validity of the ASV titration method. We are confident that Shuman and Woodward's mathematical treatment is sound *if* the stated assumptions are met. However, the validity of the assumptions has not been demonstrated for any pure ligand nor for natural water organics. On the other hand, it should be noted that the Shuman and Woodward method is simplistic in implying that one can characterize metal complexes with natural water dissolved organic matter (DOM) or soil fulvic acid (FA) in terms of a single β'. Graphical analysis of complexometric titration data by Scatchard plots invariably has shown the presence of at least two types of ligand groups (with two characteristic constants) in DOM and soil FA (*7–10*). Scatchard-derived stability constants, though an improvement on the idea of a single β', are themselves a simplification of reality, and some have criticized this approach as "curve-fitting". As Langford (*11*) pointed out, with polyelectrolytes like natural water DOM and soil FA, it is more appropriate to think in terms of stability functions than stability constants.

Despite Bhat and Weber's statement that "Cu^{2+}–SFA complexes are not reduced ($i_{red} = 0$)," no evidence (or even mention) of the lack of complex reduction is found in the reference they cited (their ref 4). Conversely, our work (*12, 13*) has demonstrated that copper–organic complexes for two humic-rich surface waters in Florida and for polyaspartic acid (mol wt 5400) are completely reducible, albeit at rates somewhat slower than that for ionic copper. Buffle and co-workers (*14, 15*) reported similar results for Pb–humic complexes. These findings may explain the typical titration curve obtained by using the ASV method (including, perhaps, an accurate C_L); however, calculations of β' for these samples are not accurate since one of the stated assumptions of the ASV method is that the complex be nonreducible (*5, 6*).

It is obvious that many assumptions must be met for the ASV–titration method to yield an accurate β' value. In general, the validity of these assumptions can be tested experimentally for defined systems containing a single, known ligand. However, for complicated mixtures of unknown ligands, such as occur in natural waters, the validity of the assumptions has been essentially a matter of faith among users of the ASV–titration method. To restate our conclusion, the ASV method should not be used with environmental samples until the procedure has been shown to be accurate when using copper as the titrant. To date, no validation of the procedure has been reported.

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Isotopic Analysis of Iodine by Multiphoton Ionization

Sir: The problem of monitoring ultralow levels of various radioisotopes in the environment is of current interest. In particular, ^{129}I with a half-life of 17 million years, can be used as a tracer of radioactive contamination in the environment over an extended period of time (*1–8*). ^{129}I is produced as a major product ($\sim 1\%$) in the fission process and soon mixes with the natural isotope, ^{127}I, in the environment. Thus monitoring ^{129}I can be accomplished through the detection of the mixed molecule $^{127}I^{129}I$. The I_2 samples for analysis are collected from the products of burning vegetation (*9*). The sensitivity desired for analyzing these gaseous samples is on the order of 10^7 atoms/cm³ of ^{129}I in 10^{15} atoms/cm³ of ^{127}I, i.e., an isotope detection ratio of 1:10^8. Radioactive counting techniques are not practical for detection of ^{129}I because of its low specific activity and low-energy decay products. The present method of analysis is based upon neutron activation. This technique is typically capable of a discrimination of only between 1:10^5 to 1:10^6 of ^{129}I:^{127}I under real experimental conditions (*9*) although it may be extended with great care. This is due to background radionuclides generated in the neutron activation process. The background contaminants can be chemically removed before activation to produce a discrimination on the order of 1:10^9 (*10–14*). However, the time scale of this analysis including the purification process and the decay counting procedure is on the order of several weeks. This method is therefore extremely time-consuming and cannot be used for real-time measurements. It also requires access to a high flux reactor and poses the danger to personnel of handling hazardous radioactive samples. Thus, a real-time or rapid measurement technique which can handle the isotopic discrimination requirements would be preferable. We demonstrate a detection scheme using two-color laser multiphoton ionization which is able to discriminate 2–3 parts of ^{129}I in 10^4 parts of ^{127}I in a bulb at room temperature. The use of mass spectrometry in conjunction with this technique promises to reduce this limit to better than 1:10^8.

