CHEMISTRY

Analytical Titans Gather at Pittcon to Predict the Future

Scientists love to use tools—and they love to make them too. There's a certain machismo behind efforts to dream up instruments that can produce faster and more sensitive analyses of smaller and smaller samples. Nowhere is this spirit showcased better than at the annual Pittsburgh Conference, known as Pittcon, a technocircus of inventors, makers, and users of the latest, most sensitive, most precise, most accurate, and most expensive measuring tools on Earth. And at this year's Pittcon, which will be held 7-12 March in Atlanta, participants will go beyond even the normal technical muscle-flexing. The meeting features a panel of blue-ribbon academic analytical chemists who will project the feats of molecular analysis that lie in the future. "I told these people to think of far out ideas," says symposium organizer and speaker Allen Bard of the University of Texas at Austin.

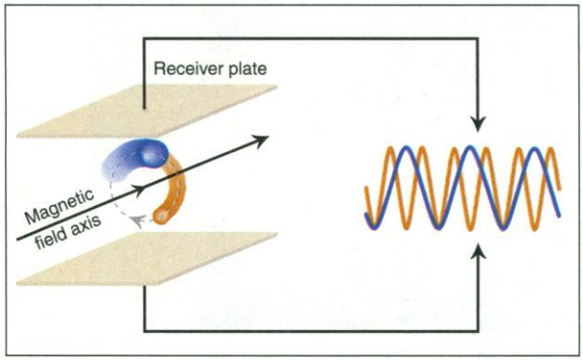
Anything coming from Bard's panel is guaranteed to sound far out to those unfamiliar with the language of analytical chemists, whose lexicon is so formidable and fat with acronyms that it's Greek to almost everyone else. Look behind the acronyms, though, and the new analytical techniques seem less outlandish than inspired: an array of ingenious schemes for making molecules move, jiggle, and flash in ways that reveal their identities, even when only a few copies are present in a sea of contaminants. And the techniques seem still less outlandish when you realize that most of the panelists won't be looking far beyond their most recent research papers. Indeed, as symposium speakers paint it, the future of analytical chemistry looks much like an improved version of the present.

Mass spectrometry gets married—again

Mass spectrometry, for example, has long been a mainstay of analysis for everyone from synthetic chemists to food scientists, and it's been continuously evolving. In one symposium talk, David Hercules of the University of Pittsburgh will argue that the technique is poised for what he identifies as its third major advance. Like many past advances, this one hinges on a marriage.

By itself, a mass spectrometer is essentially an exquisitely sensitive balance that measures the masses of charged, or ionized molecules or molecular fragments. This is commonly done by passing the ions through a magnetic field, which steers them into a detector: The heavier the ion, the stronger the magnetic field it takes to deflect it. So by sweeping through a range of magnetic field strengths, analysts can detect a range, or spectrum, of molecular masses—a clue to the sample's molecular makeup.

So far, so good. But if a sample contains too many different molecules, its mass spectrum can be tough to interpret—and that has inspired the development of various strategies for separating the components of a complex sample before they are fed into a mass spectrometer. In the 1960s, researchers began hooking mass spectrometers to the end of gas chromatographs (GC). The second major advance, in the early 1980s, came when researchers married mass spectrometers with liquid chromatographs (LC), which can handle larger, less volatile, and less stable compounds than a GC. But even GC-MS and LC-MS together can't cover the entire gamut of molecules—large biological molecules, for example, are out of their league.



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Molecular weigh station. In ICR-MS, molecules whir past detectors at different speeds, producing unique signals.

So Hercules is trying to find a new partner for MS. His proposed match: planar separation techniques such as thin layer chromatography (TLC), a widely used and extremely sensitive separation technology. There's a catch, however: The separated compounds stay on the TLC plate rather than leaving the end of a column as in GC and LC, and getting them into the mass spectrometer for analysis has been a challenge.

One solution Hercules and several other groups have been developing relies on a laser microprobe, which can be finely steered and focused to vaporize tiny regions on TLC plates. By blasting a series of tiny spots across the separation surface, the microprobe can feed the material piecemeal into the mass spectrometer, conceivably yielding information on hundreds of different compounds, each present in a quantity as small as a femtomole, or a hundred million copies—in molecular terms, a mere handful.

