Analytical Chemistry: Using Lasers to Detect Less and Less

The minimum number of atoms or molecules that can be detected is one. This limit, which represents the ultimate sensitivity of any analytical technique, has now been attained by two groups of researchers using lasers to "tag" selected atoms in such a way that they could be counted. Numerous other groups, while not reporting such spectacular results, have been busy extending the sensitivity of laser-based analytical techniques as applied to a broad spectrum of atoms and molecules, thus hastening the day when these methods will join the repertoire of analytical chemical tools alongside such standbys as atomic emission spectroscopy and atomic absorption spectroscopy.

It has been known for many years that lasers could be used for extremely sensitive chemical analysis. Among the most promising ways to use lasers for such purposes has been laser-induced fluorescence. In this technique, the intensity of the fluorescence induced by the absorption of laser light whose frequency is tuned to excite electronic transitions in atoms or molecules can be quantitatively related to the number of absorbing particles. The narrow spectral width possible with today's tunable dye lasers ensures that only the species being analyzed is excited, and the high intensity of laser light means that enough particles are excited to result in a measurable signal.

An especially striking illustration of this promise was reported 3 years ago by William Fairbank, Jr. (now at Colorado State University), Theodor Hänsch, and Arthur Schawlow of Stanford University, who measured the vapor pressure of sodium over a range of nine orders of magnitude. At the lowest temperature measured, the investigators detected sodium at a concentration of 100 atoms per cubic centimeter, but because of the low gas volume sampled by the laser, only about five atoms were actually responsible for the fluorescence signal.

Even earlier, Richard Zare (now at Stanford) and his colleagues at Columbia University were obtaining attention-getting results in their laser-induced fluorescence studies of chemical reactions in molecular beams. The researchers were able, for example, to detect barium oxide molecules in selected vibrational and rotational states at a density of 5×10^4 molecules per cubic centimeter with the use of a pulsed dye laser.

The first description of single-atom detection was published last March by Samuel Hurst, Munir Nayfeh (now at Yale University), and Jack Young of the Oak Ridge National Laboratory (ORNL). The investigators were able to count a single atom of cesium against a background of 10^{19} argon atoms and 10^{18} methane molecules in a gas-filled chamber. In addition to demonstrating the ultimate analytical sensitivity, the Oak Ridge team and several co-workers have extended their technique to measuring the concentration fluctuations of a gas, diffusion of atoms in a vapor, and the kinetics of a chemical reaction. Even more recently, the Oak Ridge group has observed single atoms of cesium produced when the californium nucleus fissions. Investigators at other institutions are or will be assembling their own instruments to use the technique for the study of the vapor pressure of nonvolatile elements at ambient temperatures, the detection of neutrinos produced by nuclear reactions in the sun, and the observation of other rare events.

Resonance ionization spectroscopy is the name given to the basic process behind single-atom detection by the Oak Ridge researchers. In essence, single-atom detection combines the capability of a pulsed, tunable dye laser to ionize selectively a desired population of atoms and of a well-designed gas-filled proportional counter to detect voltage pulses deriving from the presence of only a single free electron in the counter.

Detecting Single Atoms with a Laser

Resonance ionization spectroscopy can be viewed as a sequence of steps in which two or more photons from one or more laser beams are needed to ionize an atom. In the simplest case, two photons from the same laser beam are used. The first photon excites an atom from its ground to an excited state, and the second photon then causes the selected population of excited atoms, and only these, to be ionized. The entire process can take place inside a proportional counter, and it is the free electron created by the absorption of the two laser photons that sets off the proportional counter. The size of the voltage pulse in the counter is a measure of the number of atoms ionized by the laser pulse. Use of a sufficiently powerful laser ensures that all the selected atoms are indeed ionized, so that the method is quantitative.

By measuring the number of free electrons rather than fluorescence, the Oak Ridge technique bypasses a principal limitation of laser-induced atomic fluorescence—scattered laser light. Scattered light is neither absorbed by, reflected from, nor transmitted unaffected through a sample but is scattered at various angles with respect to the laser beam. Because of its high intensity, scattered light can overwhelm weak fluorescence signals. The high intensity results because everything in a sample chamber is a potential scatterer, whereas only a few particles emit fluorescence.

