Pulsed laser desorption method for volatilizing thermally labile molecules for supersonic jet spectroscopy

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A technique using pulsed laser desorption is described as a means of volatilizing nonvolatile and thermally labile molecules into the gas phase for entrainment in supersonic jet expansions. By carefully controlling the laser desorption power and by using a glycerol matrix to provide a thin even film of sample, repetitive desorption can be achieved over an extended period for performing jet spectroscopy. Using resonant two-photon ionization spectroscopy while monitoring only the molecular ion in a time-of-flight mass spectrometer, cold sharp spectra are observed for molecules which are difficult to volatilize by conventional heating methods.

INTRODUCTION

The supersonic jet technique has been shown to be a powerful method for providing internally ultracold molecules for gas phase spectroscopy.[1] In the past, this technique has generally been limited to molecules with reasonable volatility which can be easily heated into the gas phase for expansion with a light carrier gas. There have been several recent attempts to develop methods to extend the advantages of jet spectroscopy to nonvolatile and thermally labile molecules. This is especially significant since this will extend the technique for the study of a broad class of molecules of biological and pharmaceutical importance.

Direct heating methods have been used successfully for some time for volatilization of relatively stable large polynuclear aromatic hydrocarbons (PNAHs) with high melting points (> 200 °C) using hot pulsed or continuous ovens[2,3] or high-temperature gas chromatography[4–6] for injection into supersonic jets. However, a method recently used by Levy and coworkers to study more labile species has involved initial thermospray deposition of a thin layer of material followed by heating into a jet of He carrier gas.[7] This method has been used to study the amino acid tryptophan and several related compounds including tryptamine, 3-indole acetic acid and 3-indole propionic acid. Using either laser-induced fluorescence (LIF) or resonant two-photon ionization spectroscopy (R2PI) ultracold, sharp spectral features were obtained for the origin regions of each of these compounds.

In other work, supercritical fluids have been used as a means of solubilizing nonvolatiles for subsequent jet injection. These fluids have the property that certain molecules can dissolve in them like a liquid, but then can expand like a gas upon introduction into vacuum, i.e., a supersonic jet. This method has been used by Imasaka and coworkers to inject several relatively nonvolatile polynuclear aromatics at reduced temperature (< 200 °C) into a supersonic expansion of Ar with spectroscopic analysis by LIF.[8] Lubman and coworkers have recently utilized direct supercritical expansions of CO_2 and N_2O at temperatures as low as 40–70 °C to inject biological and pharmaceutically related compounds such as benzimidazole and iminodibenzyl[9] as well as PNAHs such as pyrene and tetracene[10] into a jet, and resulting cold spectral features have been obtained using R2PI

spectroscopy in a mass spectrometer for several of these species. More recently, several groups have used liquid injection from hot methanol or water to dissolve various small biologicals for direct jet injection[9] or for entrainment into He or Ar carrier gases.[11] Using this method, amino acids and various small pharmaceuticals have been detected using R2PI in a mass spectrometer.

An alternate method for entraining nonvolatiles into jet expansions is laser desorption, where a high-powered pulsed infrared laser is used to induce a rapid heating that desorbs molecules from a surface before they have time to decompose.[12] In the desorption process, both ions and neutrals are formed in a ratio that depends to a first approximation on the surface temperature induced by the laser. The actual number of ions produced in the desorption process is generally quite small (< 1%) compared to the large number of neutral species that result at power densities below 10^8 W/cm^2 (Ref. 13). In the work described herein, a relatively low-powered laser ($\sim 10^6$ W/cm^2) has been used to generate mainly neutral species from a glycerol matrix on a surface. Desorption from a glycerol matrix has previously been used in fast atom bombardment mass spectrometry (FABMS) in order to provide an even, renewable sample.[14,15] Likewise, the use of a glycerol matrix provides excellent pulse-to-pulse stability in the laser desorption process. Following desorption the molecules are entrained into a pulsed supersonic jet and swept into a mass spectrometer for study by R2PI spectroscopy. We demonstrate the ability to obtain cold, sharp spectral features for small biological molecules using a very simple desorption setup. This method avoids decomposition which can be induced by direct heating methods and obviates the need for injection of large amounts of liquid or gas and the use of high pressure inherent in the supercritical fluid or liquid injection methods. In addition, this method has been shown to be more versatile in terms of the size of the molecules which can be volatilized, such as large peptides, as compared to other available methods.[16–18]