Multiphoton ionization occurs when ionization follows the absorption of more than one photon by a molecule in the presence of an intense visible or UV light source. As the laser source is tuned to an allowed n-photon transition, this process is greatly enhanced. This is referred to as REMPI, i.e., resonance enhanced multiphoton ionization. In the case of REMPI, ionization occurs via a real intermediate state. Since the density of states above the lowest energy state populated is usually quite high, subsequent absorptions are often resonant or nearly resonant. When the laser is not tuned to a real

state, the probability for multiphoton ionization is nearly negligible for modest laser powers. REMPI affords us a selective means of resonantly ionizing $^{127}I^{129}I$ and not $^{127}I_2$, based on isotopic spectral shifts. Thus we can achieve a certain discrimination factor by the ionization process itself. A quadrupole mass spectrometer can provide a typical abundance separation of $>10^4$ between half mass units. If our laser multiphoton ionization technique can provide another factor of 10^4, we can in principle obtain at least a factor of $>10^8$ discrimination.

In what follows, we demonstrate first the ability to distinguish between different isotopic forms of I_2 in an ionization cell at room temperature, based upon their different spectra using a two-color multiphoton ionization scheme. These experiments were performed under bulb conditions because of the limited amount of ^{129}I-containing sample prepared and the problems inherent due to the handling of radioactive materials. Further experiments were performed under molecular beam conditions on $^{127}I_2$, in order to investigate the mechanism of ionization.

EXPERIMENTAL SECTION

A two-color pumping scheme was employed to ionize I_2 in which the two light beams of differing wavelengths overlap in space. The laser system used was a Quanta-Ray DCR-1A Nd:YAG pumped dye laser system. In one case the two wavelengths were the green ($\lambda = 532$ nm) second harmonic of one Nd:YAG laser and the UV third harmonic ($\lambda = 355$ nm) of a second Nd:YAG laser. In other experiments a green beam ($\lambda = 640$–530 nm) obtained by pumping various dyes in a Nd:YAG pumped dye laser was overlapped with the third harmonic of the same Nd:YAG laser. In several cases an intracavity etalon (ICE) was placed inside the cavity of one or both of the Nd:YAG lasers in order to narrow the line width of the 1064-nm fundamental output. The resulting line widths for the second and third harmonics were 0.08 cm^{-1} and 0.12 cm^{-1}, respectively, as measured by a Fabry-Perot spectrum analyzer. The ICE was scanned over the output profile of the Nd:YAG laser by tilting it by mounting a gear on the ICE turning shaft and then using a simple clock motor to turn the gear.

We attempted to check isotopic discrimination using the two-color multiphoton ionization technique. We obtained isotopic mixtures of I_2 samples from McClelland AFB, Sacramento, CA. The samples were prepared by standard methods (method for preparation furnished by Oak Ridge Isotope Sales) from solutions of $K^{129}I$. Dilutions were prepared volumetrically in the liquid phase. The gaseous I_2 prepared from the solutions then reflected the relative concentration of ^{127}I and ^{129}I present in the solutions. Pyrex ionization cells were used to analyze the samples. The cells contained a pair of stainless steel electrodes ~ 1 cm apart. One of the plates was biased at -150 V and negatively charged species were collected. The current was integrated by the Quanta-Ray dual-gated amplifier (DGA-1) and displayed on a strip chart recorder. The vapor pressure of I_2 in these cells was the saturation pressure at room temperature, namely, ~ 0.3 torr.

In order to understand the mechanism of ionization, we performed further laser experiments on $^{127}I_2$ in an effusive molecular beam. The experimental apparatus used for the beam experiment is shown in Figure 1. The molecular beam was formed by heating a vial of I_2 with heating tape to 40–50 °C. The iodine entered the excitation–detection chamber through a long heated tube with a 1 mm diameter aperture which served as an injection probe. The pressure of I_2 in the chamber was controlled by a stainless steel needle valve. The beam was introduced approximately 1.5 cm from the excitation zone to maximize the density. The molecular beam was crossed at right angles by our laser excitation beam inside an ion extraction cage (15). The ionized molecules and atoms were then deflected by a low-voltage focusing electrode into the quadrupole mass spectrometer (EAI Quad 200) where they could be detected and mass discriminated. The base pressure attainable in the excitation–detection chamber is 2×10^{-8} torr when the system has been baked out.

