

# An In Situ Silver Cationization Method for Hydrocarbon Mass Spectrometry

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We have developed a novel cationization method for the analysis of long-chain hydrocarbons via UV laser desorption mass spectrometry. In this technique we electrospray a thin coating of  $\text{AgNO}_3$  over a sample and perform UV laser desorption to produce  $\text{Ag}^+$  cationization of sample molecules. Use of this technique in our microscope/TOF-MS allows us to determine the spatial distribution of the species we detect in the sample. We demonstrate 8- $\mu$  spatial resolution, and submicron resolution is possible in principle. In mixed samples containing aromatic and aliphatic compounds, the aromatic compounds ionize directly and do not form adducts, and thus give single peaks as opposed to doublets from silver cations. This enables distinction between aromatic and aliphatic compounds that are in the same sample. (J Am Soc Mass Spectrom 2005, 16, 437–440) © 2005 American Society for Mass Spectrometry

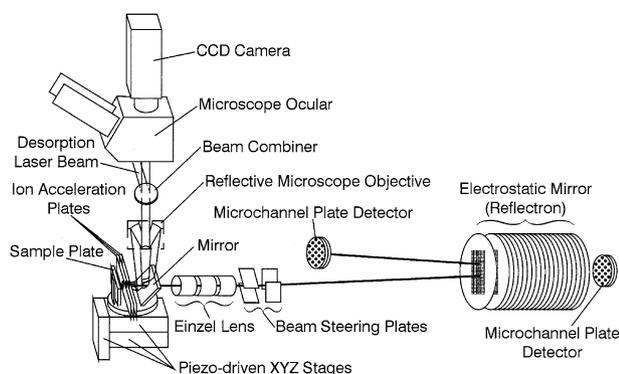
Saturated straight-chain hydrocarbons pose a considerable challenge regarding their direct analysis by means of mass spectrometry. While electron impact spectra do show characteristic fragmentation patterns for such compounds, it is difficult, if not impossible, to determine parent molecular weights, especially for components within a mixture. The strong tendency of these compounds to undergo fragmentation upon ionization causes the parent ion to appear with only minimal intensity [1, 2]. One can increase the relative intensity of the parent ion by lowering the electron energy, but this reduces the ionization efficiency and also necessitates cooling of the source [1]. In addition, branching of the carbon chain, or the presence of functional groups such as carbonyls, carboxylic acid groups, or hydroxyls further destabilizes the molecular ion to the point where it appears with even lower intensity than that of the corresponding alkane or not at all [1, 2]. The relatively low vapor pressure of these compounds and their tendency to crack when heated makes them poor candidates for chemical ionization, and their low solubility or miscibility in commonly used matrices precludes the use of fast atom bombardment. Multiphoton ionization methods are unsuitable because of the relatively large energy gaps between the ground and first excited electronic states in these compounds. While single-photon ionization of such compounds is possible, provided one can generate intense enough beams in the vacuum ultraviolet, the molecules fragment unless they are introduced via a supersonic jet

[3, 4]. Here we present an alternative method, based on silver cationization, for ionizing such compounds, implemented such that it allows for analysis of unperturbed samples with micron spatial resolution.

Grade and Cooks found that they could cationize several aromatic organic molecules through the formation of metal ion adducts by mixing metal salts into the matrix they used to perform SIMS [5]. Zackett et al. used laser desorption (LD) to generate silver cation adducts of sucrose by placing the sucrose on a silver foil support in the presence of ammonium chloride [6]. Cromwell et al. performed laser desorption of perfluorinated polyethers (PFPEs), and used a second laser to ablate a plume of metal ions from the sample substrate to form metal cation adducts [7]. Kahr and Wilkins used a layer of silver nitrate sprayed on the sample probe, over which the sample solution was sprayed, to obtain mass spectra for a set of hydrocarbon polymers [8]. Lai et al. performed TOF-MS analysis of some large biomolecules cationized by laser ablation of thin silver film, or by laser ablation of silver particles into which the molecules were incorporated [9]. Chen and Li performed LD with two long chain alkanes ( $\text{C}_{28}$  and  $\text{C}_{36}$ ), in mixtures with a variety of metal powders, to obtain the metal-cationized species [10]. Pruns et al. used silver trifluoroacetate addition to paraffin samples for MALDI analysis [11]. Dutta and Harayama analyzed oil samples by laser mass spectrometry after depositing them on a sample prepared with silver nitrate. In the approach described here, we take an original sample without any further preparation, and we use electrospray to deposit a layer of  $\text{AgNO}_3$  over it [12]. We then perform laser desorption, with 248-nm laser light, to produce silver cation adducts of the saturated hydrocarbons in the sample. We have found that aromatic