To detect a single cesium atom, the Oak Ridge researchers placed a source of cesium vapor inside a cylindrical proportional counter. By moving the source farther and farther from the laser beam (which was parallel to the axis of the cylinder), the investigators eventually reached a point where the probability that only one cesium atom is in the laser beam during each pulse is about 0.1. Under these conditions, it is known from random statistics that, if a signal is observed, it comes from only a single atom. Measurements made at various source distances resulted in the detection of concentrations of cesium ranging from 0.1 to 10^8 atoms per cubic centimeter.

According to Hurst, the two-photon method applied to cesium works only for a few alkali-metal atoms, if only commercially available lasers are used, because the energy of two photons is not sufficient to ionize most other elements. If two lasers are used, the method can be extended to more than one-half of the known elements. When both lasers are fired simultaneously, an atom can absorb one photon from each and thereby reach a much higher energy level. A third photon from either of the lasers completes the ionization process. The feasibility of this scheme has been demonstrated by the Oak Ridge group with the lithium atom and by Santos Mayo and Tom Lucatorto of the National Bureau of Standards (NBS), who applied it to the problem of detecting sodium.

The second single-atom detection technique that has been reported employs laser-induced fluorescence. Jerry Gelbwachs, Chris Klein, and John Wessel of the Aerospace Corporation, El Segundo, California, detected just a single sodium atom in an argon-filled cell at 1 atmosphere. Similar experiments with nickel and platinum have had less spectacular outcomes (for reasons to be ex-

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plained below) with detection limits of about 10^6 atoms per cubic centimeter.

The Aerospace investigators emphasize three ingredients critical to the success of their technique, which they have christened saturated optical nonresonant emission spectroscopy or SONRES. The first ingredient is called nonresonant emission, which simply means that the laser-induced fluorescence observed has a different frequency from that of the incident laser light. In order to achieve nonresonant emission, the investigators relied on the occurrence of collisions between atoms excited by the laser to a particular quantum state and other atoms or molecules in the gas-filled cell. The collisions can either impart to or remove a small amount of energy from the excited atoms, thus transferring them to a different quantum state. The atoms decay back to their ground state by emitting photons (the fluorescence) that have a slightly higher or lower frequency than the laser. The second ingredient is called saturation. Saturation occurs when the laser light is so intense that all the selected atoms are either absorbing or emitting photons at any given time. The third ingredient is the use of a continuous-wave (cw) rather than a pulsed laser.

Nonresonant emission ensures that scattered laser light does not interfere with the fluorescence signal, since the signal and the scattered light occur at different frequencies. Saturation means that each atom can contribute several million photons per second to the fluorescence process, thereby accounting for the extreme sensitivity of the technique. The use of a cw laser further enhances the sensitivity because a pulsed laser is only "on" a very small fraction of the time each second, thus reducing the average fluorescence signal. According to Gelbwachs, it was the use of a pulsed laser that accounted for the poorer detection limits for platinum and nickel as compared to sodium.

In their single-atom detection experiment, Gelbwachs, Klein, and Wessel used photon-counting techniques (a mode of operation of a conventional photomultiplier tube with special electronics that respond to pulses induced in the tube by individual photons). These investigators essentially repeated the earlier Stanford experiments of Fairbank, Hänsch, and Shawlow with the important exception that the sodium was in an argon-filled rather than an evacuated chamber. The minimum concentration of sodium detected was 180 atoms per cubic centimeter, but the average number of atoms contributing to the fluorescence each second was 0.2 because the gas volume illuminated by the focused laser beam was much less than 1 cubic centimeter. This result is the basis for the claim of single-atom detection. The estimated concentration that could have been detected was 10 atoms per cubic centimeter.

Comparison of resonance ionization spectroscopy and SONRES is difficult, in part because each may end up being used for different problems. At Oak Ridge, Hurst emphasizes the dynamic capabilities of resonance ionization spectroscopy, as well as the capability to analyze large volumes of 1 to 10 cubic centimeters. For example, because of the use of a pulsed laser and a detector that counts each ionized atom immediately after ionization, time-dependent phenomena such as density fluctuations and other statistical processes can be investigated. SONRES, with either a pulsed or a cw laser, measures the average fluorescence over a given time interval, and only steady state information is obtainable. In contrast, Gelbwachs points out that SONRES has already been shown to be adaptable to flame spectroscopy and may therefore be easier to apply to trace element analysis. In flame spectroscopy, solid or liquid samples are vaporized into their constituent elements by the flame.

