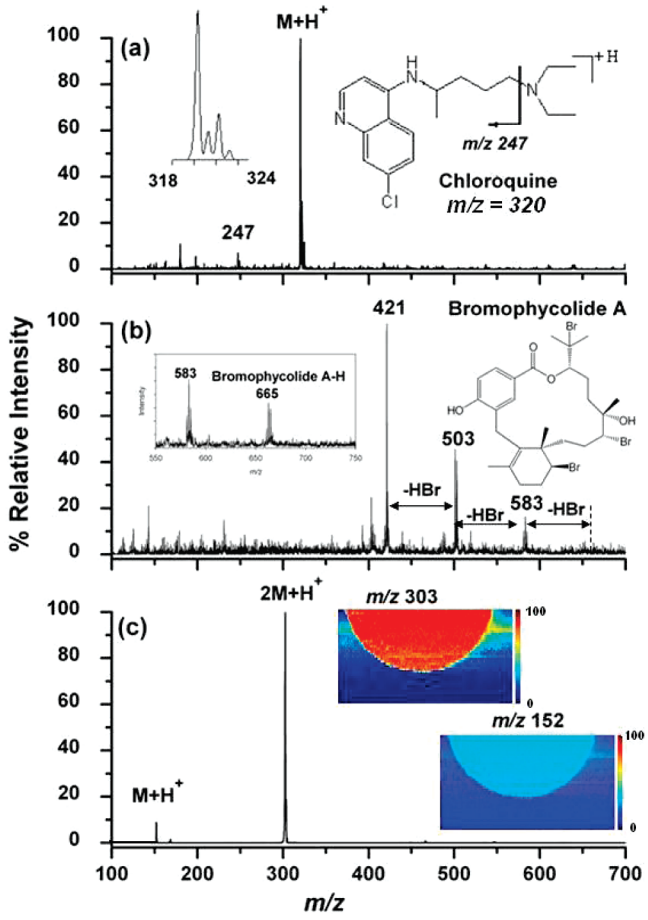

Figure 3. IR-LAMICI MS analyses of (a) a counterfeit artesunate antimalarial drug tablet collected in Cameroon; (b) a red macroalga, *Callophycus serratus*, showing the presence of bromophycolides, and (c) a Tylenol tablet (325 mg of acetaminophen). The inset in part b shows a typical negative ion mode DESI MS spectrum of a similar tissue sample, with the peak at *m/z* 665 corresponding to [bromophycolide A/B − H]$^-$ and the peak at *m/z* 583 to [bromophycolide A/B − HBr]$^-$ and/or [bromophycolide E − H]$^-$). The insets in part c show false-color scale IR-LAMICI MS images of the distribution of acetaminophen monomer and dimer ions on the tablet.

improvement of ion transmission in IR-LAMICI could result in large gains in sensitivity.

The potential of IR-LAMICI for direct MS analysis and imaging was investigated by using a variety of samples previously tested in our laboratory by DART and/or DESI. Figure 3a shows the IR-LAMICI spectrum obtained for a counterfeit artesunate antimalarial tablet collected in the Republic of Cameroon. The signal at m/z 320 was identified as the protonated chloroquine molecule, a wrong active ingredient previously detected in this sample by both DART and DESI. This assignment was verified by the observed isotopic abundance pattern (shown as inset) and by tandem mass spectrometry (MS/MS) experiments (data not shown). The signal at m/z 247 resulted from the loss of $C_4H_{11}N$. Peaks corresponding to the correct active ingredient, artesunic acid, were not detectable.

In a separate set of experiments, tissue samples of the red macroalga *Callophycus serratus* previously investigated by DESI[12] were probed by negative ion mode IR-LAMICI (Figure 3b). The obtained mass spectrum shows the presence of several ionic fragments originating from HBr losses from the deprotonated bromophycolide A molecule, a secondary metabolite involved in surface-mediated chemical defense mechanisms. Comparison with a typical DESI MS spectrum of similar tissue (insert) where the intact bromophycolide [M − H]$^-$ was readily observed suggested that during IR-LAMICI the amounts of internal energy deposited on analyte molecules are larger than in DESI. However, a more extensive optimization of the numerous variables involved in IR-LAMICI (e.g., laser pulse length, laser power, metastable flux) would be necessary for a more thorough comparison to other, more established, ambient MSI techniques.

The full potential of IR-LAMICI was best showcased in MSI experiments. The successful coupling of IR-laser ablation with metastable-induced chemical ionization resulted in a ambient air plasma-based small molecule imaging platform for MS. The IR-LAMICI spectrum of a Tylenol (325 mg) tablet showed peaks at m/z 152 and 303, corresponding to the protonated acetaminophen molecule and dimer, respectively (Figure 3c). The protonated dimer was found to be more intense than the protonated monomer, likely due to two factors. First, laser ablation generates significantly large amounts of neutral sample material off the sample surface, thus favoring the formation of dimeric species at higher acetaminophen concentrations. Second, the use of an ambient temperature metastable flux, rather than a heated one as in DART, coupled to collisional cooling promotes more extensive clustering. Similar effects have been observed when comparing atmospheric pressure and vacuum matrix assisted laser/desorption ionization (MALDI).[13] Assuming uniform distribution of acetaminophen in the tablet and a 10 μm laser depletion layer, the limit of detection was estimated to be 15−30 pg. The insert in Figure 3c shows chemical images constructed from the signals at m/z 152 and 303, corresponding to the protonated acetaminophen monomer and dimer, respectively. Images were constructed from individual surface raster scans with an in-house written MATLAB program (version R2008a, Math-Works, Inc., Natick, MA). The total image construction time, which includes image data collection and processing, was approximately 40 min for a 14 by 7 mm area. The images show a relatively uniform distribution of acetaminophen on the tablet surface, indicative of a good tablet formulation practice. The left-to-right streaking effect seen beyond the curved edge boundaries of the tablet images is a result of some of the ablated unionized material depositing on the inner walls of the mass spectrometer inlet capillary. This material is ionized while scanning the subsequent surface pixel. Despite this secondary effect, lateral resolution in these images was sufficiently high to accurately describe the sharply curved tablet edges. The main advantage of IR-LAMICI is that the resolution of a chemical image is determined by the crater size produced with each laser pulse but not by the size of the metastable gas jet. This is important in acquiring spatially resolved chemical information for micrometer-sized surface features.

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We expect that IR-LAMICI will make small molecule ambient MSI more accessible to the end user and enable new applications in the life sciences. As shown by finite element simulations, much improvement is still possible in terms of ion transmission, which should result in greater sensitivity and better detection limits.

ACKNOWLEDGMENT

This work was supported by the Bio-Imaging Mass Spectrometry (BIMS) Center at the Georgia Institute of Technology and by ARRA NSF MRI Instrument Development Grant No. 0923179 to F.M.F. We thank Dr. Julia Kubanek and her group for providing algae specimens and Dr. May Wang's group for the MatLab software interface.

SUPPORTING INFORMATION AVAILABLE

Isomeric perspective of the IR-DART computational fluid dynamic simulations. This material is available free of charge via the Internet at http://pubs.acs.org.

Received for review December 19, 2009. Accepted February 11, 2010.

AC902905V