Fragment-Free Mass Spectrometric Analysis with Jet Cooling/VUV Photoionization

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We show that it is possible to obtain fragment-free mass spectra of large molecules by a combination of laser desorption, jet cooling, and VUV single-photon photoionization. The ability to obtain parent molecular masses is particularly important for the analysis of mixtures, such as combinations of fully saturated hydrocarbons. By varying the cooling conditions, we can also achieve partial fragmentation in order to obtain further structural information. The use of different wavelengths provides additional selectivity between aromatic and aliphatic compounds.

In most standard implementations of mass spectrometry, one mostly obtains fragmented mass spectra for two reasons: (i) many compounds tend to decompose when heated in the inlet of a mass spectrometer and (ii) electron impact ionization has its highest cross section at 70 eV. At that energy, enough excess energy is transferred to the molecule to generate fragmentation of the ion. Such fragmentation can be very useful in providing structural information. For this reason, there exist extensive libraries of mass spectral data at the standard ionization condition of 70-eV electron impact ionization. However, there are also two disadvantages to this approach. First, the analysis can become very difficult in the case of complex mixtures. Second, it is often the case that different species of the same basic molecular type yield practically identical fragmentation patterns. For example, most alkanes, as well as many related compounds, produce virtually the same mass spectrum independent of their number of carbons. In such a case, positive identification of the molecule requires a technique by which one can measure the parent molecular mass. This is even more important when one wants to analyze a mixture of such compounds.

A number of novel mass spectrometric techniques have emerged that produce parent molecular masses by employing alternative approaches to sample introduction and to ionization. Field ionization has been quite successful but impractical for routine analysis. Fast atom bombardment matrix-assisted laser ionization, and electrospray ionization have gained widespread use for analysis of larger molecules, particularly biomolecules. Each technique has its own advantages and disadvantages. The former include the ability to handle very large molecules, with masses up to several hundred thousand daltons. The latter include various requirements for sample preparation or limitations on possible mass resolution. Another approach is based on the incorporation of a molecular beam in the mass spectrometer inlet. Amirav et al. have shown that electron impact ionization spectra can be greatly simplified by internally cooling the molecules in a jet expansion prior to ionization. We combine this advantage of jet cooling with both laser desorption and photoionization. This approach has been reported before with multiphoton ionization, usually with emphasis on spectroscopy and applied to aromatic molecules. The limitation to aromatic compounds follows from the fact that commercially available lasers require molecular absorption at visible or near-UV wavelengths. Therefore many aliphatic compounds are excluded, reducing the practical usefulness of the method for analytical purposes. We overcome this limitation by using vacuum ultraviolet (VUV) single-photon ionization.

On the face of it, one may expect single-photon VUV photoionization to serve as a general soft ionizer. In principle, one can tune the wavelength such that the absorbed photon energy exceeds the ionization potential by only a few tenths of an electronvolt, which is less than the bond energies of the ion. In practice, however, one needs to also account for the internal energy of the molecule. For larger molecules, with many degrees of freedom, single-photon ionization may not be sufficient. Therefore, we have studied the use of VUV wavelengths for improving the mass spectra of larger molecules. Our results are consistent with those of previous experiments. We show that the use of VUV wavelengths, in combination with jet cooling, can provide fragment-free mass spectra. The appearance of fragments is reduced, allowing the analysis of complex mixtures. This approach may be particularly useful in the analysis of larger molecules, such as proteins and other biologically important compounds.

References

of freedom to be multiplied by \( kT \), this can add at least several tenths of electronvolts, such that the ion in most cases still fragments. By employing jet cooling, we reduce the internal energy and we find that this approach dramatically reduces fragmentation. The result of this combination is indeed a rather general soft ionizer for fragment-free mass spectrometry. We will first describe the technique and then provide a number of examples.

**EXPERIMENTAL SECTION**

A schematic outline of the experimental setup appears in Figure 1. Material is laser desorbed from a sample probe in front of a pulsed nozzle. The desorption laser is usually a Nd:YAG laser operated at its fundamental wavelength of 1064 nm. At this wavelength, one does not expect photochemical interaction with any of the materials, we desorb. We have, however, also in many cases successfully used the second and fourth harmonic as well as a 248-nm KrF laser for desorption. Laser desorption involves heating of the substrate rather than the adsorbate. Therefore, it is typically desirable to match the wavelength of the desorbing light with the absorption characteristics of the substrate. We routinely use graphite as a substrate, although we have also successfully used metal substrates. Typical laser fluences are of the order of 1 mJ/cm\(^2\) or less. The laser is focused to a spot of the order of 0.5-mm diameter within 2 mm in front of the nozzle. This is important because in a supersonic expansion most of the cooling takes place close to the nozzle by collisions with the drive gas along a distance of about 10 nozzle diameters. Beyond that distance, pressure in the beam already decreases enough to rapidly decrease the number of collisions. The nozzle consists of a pulsed valve with a nozzle diameter of 1 mm. We usually operate with Ar as a drive gas at a backing pressure of about 5 atm. For larger compounds, we have found that it is advantageous to use a heavier drive gas such as Kr or Xe in order to obtain optimal cooling. In earlier work, we optimized the geometry for effective entrainment by mapping entrained perylene with laser-induced fluorescence.\(^{(20)}\) In that work, we found that it is possible to entrain a portion of the desorbed material on the axis of the supersonic beam, such that the ionizing laser downstream can interact with a fraction of about 10\(^{-5}\) of the desorbed material.