Making a Difficult Choice

One person who has already had to choose between the two techniques is Andrzej Miziolek of the Scripps Institution of Oceanography. Miziolek wants to measure the vapor pressure of nonvolatile elements at ambient temperature as part of a project to determine the sources of the heavy metals that are known to be present in the atmosphere. The decision was in favor of resonance ionization spectroscopy, but just barely. The deciding factor was the frequency range of commercially available tunable dye lasers. The present lasers permit a wider variety of elements of the type Miziolek is interested in to be studied by resonance ionization spectroscopy.

Analytical chemists, who more often than not work with commercial instrumentation rather than with machines they build themselves, realize that single-atom detection represents primarily the best performance obtainable under optimum and somewhat artificial conditions. Resonance ionization spectroscopy, for example, is still so new that its potential has hardly been explored. But of the applications that have been or are planned to be made, it can fairly be said that they are sophisticated basic research, not routine trace-element analysis of the sort that many analytical chemists spend much of their time doing. No one has yet shown, for example, how to vaporize real-world samples, such as seawater that contains many chemical species other than those being analyzed, inside a proportional counter (or other electron multiplier-type detectors). Nor has anyone shown how to relate the concentration in the counter gas to that in the actual sample, which is the quantity ultimately of most interest in quantitative analysis.

One example of this sort of difficulty is being faced by Mayo and Lucatorto at NBS, who want to study sodium contamination of silicon, a problem of interest in the manufacture of microelectronics. They plan to vaporize silicon with a powerful infrared laser, but they worry that oxygen, which is present in all silicon, may combine with the sodium atoms before they can be ionized. If most of the sodium is removed in this way, the effective sensitivity of the technique would be greatly reduced. (Hurst and his colleagues have already studied the kinetics of the reaction between cesium and oxygen by resonance ionization spectroscopy and conclude that, in this case, reaction with oxygen ought not to be a limiting problem.)

James Winefordner and his co-workers at the University of Florida have been pioneers in applying laser-induced fluorescence in more workaday circumstances, such as atomic fluorescence flame spectroscopy. In fact, virtually all of the variations of laser-induced fluorescence, including those applied in SONRES, have been explored at Florida in the context of flame spectroscopy.

In the earliest experiments, the detection limits obtained at Florida and in other laboratories were often poorer than those found with analytical techniques, such as atomic absorption spectroscopy. More recent work, however, has changed this situation and gives perhaps the most direct view of the near-term role of the laser in quantitative analysis. Stephan Weeks (now at NBS), H. Haraguchi, and Winefordner at Florida have made a systematic investigation of laser-induced atomic fluorescence as applied to elemental analysis using a pulsed dye laser and a variety of vaporization methods (flame, furnace, and so on). By carefully optimizing their apparatus, they were able to obtain concentrations of some 23 elements at a level of 1 nanogram per milliliter (a standard concentration unit referring to the weight of element contained in a liquid sample) or less. These detection limits are improved from 10 to 200 times as compared to those obtained previously. While not yet superior in every case to other analytical methods, the investigators concluded that further improvement is possible and that laser-induced atomic fluorescence spectroscopy has a good chance of becoming the method of choice when high sensitivities are needed. Winefordner adds that a commercial instrument could appear within a year.

Laser-induced atomic fluorescence flame spectroscopy has also been carried out at Aerospace using SONRES. Under the turbulent conditions of the flame and with the use of a pulsed dye laser rather than a cw dye laser, Gelbwachs, Klein, and Wessel were not able to achieve quite so high a sensitivity as that obtained in the ideal environment of a clean argon-filled cell, but the concentration reported (10^5 sodium atoms per cubic centimeter, which approximately corresponds to a concentration of 0.1 picogram per milliliter) is still 50 times lower than the next best flame method can detect.

A technique that bears some resemblance to SONRES has been developed at Sandia Laboratories by J. P. Hohimer and P. J. Hargis, Jr. These researchers used a graphite furnace to vaporize solutions containing cesium or thallium. In atomic absorption spectroscopy, for example, use of such flameless atomizers has resulted in detection limits 100 times better than those when flames were used. Detection limits obtained at Sandia for cesium and thallium were 20 picograms per milliliter and 0.5 picogram per milliliter, respectively.