The laser-induced fluorescence spectrum of I_2 was obtained either by scanning the ICE over the bandwidth of the second harmonic or by using a tunable dye laser. The signal was viewed
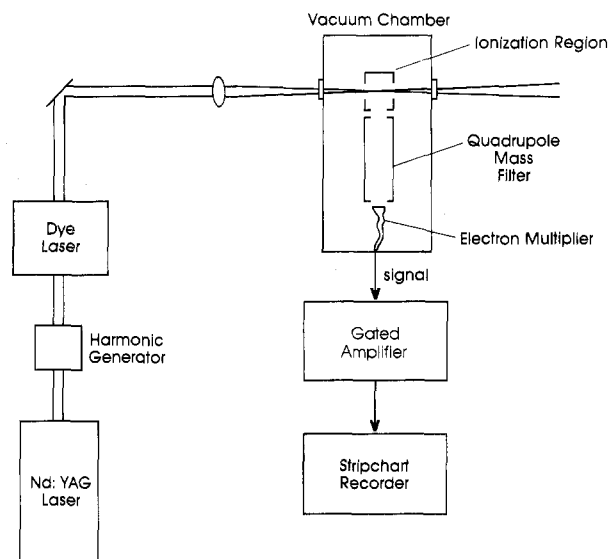

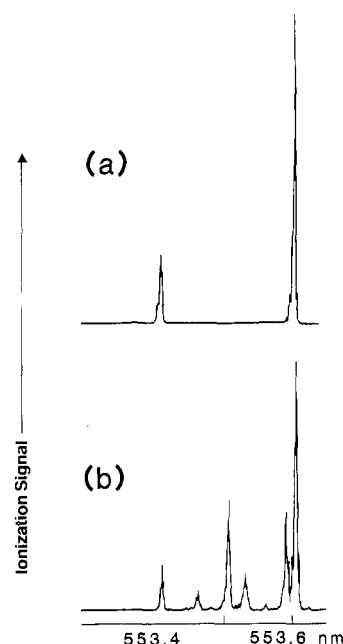**Figure 1.** Experimental setup used in molecular beam ionization experiments.

Figure 2. Two-color MPI spectrum of (a) $^{127}I_2$ and (b) a 1:20 mixture of $^{127}I^{129}I:^{127}I_2$ in the region 553.4–553.6 nm at a particular intracavity etalon setting for the third harmonic.

by a photodiode. Colored glass filters were used to block scattered light. The signal was amplified and then signal averaged by use of the DGA-1.

RESULTS AND DISCUSSION

Several isotopic mixtures obtained from McClelland AFB were analyzed by the two-color multiphoton scheme. The spectra of $^{127}I_2$ (Figure 2a) and 1:20 mixture of $^{127}I^{129}I:^{127}I_2$ (Figure 2b) are quite different, as expected. These spectra were taken by using the dye laser as the green pump beam combined with the line-narrowed third harmonic of the Nd:YAG laser. Figure 3 shows a comparison of a spectrum of $^{127}I_2$ with and without an ICE. The spectrum becomes complicated and isotope discrimination becomes difficult without the ICE. In addition, for each position of the ICE the spectrum obtained by scanning the dye laser appears different.

Because the spectrum of $^{127}I^{129}I$ differs from that of $^{127}I_2$ by the isotope shift, it is possible to excite selectively one
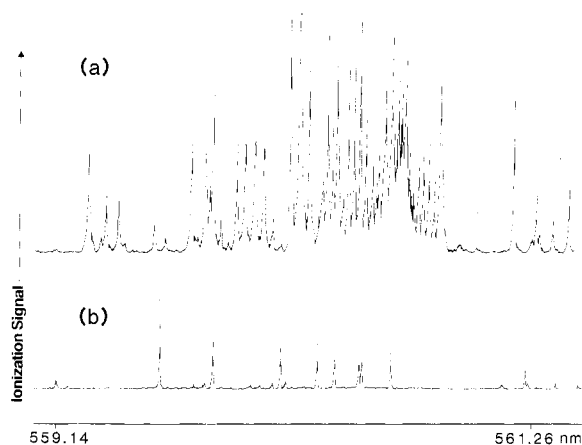
Figure 3. Two-color MPI spectrum of $^{127}I_2$ (a) without an Intracavity etalon and (b) with an Intracavity etalon. The vertical scales are arbitrary but at the same gain setting.