Downstream the beam is intersected by the ionization lasers inside the source region of a reflectron time-of-flight mass spectrometer (Jordan Co.). Excimer lasers and a dye laser, used for two-photon ionization, intersect the beam at right angles. A VUV beam is aligned collinearly with the gas beam. This arrangement ensures maximum overlap and is made possible by the fact that ions are extracted at right angle to the beam.

The VUV light is generated by sum frequency mixing in Hg vapor.\(^{(21)-(23)}\) This scheme, as indicated in the inset to Figure 1, produces 125.14-nm photons. While this approach is more complex than producing the same wavelength as the ninth harmonic of a Nd:YAG laser, it has the advantage of higher possible fluences, typically of the order of 10\(^{13}\) photons/cm\(^2\) per pulse. The 625.7-nm light from a YAG pumped dye laser is frequency doubled and tuned to a 156.4-nm resonance in Hg. The frequency-doubled light is focused in a heat pipe type Hg cell together with the fundamental, which scatters at the resonant level in the Hg to produce the sum frequency of 125.14 nm. A second advantage of this approach, which we have not exploited yet, is the potential of producing tunable VUV light by varying the wavelength of the red light, while keeping the frequency-doubled UV light fixed at the resonant wavelength. With this refinement,

which requires two dye lasers, one can in principle tune the ionizing light precisely to the ionization threshold.

RESULTS

Cooling and Photoionization. Figure 2 serves to demonstrate the role of cooling in photoionization of hydrocarbons. It shows mass spectra of n-C_{38}H_{78} obtained under different conditions as follows: (a) laser desorbed, jet cooled, photoionized; (b) laser desorbed, not jet cooled, photoionized. Figure 2c shows the 70-eV electron impact library spectrum for comparison. The effect of cooling becomes clear by comparing spectrum a and spectrum b. Without cooling, the result is almost identical to conventional electron impact ionization with extensive fragmentation and no parent molecular peak, while with jet cooling, we can observe the parent ion without fragments. This effect has been discussed extensively before.\textsuperscript{24} Once most of the internal energy is removed by jet cooling, the only energy still available for breaking bonds in the ion derives from the excess ionization energy. Part of the excess energy is carried off by the photoelectron; the remainder is available as internal energy of the ion. In the case of electron impact, as shown by Danon et al., jet cooling also significantly reduces fragmentation, such that even at 70-eV electron energy the parent molecular ion can be observed. Reducing the electron energy from 70 to 18 eV further reduces fragmentation, albeit at the expense of ionization efficiency.\textsuperscript{24} With photoionization, one could in principle tune the photon energy to the threshold for ionization, to reduce the excess energy essentially to zero. In our case, at 9.9 eV, the energy is already within a few tenths of the ionization potential, which is between 9.5 and 9.8 eV for most molecules discussed here.

To discuss the issue of fragmentation further, Figure 3 shows mass spectra of squalane, a branched C_{30}H_{62}. Spectra a and b result from 125-nm photoionization with optimal cooling and with partial cooling, respectively. Spectrum 3c shows the 70-eV library spectrum. The bonds in the branched alkane are weaker than those in the straight isomer. Nevertheless, at conditions of optimal cooling and 9.9-eV ionization energy, we obtain exclusively the parent molecular ion. In fact, we can also observe dimers and trimers that are formed in the jet expansion. The appearance of dimers and trimers is further evidence that the peak observed at the parent mass represents an unfragmented molecular ion. Thus, the observation of clusters can serve as additional information for mass spectral analysis. We achieve partial cooling in our experiments by detuning the delay times such that we ionize molecules that have not been entrained in the highest pressure part of the pulsed jet. We do not know what the temperature is in that case; we can only make the statement that the cooling is incomplete. The resulting photoionization mass spectrum shows a distinct fragmentation pattern. The branched alkane breaks at the branches in a nonrandom pattern. All peaks that are marked with their mass number are the result of breaking at a branching point. Note that fragments that result from breaking toward the middle of the chain have a larger abundance than those that result from breaking at a branch on the side toward the end of the molecule. The broad peaks in the spectrum are the result of metastable decay in the flight path of the reflectron time-of-flight mass spectrometer. At 70-eV electron impact ionization, with cooling the result is similar but with important differences when one analyzes the fragmentation in detail.\textsuperscript{12} At 70-eV electron impact ionization without cooling, the bond breaking near the branches constitutes only a minor part of the mass spectrum. A detailed discussion of the unimo-

molecular decomposition dynamics will be published elsewhere. Here we merely wish to point out the fact that, in addition to parent molecular information, we can also obtain structural information from photoionization with partial cooling. For example, the fragmentation pattern in this case clearly distinguishes between the branched and the unbranched \( n \)-alkane of the same mass. This introduces an isomer specificity that is practically absent in conventional electron impact ionization mass spectrometry without cooling.