A second group at NBS is working on a laser-analytical technique that relies on ionization rather than fluorescence to tag atoms of interest in the context of flame spectroscopy. Richard Keller (now at the Los Alamos Scientific Laboratory), John Travis, and their co-workers at NBS first observed what they called an optogalvanic effect when laser light was absorbed by the gas in a hollow cathode lamp. More recently, Gregory Turk, Travis, James DeVoe, and their colleagues at NBS have applied the observation, now renamed laser-enhanced ionization in flames, to elemental analysis. The detection limits obtained vary widely among the half-dozen elements that have been analyzed, but in the best cases are more than ten times better than those of other flame techniques. They could detect sodium at a level of about 50 picograms per milliliter, which corresponds to about 5×10^6 sodium atoms per cubic centimeter in the flame, for example. Because laser-enhanced ionization is little more than a year old and hardly optimized, Travis thinks that considerable increases in sensitivity can still be achieved in the future.

The mechanism responsible for the effect they observe is suggested by the name of the technique, although it is not yet understood in detail. What the researchers see is a change in the conductivity of the gas in the flame when laser light is absorbed by atoms there. Apparently, the laser excites atoms to a high energy level which can then be easily ionized by the high temperature of the flame.

There is also substantial interest in applying laser-induced fluorescence to condensed media, such as solutions, without first vaporizing them. An instance in which this would be desirable might arise in measuring the concentration of a particular molecular species in a sample. In this case, an elemental analysis would not be of much help. Because of the plethora of vibrational and rotational states in molecules, almost all fluorescence is nonresonant even without collisional effects. Nonetheless, condensed media present special problems of their own, and workers in the field jokingly refer to detecting impurities in gases as easy by comparison. In impure solutions, for example, fluorescence from other species can interfere with that being sought. Thus, detection limits of condensed media techniques are generally poorer than those of the gas phase.

Measuring Aflatoxin Contamination

An exceptionally timely application of laser-induced fluorescence is being carried out by Gerald Diebold, Noga Karny, and Zare of Stanford in collaboration with Larry Seitz of the U.S. Grain Marketing Research Center, Manhattan, Kansas. These researchers are trying to measure the degree of contamination of corn by aflatoxins, which are mold metabolites that have been shown to cause tumors in laboratory animals. By combining thin-layer chromatography, high-pressure liquid chromatography, and laser-induced fluorescence, they have succeeded in detecting a particular aflatoxin to a level of 100 parts per trillion (weight of aflatoxin per weight of corn). In an earlier experiment at Columbia, Diebold and Zare had reported a detection limit of 0.75 picogram when standards of aflatoxin were used as samples. The number of aflatoxin molecules in the 4-microliter detection volume was estimated to be less than 10^7 in the latter experiment.

The essence of the Stanford technique is the replacement of conventional liquid chromatography detectors with laser-induced fluorescence. To accomplish this, it was necessary to convert the poorly fluorescing aflatoxin molecules to a more fluorescent form by treating the sample with hydrochloric acid. On passing through the liquid chromatography column, the aflatoxin molecules have a characteristic time for passage and are in effect "bunched together" by the chromatography process. The researchers used a cw ultraviolet laser to irradiate the liquid droplets continuously emanating from the end of the chromatography column. But only at the time corresponding to the duration of the aflatoxin transit through the column was the characteristic fluorescence detected.

According to Zare, contaminants in the corn interfered with the fluorescence from the aflatoxin molecules, so that the detection limit achieved earlier with pure samples was not attained. Nonetheless, the limit that was reached is some 50 times better than that of previous methods. Zare is so enthusiastic over the prospects of tagging molecules of all sorts by making them efficient fluophors that he thinks this procedure in combination with laser-induced fluorescence could replace conventional methods of detection in other standard analytical techniques, such as radioimmunoassays.

Analytical chemists emphasize that any new technique must undergo a years-long breaking-in period between the first spectacular results and its appearance as a commercial instrument. During this development period, the performance limits of the technique under many conditions, the cost of the instrumentation, and a convenient mode of operation must all be determined and optimized. Most of the laser-based techniques are just in the beginning stages of this process. One large obstacle to eventual widespread adoption may be cost. At present, pulsed, tunable dye laser systems present a $30,000 price tag to their users, about double the cost of an automated flame atomic absorption spectrometer. A second obstacle is the limited frequency range available in commercial tunable dye lasers. Dye lasers emit light mainly in the visible range (although there are now some ultraviolet pulsed dye lasers), but the energy levels of many atoms and molecules are well into the ultraviolet. Rounding out the limitations is that dye lasers are complicated to operate in comparison with other instruments.

Observers agree that the cost and complexity of lasers will limit their use for routine quantitative analysis for some time to come, but as investigators want increasingly better detection limits, laser-based techniques will come into their own.—Arthur L. Robinson