isotopic molecule in the presence of the other. A two-color scheme is preferable to a one-color scheme for two reasons. First, it can provide additional selectivity of the wanted isotope. Second, a one-color scheme in the green is relatively specific but very inefficient because it requires absorption of six or more photons (16). (A resonant one-color scheme in the UV, e.g., at λ = 266 nm, causes ionization of organic background.) We have demonstrated, by correct choice of the proper combination of wavelengths, the detection of $^{127}I^{129}I$ in a 1:100 mixture of ^{129}I:^{127}I by multiphoton ionization of saturated I_2 vapor contained in a room-temperature bulb. Under these conditions the signal to noise is 30:1 to 60:1. Thus, we estimate that $^{127}I^{129}I$ could be detected with unity signal to noise at a ^{129}I:^{127}I ratio of 2–3:10000 in a bulb. This estimate was experimentally verified in a separate dilution experiment in which we could detect $^{129}I^{127}I$ in a ^{129}I:^{127}I mixture of 5:10⁴, with a signal to noise ratio of at least 2. Collisional effects limit the results because at 0.3 torr of I_2 a background ion signal which is two-color dependent appears when the laser is tuned off resonance. *This effect is not seen under molecular beam conditions.* Therefore, in a vacuum system using a quadrupole mass spectrometer we should in principle be able to discriminate $^{127}I^{129}I$ from $^{127}I_2$ in an abundance ratio on the order of 1:10⁸ or better with our selective laser ionization source. Since present commercial quadrupole mass spectrometers can provide an isotope abundance discrimination of at least 1:10⁵ between mass 127 and 129, this estimate is not unreasonable (17).

Under molecular beam conditions we were able to detect $^{127}I_2$ at the base pressure of the chamber, i.e., 2×10^{-8} torr. This corresponds to $\sim 1.5 \times 10^8$ molecules/cm³. The $^{127}I_2$ signal at this detection level could be uniquely distinguished from background not only by the quadrupole mass spectrum but by the fact that the $^{127}I_2$ signal is specifically dependent upon the presence of the two laser wavelengths. At a pressure of 1.0×10^{-6} torr of $^{127}I_2$ we detect a peak signal which is typically 150 mV across 10⁶ Ω, i.e., a collector current of 10^{-7} A. Since the measurement of 10^{-12} A of pulsed current is quite reasonable, we could in theory detect 10⁵ molecules/cm³ of $^{127}I_2$. Our present quadrupole has approximately 1% transmission at mass 127. A system with a higher transmission could increase the detection limit. The use of ion counting techniques with background subtraction may yet further extend the limits of this technique.

We chose a two-color, two-level resonant ionization scheme for iodine in order to achieve enhanced isotopic discrimination. The mechanism of ionization was investigated in order to understand and possibly be able to optimize the analytical potential of the technique. It was originally expected that
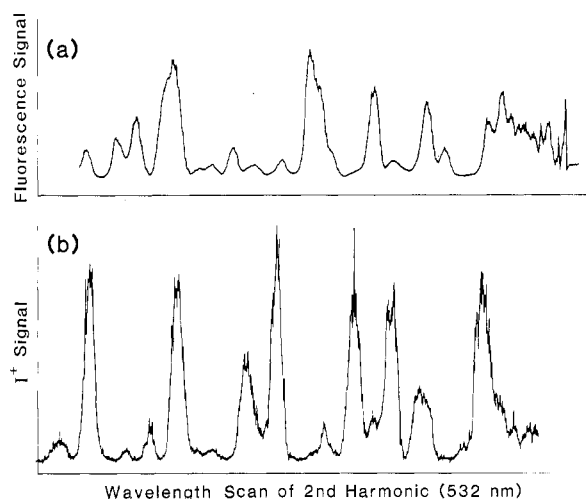

Wavelength Scan of 2nd Harmonic (532 nm)

Figure 4. (a) Fluorescence spectrum of $^{127}I_2$ taken by scanning the Intracavity etalon over the bandwidth of the second harmonic of the Nd:YAG laser. (b) Two-color ionization spectrum taken over the same region by scanning the Intracavity etalon over the bandwidth of the second harmonic while the third harmonic from another laser was maintained broad band.

the second harmonic of one Nd:YAG laser (λ = 532nm) would pump I_2 to the $B(^3\Pi_{ou+})$ state and the third harmonic (λ = 355 nm) of a second Nd:YAG laser would pump I_2 to the $F(^3\Pi_u)$ state. A second UV photon would then be sufficient to cause ionization (IP = 9.28 eV). This was the expected sequence of results based upon the work of Mulliken (18) and Williamson and Compton (19). It was indeed found that this combination of wavelengths could ionize a molecular beam of iodine. The laser beams were unfocused with a beam diameter of 6 mm. If either beam was blocked, the signal disappeared within our detection sensitivity. For further verification of this mechanism, the intracavity etalon was scanned over the full bandwidth of the second harmonic (~ 4 cm⁻¹) in order to obtain a laser-induced fluorescence spectrum of room temperature I_2. In addition, we scanned the etalon over the second harmonic and maintained the third harmonic broad band (~ 6 cm⁻¹) in order to record the ionization spectrum of I_2. As shown in Figure 4, the ionization spectrum obtained differs completely from that of the I_2 B–X fluorescence spectrum. This was true in the bulb and in the molecular beam. Strong ionization signals were often obtained in cases where there was no fluorescence; there was absorption of green photons, but no observable fluorescence from the state absorbing the photons.