I. EXPERIMENT

A. Apparatus

The supersonic beam time-of-flight mass spectrometer (TOFMS) setup is similar to that described in previous ex-

periments.[16,19] It consists of a TOFMS mounted vertically in a stainless-steel six-port cross pumped by a 6-in. diffusion pump (see Fig. 1). The pulsed supersonic molecular beam expands into the acceleration region of the TOF and a laser beam perpendicular to both the supersonic jet and flight tube ionizes the sample. The acceleration region and TOF flight tube are enclosed in a liquid N_2-cooled cryoshield and are differentially pumped by a 4-in. diffusion pump. In this configuration, a background pressure of $\sim 10^{-7}$ Torr is obtained in this region, and the background due to pump oil and other contaminants becomes negligible. The supersonic beam source is a stainless-steel pulsed valve (0.5-mm aperture diameter), based on the magnetic repulsion principle (R. M. Jordan Co.) which can provide gas pulses of $\sim 55\,\mu s$ FWHM at "choked flow" at 10-Hz pulse rate, thus substantially reducing the duty cycle needed for pumping. In these experiments in which CO_2 is used as the carrier gas, the average background pressure remains at $\sim 1 \times 10^{-6}$ Torr since CO_2 is efficiently cryopumped by the LN_2 trap.

B. Sample preparation and introduction

Sample introduction was performed by desorbing the compound of interest from the surface of a $\frac{1}{8}$-in.-diam rod made of machinable Macor™ ceramic (Corning Glass Works, Corning, NY). Other surfaces were also tested including various metals, Teflon, glass, quartz, etc., however, this particular material was found to provide minimum decomposition even for the most labile compounds studied.[20] In previous studies, the sample was deposited on the face of the rod simply by dissolving in benzene or methanol and coating the surface with the resulting mixture. However, the key to these spectroscopic experiments is obtaining shot-to-shot stability which can be optimized by dissolving the material in a high-viscosity fluid such as glycerol or silicone diffusion pump fluid. Generally $\sim 100\,\mu g$ of sample is used in these experiments, and the glycerol matrix causes the sample to form a very even, thin layer from which pulse-to-pulse stability appears quite good, i.e., $\sim < \pm 5\%$. The main sourc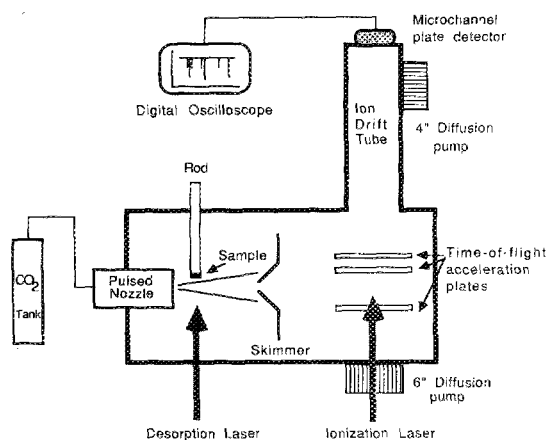e of signal instability appears to be due to the pulse-to-pulse variation of the ionization source. In FAB/MS, where a glycerol matrix is also used, the glycerol often provides interfering fragments in the mass range less than 200 amu. However, in the experiments described herein, the matrix is transparent to the laser radiation and no interference is observed.

The position of the ceramic rod with respect to the beam is critical for obtaining the optimal cooling. There are two dimensions to consider here: (1) the distance of the rod from the molecular beam axis and (2) the distance of the rod from the nozzle orifice. In the first case, the best cooling was observed when the rod was as close to the supersonic beam as possible without destroying the jet. This was performed in our work by including a volatile sample such as aniline in the jet and moving the rod toward the beam until the molecular ion peak intensity started to decrease. As the rod was moved further, generally a dramatic decrease occurred as the rod began to interfere with the jet. In the second case, the distance between the center of the rod and the nozzle in our experiments was ~ 5.5 mm. This distance provided excellent cooling, while if this distance was decreased, a significant decrease in signal was observed with no noticeable increase in cooling. This appears to occur since at shorter distances, where the carrier density was very high, it was difficult to efficiently penetrate the beam without causing shock waves, whereas at longer expansion distances, where the carrier density was lower, sample penetration appeared to be much more efficient. The use of CO_2 vs Ar or He provided an increased number of collisions at longer expansion distances for enhanced collisional cooling.[22]