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**Figure 1.** Schematic of the microscope-laser desorption/ionization-reflectron time-of-flight mass spectrometer.

molecules mixed in with the straight-chain hydrocarbons preferentially undergo two-photon ionization and do not readily form silver ion adducts. This technique thus offers three advantages over existing methods. (1) It preserves the physical arrangement of the sample on the substrate, so that we may determine the spatial distribution in the sample. (2) It allows us to prepare the sample *in situ*, that is, without redissolving it or performing chemistry on it. (3) We can distinguish between aliphatic and aromatic compounds that are mixed together in a single sample.

## Experimental

**Figure 1** shows a schematic of the microscope/laser desorption/reflectron time-of-flight mass spectrometer. This instrument has been described previously, but the ion source has since been modified [13].

To perform desorption (and ionization) we use a KrF ultraviolet waveguide laser (Potomac Photonics, model GX-500, Lanham, MD). This laser emits light at 248 nm, in 50-ns pulses with a maximum energy of 10  $\mu$ J per pulse. The ion source of the mass spectrometer is designed so that one may view the sample under a microscope while it is in place for acquisition of a mass spectrum, and so that the desorption laser beam is tightly focused to desorb and ionize over a small region of the sample. We accomplish this by using a reflective objective as our focusing element. This type of objective is achromatic, so it allows simultaneous focusing of UV laser and visible light, and combines a large working distance with a large numerical aperture. We introduce the laser beam along the optical axis of the microscope by means of a dielectric mirror, coated for 248-nm light. A second mirror, placed between the ion acceleration plates and the einzel lens, folds the optical axis by 90° to intersect with the sample. Based on measurement of a damage track made in a test sample, we estimate the diameter of the laser spot on the sample to be 8  $\mu$ m. We estimate that the maximum energy density per pulse delivered to the sample is on the order of 3 J/cm<sup>2</sup>.

The sample is mounted on a plate that sits on an assembly of three translation stages, mounted for travel

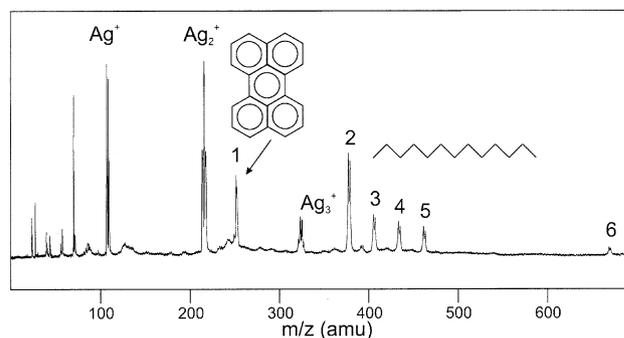
along mutually perpendicular axes, and driven by piezoelectric inchworm motors. The stages can move with a precision of 0.5  $\mu$ m. Because the various molecules we used in this study could be heated without decomposition, we used an evaporative coating technique to prepare the analyte samples. We measured the thickness of the sample layer by means of a quartz crystal microbalance.

To accomplish the AgNO<sub>3</sub> treatment we used an electrospray technique. We dissolved the AgNO<sub>3</sub> in methanol, at a concentration of 0.2  $\mu$ g/ $\mu$ l, with the aid of an ultrasonic bath. We then used a syringe pump, with a high voltage applied to the syringe needle (Hamilton, point style 3, Reno, NV), to spray the AgNO<sub>3</sub> solution onto the sample plate. The typical flow rate was about 200  $\mu$ l/h, and the typical voltage applied to the needle was about 3 kV. We obtained the best response in the cationization experiments for AgNO<sub>3</sub> coatings ranging in thickness from about 0.04 to 0.1  $\mu$ m.