Circular reasoning

Hercules' new version of mass spectrometry may open the way to sharper analytical snapshots, but another mass spectrometry aficionado, Fred McLafferty of Cornell University, is striving for a veritable motion picture. He'd like to be able to study changes in the mass of large biological molecules as they break down or react, and yet another hyphenated version of mass spectrometry—ICR-MS, for Ion-Cyclotron-Resonance Mass Spectrometry—is key to his hopes.

In other kinds of mass spectrometry, the molecules of interest take a one-way trip into the detector, but ICR-MS preserves the molecules so that they can be modified. Instead of simply deflecting charged molecules with a magnetic field, ICR-MS magnetically traps them in circular orbits within a miniature cyclotron. As the charged particles whir past metal plates along the periphery of the cyclotron chamber, they induce sinusoidal electrical signals, whose amplitudes reflect the molecules' concentrations. And because more massive molecules orbit more slowly in a magnetic field, the frequencies of the signals reveal the molecular masses. In the device McLafferty and his colleagues have built, those frequency differences can betray differences in molecular weight equivalent to a single hydrogen atom in a large protein.

What's more, says McLafferty, because the molecules are trapped, "you can introduce reagents into the cyclotron chamber and react them with the sample molecules," all the while monitoring the changing masses of the orbiting molecular species. A protein chemist, for example, might add a reagent that would chop protein molecules into smaller and smaller fragments. By precisely measuring the mass of each fragment, says McLafferty, "we [could] do automated sequencing of proteins" at a clip of a million amino acids per day, starting with subfemtomole samples. Or, if an analyst wanted to learn about a protein's three-dimensional shape, he might measure its mass in the presence of deuterium (heavy hydrogen) atoms. The deuterium would take the place of some of the ordinary hydrogens in the protein, making it heavier. The exact mass increase would yield clues to the protein's shape by revealing how much of it was exposed to the deuterium.

Molecules on the march

Another workhorse analytical method is electrophoresis, in which an electric field separates the molecular components of, say, a

sample of urine or blood or a cell extract by coercing them to migrate through a medium of some kind, each at its own characteristic rate. Milos Novotny has been looking into the future of electrophoresis—and he sees tiny flashes of fluorescence.

Novotny, a highly decorated analytical chemist at Indiana University, has been pushing the envelope of so-called capillary electrophoresis, first introduced about 10 years ago by one of Novotny's graduate students at the time, James Jorgensen. Like all electrophoretic methods, the capillary technique relies on the fact that molecules migrate at rates that depend on their electric charges. But because its venue is tiny capillaries, which quickly dissipate heat, rather than larger structures such as gels, capillary electrophoresis allows researchers to apply much higher electrical fields. That, in turn, means faster and more sensitive separations.

Novotny's glimpse of the future came when he decided to extend CE's reach to smaller samples. That's not easy, because the minute fractions that emerge after the separation can easily escape detection. To amplify their signals, Novotny is now combining CE with a supersensitive detection technique called laser induced fluorescence (LIF). The idea is to tag certain molecules in a sample with a fluorophore—a chemical appendage that absorbs laser energy and then reemits light into a detector. The technique, Novotny says, enables researchers to "see" a substance in sub-attomole quantities, corresponding to about 100,000 molecules.

Such visibility could be especially important for molecules such as carbohydrates, which can be difficult to detect by other means, especially when present in minute quantities. Indeed, Novotny predicts that CE-LIF could become a favorite acronym in glycobiology, a little-developed field focusing on the biological roles of sugars, polysaccharides, and other carbohydrates.

Chemical cartography

Analytical chemists are not merely interested in identifying which molecules are present in a sample and in what quantities. They'd also like to be able to take a sample—a surface or even a living cell—and map it chemically, determining not only what its constituents are but how they are arranged in space. "In the next century," says Bard of the University of Texas, "there will be a growing need for high-resolution spatial analysis" in fields such as microelectronics. Bard's own role in the symposium will be to talk about a new tool for chemical map-making—a descendant of a device that is now a fixture in solid-state science, the scanning tunneling microscope (STM).