**Analytical Applications.** Figure 4 demonstrates the analytical potential of this technique. It shows a mass spectrum obtained from a roughly equimolar mixture of \( \text{C}_{30}\text{H}_{62} \), \( \text{C}_{40}\text{H}_{82} \), \( \text{C}_{50}\text{H}_{102} \), and \( \text{C}_{60}\text{H}_{122} \). The number of carbons is indicated for each peak.

![Figure 4](image)

**Figure 4.** Mass spectrum obtained from a roughly equimolar mixture of \( \text{C}_{30}\text{H}_{62} \), \( \text{C}_{40}\text{H}_{82} \), \( \text{C}_{50}\text{H}_{102} \), and \( \text{C}_{60}\text{H}_{122} \). The number of carbons is indicated for each peak.

Carbon mixture such as this can only be unambiguously identified mass spectrometrically if parent molecular peaks can be measured. As is evident from the NIST library, the conventional mass spectra of these compounds are all dominated by virtually the same fragments. With jet cooling/VUV ionization we can obtain exclusively parent molecular peaks. Although the laser desorption technique is not very quantitative, there appears to be no strong dependence of detection efficiency on molecular weight over the range from 30 to 60 carbons. Schlag et al. have predicted that photoionization efficiency will decrease with molecular size. If true, this would reduce the analytical usefulness of this technique. However, the results in Figure 4 show that, at least for the molecular range we investigated so far, there is no such limitation.

Figure 5 provides another example of the analytical importance of the ability to measure parent molecular weights. The mass spectra in Figure 5(a) and 5(b) represent the analysis of two different types of Pentaerythritol tetraester oils. These compounds are commonly used as high performance lubricants. The basic structure of the tetraester is with \( R_i = -\text{CH}_3\text{OCO(CH}_2)_{i-2}\text{CH}_3 \).

![Figure 5](image)

**Figure 5.** Mass spectra of pentaerythritol tetraester oils. (a) and (b) are two different oils. (c) is the same material as in (b) after frictional degradation. See text for details.

It is formed by the esterification of \( \text{C(CH}_3\text{OH})_4 \) with four acids, \( A_i = \text{HOOC(CH}_2)_{i-2}\text{CH}_3 \).

The numbers indicating the peaks in Figure 5 denote the total number of carbons, \( i + j + k + l \), in all four branches combined. Figure 5(a) shows the spectrum of a tetraester which must have been formed from an equimolar mixture of \( A_7 \) and \( A_8 \). The result is a binary distribution of all possible combinations of branches with \( R_7 \) and \( R_8 \), represented in peaks with 28–32 branch carbon atoms. On the other hand, the oil from Figure 5(b) must have been produced entirely differently. It is composed of a mixture of pure homobranched tetratesters, \( C(R_i)_4 \) each produced with a single acid and mixed only after esterification. In this case, \( i \) equaled 5, 6, 7, and 8, resulting in the peaks with 20, 24, 28, and 32 branch carbon atoms, respectively. Finally Figure 5(c) shows a spectrum of the same material as in Figure 5(b) after degradation as a result of having been used as a bearing oil. The additional peaks in this spectrum result from reactions involving fragmentation at the ester linkages and subsequent cross-esterification, such that mixing of branches can occur. All of these observations can only be recorded with the help of mass spectra without fragmentation. Other examples include the analysis of small polymers, for which parent masses are necessary in order to obtain reliable oligomeric distributions.

The combination of laser desorption, jet cooling, and photoionization offers another analytical advantage by providing the ionization wavelength as an additional parameter to help identify compounds. We can capitalize on the wavelength in two ways: (a) we can use single-versus two-photon ionization to distinguish between aliphatic and aromatic compounds and (b) we can use

![Analytical Chemistry, Vol. 71, No. 9, May 1, 1999](image)

resonance-enhanced two-photon ionization to selectively ionize specific compounds. As for the former, aromatic molecules can generally be two-photon ionized at convenient laser wavelengths, such as 266 or 193 nm. Most aliphatic compounds are transparent at those wavelengths. On the other hand, at 125 nm we can single-photon ionize any molecule with a ionization potential below 9.9 eV, whether it is aromatic or aliphatic.

SUMMARY

The combination of laser desorption, jet cooling, and photoionization provides a way to generate parent molecular ions for mass spectrometric analysis. We show that cooling is essential for preventing decomposition of the ion as a result of internal energy. By using single-photon ionization at 125 nm, we can ionize essentially all molecules with a vertical ionization potential of 9.9 eV or less. This includes most aliphatic hydrocarbons such as n-alkanes with more than eight carbon atoms. In addition to providing parent molecular weight analysis, this approach can offer additional information when the ionization wavelength is varied. In this respect, we note that often optical resolution can be much higher than mass resolution. Another advantage of photoionization is the fact that it is based on a relatively simple and well-understood process, while most other ionization methods for large molecules rely on involved processes too complicated for quantitative modeling. By controlling the degree of cooling, we can observe limited fragmentation, which can provide additional isomeric and structural information. The combination of jet cooling and photoionization improves molecular identification, especially in complex mixtures.

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