## Results and Discussion

**Figure 2** shows a mass spectrum of a mixture of (1) perylene (C<sub>20</sub>H<sub>12</sub>), (2) *n*-octadecanol (C<sub>18</sub>H<sub>37</sub>OH), (3) *n*-eicosanol (C<sub>20</sub>H<sub>41</sub>OH), (4) *n*-docosanol (C<sub>22</sub>H<sub>45</sub>OH), (5) *n*-tetracosanol (C<sub>24</sub>H<sub>49</sub>OH) and (6) tetracontane (C<sub>40</sub>H<sub>82</sub>). The sample was 1500 Å thick. To prepare the sample, we placed a roughly equimolar mixture of these compounds into the boat of the evaporative coating apparatus, and heated the boat to evaporate them simultaneously. We then oversprayed the sample with a layer of AgNO<sub>3</sub> approximately 0.05  $\mu$ m thick. The doublet and triplet patterns arise from the isotopic distribution for silver, which is 51.82% mass 107 and 48.18% mass 109. The doublets at masses above 330 Da correspond to the masses of the intact hydrocarbon molecules with each of the silver isotopes attached. We observe little or no fragmentation of the analyte molecules.

Generally, the (M + Ag)<sup>+</sup> peaks required a slightly higher potential on the second stage of the reflectron than did the Ag<sup>+</sup> and Ag<sub>2</sub><sup>+</sup> peaks for optimum focusing. This indicates that the adducts form in the plume of



**Figure 2.** Mass spectrum of a mixture of perylene (1), *n*-octadecanol (2), *n*-eicosanol (3), *n*-docosanol (4), *n*-tetracosanol (5), and tetracontane (6). The sample is 1500 Å thick with a 0.05- $\mu$ m-thick AgNO<sub>3</sub> overspray.

desorbed material, as opposed to the silver monomer and dimer ions, which are formed at the sample surface.

### Detection Limit and Linearity

We can make a rough estimate regarding the detection limit for eicosanol. We found that a sample layer thickness of about 50 Å yields a single shot signal-to-noise of 6. With a laser spot diameter of 8 μ this corresponds to 0.75 fmol of material.

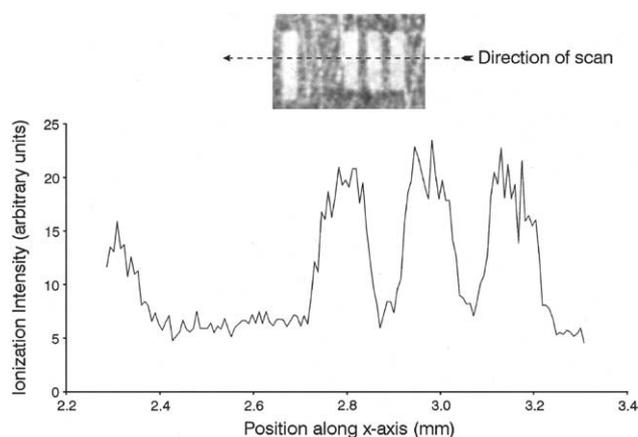
To obtain a rough idea of the range of sample thickness over which the signal for eicosanol is linear, we prepared a sample with bands of *n*-eicosanol of different thicknesses, onto which we then sprayed approximately 0.11 μ thick stripes of AgNO<sub>3</sub>. A linear regression of the resulting MAg<sup>+</sup> signal versus thickness for the 10-, 60-, and 600 Å peaks shows that their intensities lie fairly close to a straight line, leveling off close to the thickness of 600 Å. According to the estimates made above, this corresponds to a quantity of 3 pg, or 9 fmol of eicosanol per laser shot.

### Relative Efficiencies

We compared mass spectra of the straight-chain hydrocarbon hexacosane, C<sub>26</sub>H<sub>52</sub>, the corresponding alcohol, *n*-hexacosanol (C<sub>26</sub>H<sub>51</sub>OH), and the carboxylic acid, *n*-hexacosanoic acid (C<sub>26</sub>H<sub>49</sub>COOH). The sample consisted of an 800 Å thick stripe of each of these materials, covered by a layer of AgNO<sub>3</sub> approximately 0.11 μ thick. We normalized the data to the intensity of the silver ion peaks. While we clearly observe an adduct peak for the alkane, those of the alcohol and the carboxylic acid are considerably more intense, and are of comparable magnitude. As a straight, unsubstituted alkyl chain is nonpolar, it is likely that adduct formation with silver and an alkane involves ion-induced dipole interactions. Both the straight-chain alcohol and the straight-chain carboxylic acid have a polar functional group at one end, which would provide a site for an ion-dipole interaction, which is considerably stronger. This observation is consistent with the reported efficiency for characterizing hydrocarbons and lipids using Ag<sup>+</sup> coordination ion-spray [14, 15]. Assuming the cation attaches at the functional group, one might expect the cationization efficiency of functionalized alkanes to be independent of chain length, whereas the efficiency would increase with length for pure unsaturated alkanes. It would be interesting to investigate this point in more detail in future experiments, with the caveat that the chain length can also affect other aspects of the experiment, such as matrix effects in the desorption step.