An experiment in which one color is delayed in time from the other was performed in order to check whether the green or UV photon was the first absorption step for I_2. Only if the green beam preceded the UV beam was ionization obtained. Therefore, the green beam is absorbed first. The delay between the two lasers was varied between 50 ns and 6 μs and the lifetime of the intermediate state inferred by plotting the logarithm of the ionization signal vs. the time delay. The lifetime was found to be between 10 and 12 μs, which is the predicted diffusion time out of the interaction region. This result was checked for several ionization peaks, including wavelengths for which no fluorescence is observed. The B state fluorescence lifetime around 532 nm is ~ 1 μs (20). Several two-photon absorption studies have been carried out on I_2 (21–23), but we are unable to assign the intermediate state.

In order to investigate the state which absorbs 355-nm light, we placed the ICE inside the cavity of the second Nd:YAG laser. The beam from the first laser was held constant at a wavelength known to lead to ionization while the etalon was
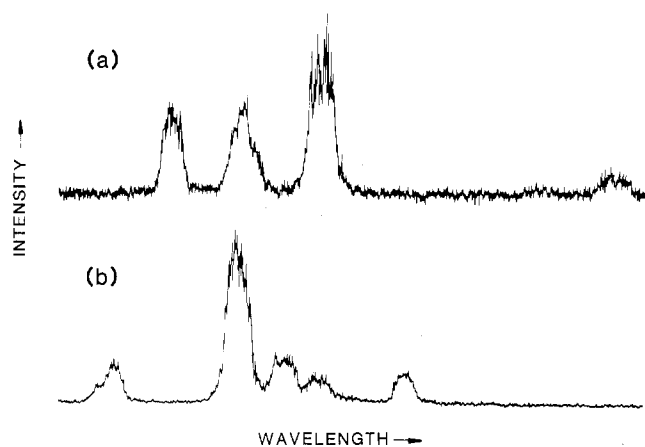
Figure 5. Two-color ionization spectrum produced by maintaining the line-narrowed second harmonic constant while scanning the third harmonic of a second laser over its bandwidth. For each different value of the green wavelength corresponding to an absorption line in Figure 4b, a different spectrum is obtained as the third harmonic is scanned. This is demonstrated for two different absorption lines in (a) and (b).

scanned over the profile of the second laser. A discrete spectrum of one or two major lines was obtained (see Figure 5). The spectrum was different for every line initially pumped by the green laser. Thus, the UV photons cause transitions to another electronic state having a discrete spectrum.

Various neutral density filters were used to obtain the power dependence of each step. The power dependence for the green photon was found to be unity. The same result was obtained for the UV photon. However, these power dependences may only indicate a bottleneck step for each photon rather than the true rate dependence.

It is interesting to note that both I^+ and I_2^+ are present in the ionization process. No other background peaks were detected over the mass range 1–500 amu. The spectra for I_2^+ and I^+ as a function of the green wavelength between 640 and 530 nm are quite different (16). Both spectra are totally different from that of the I_2 B–X fluorescence. The I^+ peaks are typically 10–100 times larger than the corresponding I_2^+ peaks. The I_2^+ spectrum is also much sparser. Using a negatively biased TOF mass spectrometer, we found no I^- at a beam pressure of 10^{-6} torr; instead, an e^- peak appears.

We conclude that the absorption of the green beam populates a long-lived intermediate which appears not to be the B state. We also know that an intermediate following the absorption of one or more UV photons has structure. Unfortunately, we are unable presently to specify in more detail the ionization pathway. Insight into the mechanism of this process allows an increased utilization of the capability of this technique for isotopic discrimination. For example, a unique spectrum in the green results from the particular frequency-narrowed third harmonic defined by the ICE. Thus each change in the position of the ICE produces a different spectrum. Of course a change in the green wavelength allows a totally different set of spectra in combination with the third harmonic beam. Thus, an almost unlimited number of possible combinations are available for maximizing isotopic

discrimination and minimizing background. We have just explored a rather restricted number of these combinations. Using this two-color scheme, we have been able to demonstrate in an ionization cell the ability to discriminate $^{127}I^{129}I$ from $^{127}I_2$ in an abundance ratio of 2–3 parts in 10^4. The use of a quadrupole mass spectrometer could extend this limit to $>1{:}10^8$.

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