The desorption laser was a Quanta-Ray EXC-1 CO_2 laser ($10.6\,\mu m$). A Scientech 365 power meter was used for measurement of the beam energy, and based upon the known beam profile the power density of the beam can be estimated. The IR beam was softly focused with a 10-cm focal length biconvex germanium lens to a ~ 2 to 3-mm spot for desorption, although the focus was adjusted for each sample molecule to optimize our results. The amount of sample desorbed per pulse depends on the power density on the surface and on the properties of the sample, particularly the melting point. In the case of catechol (mp = 106 °C), the desorption is so efficient that a low laser power was used in these experiments ($\sim 1 \times 10^5$ W/cm²), whereas for tyrosine (mp = 325 °C), a power density of nearly 5×10^6 W/cm² was used. In either case, a higher power density can be used to increase the signal intensity. However, by carefully controlling the desorption laser power, the sample can be made to last for an extended period of time for spectroscopic scans. In several spectroscopic scans in our work, a 100-μg sample has lasted for $\sim \frac{1}{2}$ h at a repetition rate of 10 Hz. This is equivalent to $\sim 3 \times 10^{13}$ molecules or ~ 0.1 monolayers desorbed per CO_2 laser pulse.

C. Ionization setup

The laser ionization (R2PI) was performed using the doubled dye output from a Quanta-Ray DCR-2A Nd:YAG pumped dye laser system. The 6-mm output beam was collimated with a combination 30-cm focal length positive lens

FIG. 1. Schematic of the experimental apparatus.

and a 10-cm focal length negative lens to produce a laser beam 2–3 mm in diameter. The power density was adjusted to obtain the desired signal level which depended upon the amount of material entrained in the jet and the efficiency of ionization of each particular compound. The nozzle-to-excitation distance was generally ~16 cm, which ensured that by the time the expansion had reached the acceleration plates, the molecular beam was well within its "free-flow" region. The actual sequence of events was controlled by several delay generators where the pulsed CO_2 laser fires first to produce desorption followed by the pulsing of the valve. The two events were synchronized in time so that the desorbed plume was entrapped into the jet expansion of CO_2 and carried into the acceleration region of the TOFMS. The flight time of the jet from the pulsed valve to this region was ~300 μs, and the laser was, therefore, set to pulse as the gas pulse arrived at this point. Laser R2PI was produced and a LeCroy 9400 digital oscilloscope was used to record the mass spectrum. The wavelength spectrum was obtained by using a SRS 250 gated integrator to monitor only the molecular ion as the dye laser was scanned.

II. RESULTS AND DISCUSSION

In Figs. 2–5 are shown cold jet spectra of several nonvolatile or labile molecules which have been entrained into the jet using the pulsed laser desorption technique described herein. In each case, the molecular ion results with only very minor fragmentation and is monitored as a function of wavelength. In Fig. 2 is shown the origin region of a jet-cooled spectrum of carbazole. This molecule has a melting point of 250 °C and is stable upon heating. It has been chosen for study since spectra have been obtained in previous work using direct heating at between 180–200 °C using CO_2 (Ref. 10) or Ar carrier[21] and using supercritical fluid expansion from N_2O at 40–45 °C and 200-atm backpressure.[9,10] The sharpness of the rovibronic structure of the jet spectrum and the signal/noise ratio obtained by the laser desorption entrainment technique is at least comparable to or better than
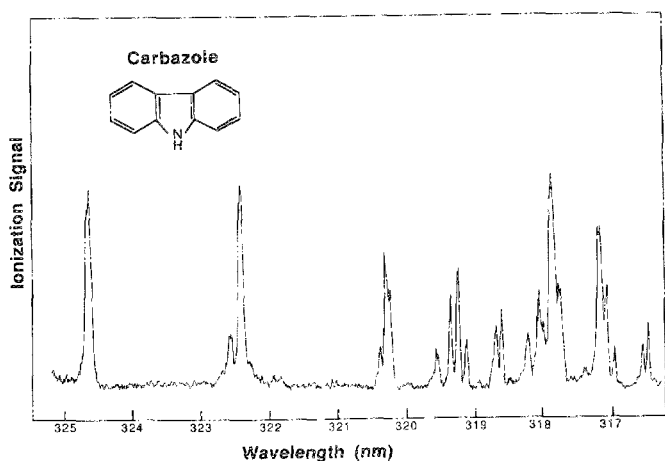

FIG. 2. Resonant two-photon ionization (R2PI) spectrum of carbazole obtained by monitoring the molecular ion in a TOFMS using the pulsed laser desorption method for sample introduction into the jet expansion. The desorption laser power density is estimated at ~5×10^5 W/cm^2.

that obtained by either of these alternate methods. In addition, the pulsed desorption technique avoids the experimental difficulties involved in hot nozzle injection or in working with high-pressure fluid expansions. In Fig. 3 is shown a jet-cooled spectrum of benzimidazole (mp = 174 °C) obtained by pulsed laser desorption. This molecule exhibits a strong origin band at 277.5 nm. The spectrum obtained is once again comparable to that obtained from expansion from supercritical N_2O at 350 atm and 50 °C (Ref. 9) and avoids the experimental difficulties involved in working with high-pressure fluid expansions.