### Aromatic versus Aliphatic Analytes

In Figure 2 the ion from perylene, an aromatic compound, appears predominantly at the parent mass, as a single peak, with a very weak cation signal, while the



**Figure 3.** Line scan across sample prepared by vapor depositing tetracosanol through a mask onto the substrate and then overspraying the entire surface with AgNO<sub>3</sub> 0.11 μ thick. The trace was obtained by gating the signal arising from the (M + Ag)<sup>+</sup> peak, and recording it as the desorption laser spot was moved across the sample (by moving the sample). The trace in the figure is an average of 10 scans, with no smoothing. The photomicrograph was taken before the sample was coated with AgNO<sub>3</sub>. After AgNO<sub>3</sub> coating, the pattern was no longer visible.

ions from the aliphatic molecules appear only as doublets, 107 and 109 u above their respective masses. We are thus able to differentiate between aliphatics and aromatics in the same sample.

On the one hand, polyaromatic hydrocarbons have considerable Ag(I) affinities [16]. On the other hand, any substituted benzene or larger aromatic molecule readily undergoes two-photon ionization when irradiated with ultraviolet laser light, especially at wavelengths of 266 nm or shorter. The two-photon ionization process happens very quickly, on the order of 15 femtoseconds or so, presumably before the molecule would have a chance to collide with silver ions in the desorbed plume. The cation thus formed would repel any other cations, and would leave the source without forming an adduct. If the silver cation adduct did form, and the aromatic chromophore were subsequently photoionized, the resulting complex should dissociate into two separate cations. Furthermore, if photon or collision induced dissociation of a cation complex were to occur, the relatively low ionization potential of the polyaromatic compound could also lead to parent cations. Lai, Owega and Kulczycki also observed predominant photoionization rather than silver adduct formation for benzopyrene [9]. In their experiment they used thin film silver substrates and/or suspensions of silver particles as a source of cations while in our experiment, we introduce the silver as ions.

### Spatial Mapping

Figure 3 shows a plot obtained by scanning across a sample of *n*-tetracosanol, which we had deposited in a pattern of 150 μ wide stripes on the sample plate. We prepared this sample by depositing the tetracosanol

through a mask placed over the surface of the sample plate, and then spraying a 0.11  $\mu$  thick AgNO<sub>3</sub> coating over the entire area. In the mass spectrometer, we moved the sample from one side to the other, so as to scan the laser beam across the stripes, recording the signal from the gated quasimolecular ion peak as we did so. We operated the laser at 4 Hz, and scanned the sample at approximately 33  $\mu$ /s, for a total of 140 shots across the scan, and approximately 20 shots across each small stripe. The illustration above the plot is a photograph of the tetracosanol stripes, with an arrow indicating the direction of the line scan. The x-axis in the plot is the position across the sample plate, in mm. The trace is an average of ten scans, with no smoothing, and shows the distribution of the tetracosanol. This demonstrates that we can use the overspray technique on a sample in which the analyte has an irregular distribution, and map the spatial distribution of the analyte molecules. The resolution of this technique is on the order of the diameter of the desorption laser spot. In principle, the spot size can be reduced to the diffraction limit, making possible spatial resolutions of the order of a micron, albeit at the expense of sensitivity.

## Conclusions

We have demonstrated a technique that allows the analysis of saturated hydrocarbons and related compounds via UV laser desorption. This technique offers three advantages over existing techniques: First, it is not necessary to perform chemistry on the sample, or to dissolve it, before analysis. Second, the sample may be prepared in situ, preserving its spatial arrangement, and enabling one to determine to within several microns where on the sample surface the various compounds are that appear in the mass spectrum. Last, aliphatic and aromatic compounds behave differently,

such that in a mixed sample one can identify which peaks in the mass spectrum arise from aliphatic compounds and which peaks from aromatic compounds.

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