Since 1989, Bard and colleagues have been developing what they call scanning electrochemical microscopy (SECM). Like other scanning probe techniques, SECM relies on an electrical signal coming from a fine stylus scanning over a sample surface. In a conventional STM, the signal derives from a quantum mechanical tunneling current between the tip and a conductive sample. Because the current increases as the tip-sample distance decreases, computers can convert variations in signal into stunning molecular and atomic landscapes, now ubiquitous in the scientific literature.

In Bard's SECM, the signals come instead from electrochemical reactions in a liquid reaction medium between the tip and the sample surface. The rate of the reaction changes as the tip approaches the surface, which enables an SECM to chart topography, much as an STM does. But there's a key difference: Since the chemical nature of the surface also affects the rate of the electrochemical reaction, an SECM—though unable to scan with the atomic precision of STM—can also gather rough chemical information about the sample.

An SECM could explore composition in far more detail, Bard and his colleagues realized, if it could create specific voltage differences between the tip and the surface. Because electrochemical reactions occur at characteristic voltages, the tip could, in effect, test the chemical makeup of patches of surface 5 microns across.

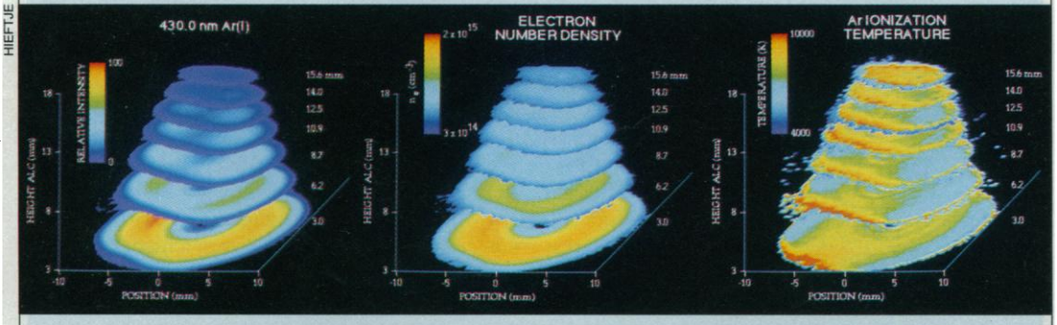
As Bard will tell his Pittcon audience, such a chemical mapper is now up and running. In place of the simple tip of earlier SECMs, the new microscope Bard devised with colleagues at Austin and the Technical University in Budapest has a glass-encased antimony microelectrode, its tip just 3 microns in diameter. By scanning the tip with different voltages, the group has already detected catalytically active areas of a surface studded with the enzyme urease and mapped the conversion of sugar to acids on a surface peppered with living yeast cells.

Oh yes, there's one more thing an SECM can do. Instead of just witnessing local chemistry, the chemical microscope can change it—by, say, electrochemically depositing or etching superfine metallic lines, something that might be useful for making future generations of micromachines and sensors. When you can take this close a look at chemistry, why be just a spectator?

–Ivan Amato

An Analytical Triptych

HIEFTJE



While other analytical chemists scheme to get ever more data about smaller samples, Indiana University chemist Gary Hieftje will be telling his colleagues at Pittcon about new ways to make the most of that information. He is now working on strategies for combining five and six kinds of analytical information about a sample—say, its mass spectrum, the wavelengths and intensities of radiation it emits or absorbs, and the time it took to snake through a chromatography column—into humanly understandable graphic displays.

The trio of images above, for example, portrays the panoply of information available from a single technique, inductively coupled plasma emission spectroscopy (ICP). In ICP spectroscopy, the energy of a plasma—an argon plasma in this case—excites the atoms in an injected sample. The excited atoms then emit light at signature wavelengths. To interpret the spectrum confidently, though, an analyst often needs to understand various properties of the plasma as well. The images shown here provide four-dimensional maps—three spatial dimensions plus a magnitude dimension encoded in color—of light emission *(left)* as well as the plasma's electron density *(center)* and temperature *(right)*. By comparing such images, Hieftje believes, analysts could get a sharper, more accurate picture of a sample's makeup than they could from separate data.

–I.A.