In Fig. 4 is shown a jet-cooled spectrum of tyramine (mp = 163 °C) obtained by pulsed laser desorption and entrainment into a jet of CO_2. In previous work, a similar spectrum was obtained by heating tyramine in a hot pulsed oven to 100 °C with subsequent expansion in Ar carrier.[16] In this case, substantial pyrolysis of the sample occurred where all but a fraction of the sample was converted to a polymeric tar. There were also several decomposition products observed in the mass spectrum for this relatively labile molecule, although a wavelength spectrum was obtained by monitoring only the molecular ion at $m/z = 137$. However, using the pulsed laser desorption method, volatilization of tyramine was accomplished with no apparent decomposition or pyrolysis products. The spectrum obtained by pulsed laser desorption appears considerably sharper and the peaks better resolved than that obtained by direct heating. The solubility of tyramine in supercritical CO_2 or N_2O below 70 °C (Ref. 10) was found to be negligible, and a wavelength spectrum could not be obtained by this method. In Fig. 5 is shown a jet cooled spectrum of tyrosine obtained by pulsed laser desorption–volatilization. In our present setup, we cannot use direct heating since the melting point of this fairly labile molecule is 325 °C, which is beyond our present capabilities, and tyrosine has proven to have negligible solubility in either supercritical CO_2 or N_2O. It should be noted that the basic structural precursor of tyrosine and tyramine is p-cresol which has its π–π^* origin at 282.97 nm. Since we are exciting the π–π^* center of the aromatic ring, one might expect that a substituent group removed from this ring would result in a minor shift of the origin transition. This indeed appears to be true where both the tyramine and tyrosine spectra are shifted only 2–3 nm to shorter wavelength than p-cresol.

It should be noted that Ar was initially used in these experiments since enhanced rotational cooling should be obtained as compared to a polyatomic such as CO_2. However, we have found that increased penetration and enhanced signal levels are obtained in CO_2 over Ar by desorbing at longer distances (~5.5 mm) from the nozzle. The increased collisional rate at longer expansion distance for CO_2 than for the monatomic carriers provides a sufficient number of collisions at this point (i.e., 5.5 mm) (Ref. 22) so that excellent cooling is still obtained provided the beam is probed at a distance sufficient to be in the free-flow region where the terminal Mach number and the optimal cooling is achieved. In addition, some estimate of the efficiency of entrainment can be made. If a typical signal on the tyramine 0–0 transition is 30 mV with a 10-ns FWHM peak width through a 50-Ω termination, and the dual microchannel plate detector has
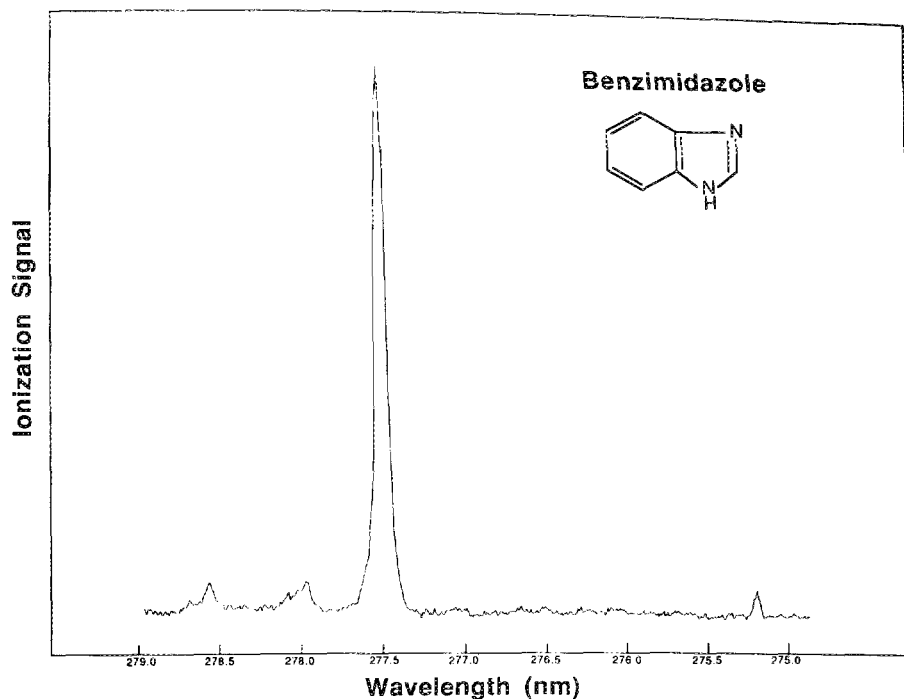
FIG. 3. R2PI spectrum of benzimidazole obtained by monitoring the molecular ion in a TOFMS using the pulsed laser desorption method for sample introduction. The desorption laser power density is estimated at $\sim 2 \times 10^5$ W/cm^2.

an estimated gain of 5×10^6 at the given working voltage, then ~ 10 ions are detected per pulse. Assuming a 0.1% ionization efficiency and that a 3-mm laser beam intersects $\sim 15\%$ of the gas pulse[23] then $\sim 10^5$ neutrals are entrained in the beam per pulse. This corresponds to only $1/10^8$ of the estimated number of molecules desorbed per pulse. Never-

theless, a remarkably small amount of sample is required (~ 100 μg) in order to obtain the spectra shown herein.

Thus, the use of the pulsed laser desorption in conjunction with the sample dissolved in a glycerol matrix provides a simple means of repetitively volatilizing molecules with low volatility into the gas phase for entrainment into a supersonic jet expansion. This method avoids decomposition due to direct heating and the technical problems involved in high-pressure injection from supercritical fluids and hot methanol.
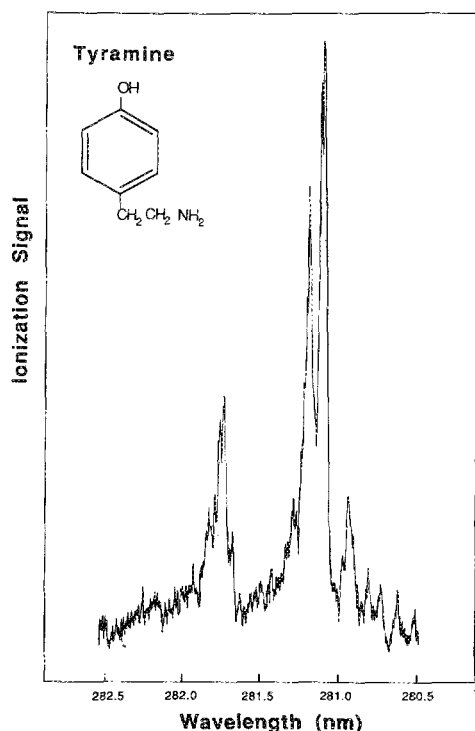

FIG. 4. R2PI spectrum of tyramine obtained by monitoring the molecular ion in a TOFMS using the pulsed laser desorption method for sample introduction. The desorption laser power density is $\sim 1 \times 10^6$ W/cm^2.
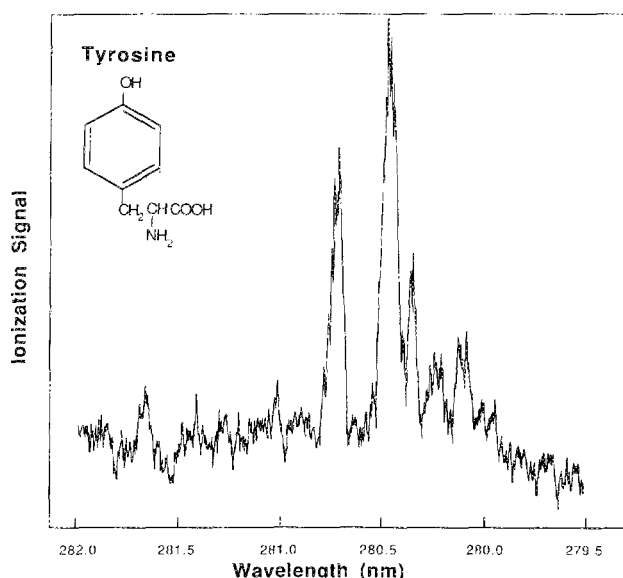

FIG. 5. R2PI spectrum of tyrosine obtained by monitoring the molecular ion in a TOFMS using the pulsed laser desorption method for sample introduction. The desorption laser power density is $\sim 5 \times 10^6$ W/cm^2.

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