Checking the Pulse of PitCon '88

At PitCon '88—otherwise known as the Pittsburgh Conference and Exposition on Analytical Chemistry and Applied Spectroscopy—exhibited instruments capitalized on improvements in existing technology rather than novel concepts or design. This year's models feature increasing automation, particularly for aspects of sample presentation. The 1988 generation of so-called hyphenated systems emphasize a combination of techniques within the same work station—gas chromatography to detect and separate organic contaminants combined with mass spectrometry to identify them, for example. And in keeping with strict environmental standards, many companies are stressing the ability of their latest instruments to detect levels of environmental pollutants in the parts per billion, rather than parts per million, range.

PitCon '88 lured more than 25,000 participants to New Orleans for the week of 22 to 26 February. Approximately 830 companies displayed their wares at 2400 booths, and it took two separate facilities to house them. Specialized symposia on advances in spectroscopic, chromatographic, and electrochemical techniques increased in number as did the number of individual talks and posters. And new this year were three general interest symposia—identifying antiques by spectrochemical techniques, cholesterol, and drug testing in the workplace.

Probing the Authenticity of Antiquities with High-Tech Attacks on a Microscale

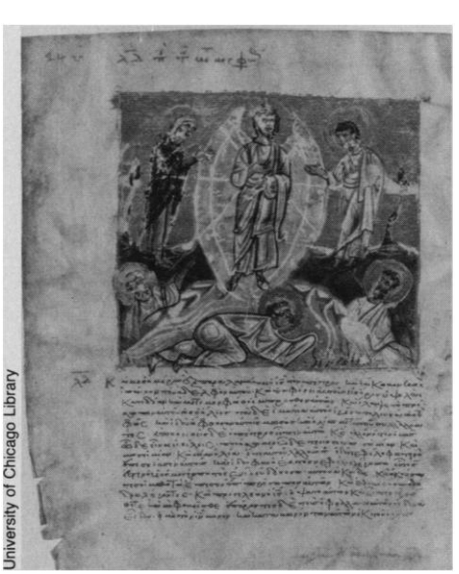
Analyzing and dating antiquities has become an art form in and of itself. But an underlying problem in many cases is how to analyze the art object without damaging it. The solution seems to lie in a combination of scholarly research and the methodological repertoire of the modern analytical chemist, who can apply high technology to a microscopic sample of an artifact that is thousands of years old. Participants in the symposium entitled "Identification of Antiques by Spectrochemical Techniques" described their approaches for determining the composition or authenticity of ancient art objects.

Some analytical chemistry techniques are used to determine the composition of art materials and yield clues as to how the object was crafted. Others are used exclusively to date and authenticate an object. Still a third category of techniques accomplishes both. By analyzing the composition of particles that are sometimes so small that they are invisible to the naked eye, researchers can analyze both the composition of the sample and judge its authenticity.

"In 1818 Sir Humphrey Davey presented a paper to the Royal Society on the colors used by the ancients in painting," says Patricia Lang of Ball State University in Muncie, Indiana. "He prided himself on not damaging the piece and said that he used mere atoms in his analysis. So it is not surprising, some 170 years later, that scientists are still trying to sample and analyze an art object without damaging it."

Lang analyzes paint fragments from paintings or manuscripts to determine their authenticity by using Fourier transform infrared spectrometry or Raman spectrometry coupled with microscope accessories. She reported preliminary data on her analysis of illuminated manuscripts from the Byzantine Empire in the 12th century. "Even though this was the Dark Ages in Western Europe, it was one of two cultural high marks in the Byzantine Empire, where the art was primarily in the form of illuminated manuscripts," she says. Monks painted these manuscripts and they describe the life of Christ.

"The Byzantine paint samples are a mix containing many components," said Lang. This makes its composition more difficult to analyze and Lang uses spectral subtraction to detect the individual components. For example, a yellow pigment contained kaolin (a fine white clay), calcium carbonate (the major ingredient in chalk and limestone), and egg yolk. The egg yolk helped the pigment adhere to the parchment paper and the calcium carbonate made the color more opaque. Lane also found pigments that contained white lead, ultramarine blue, and a red pigment that she thinks is cochineal, which comes from a scale insect that only inhabits warm, dry climates.



Illuminated manuscript. *The manuscript is from the 12th-century Byzantine Empire.*

Victor Bortolot, founder of Daybreak Nuclear and Medical Systems in Guilford, Connecticut, has a completely different approach to dating art objects. "Thermoluminescence dating is a technique applied to inorganic art objects like pottery or to other mineral materials that have been heated past 500°C at the time of interest, for example, when the pottery was fired," he says. "It should be compared to radiocarbon dating." The latter is used to determine when organic materials were alive and is limited to approximately the last 30,000 years because the signal decreases with time. But thermoluminescence (TL) of an inorganic object that has been fired increases with time.

"Thermoluminescence is the light emitted as an object is heated," says Bortolot. "The effect of TL is induced by radiation." The basic principle of the technique, he says, is to measure the accumulation of a dose of radiation since an object was last heated up. When natural crystals form, they absorb ionizing radiation from the environment which then serves to generate electrons that become trapped in the irregularities of the crystal. If inorganic material containing such crystals is heated, the electrons are freed, photons are produced, and the result is thermoluminescence. The amount of light given off is proportional to the radiation dose. When Bortolot uses TL to authenticate the approximate age of an art object, he takes several small powder samples of the object and subjects them to a known dose of beta radiation. To approximate the age of the fired object, he compares the TL acquired in samples that receive the known calibration dose of radiation to the TL in other samples from the same object.

TL dating is most accurate if several samples from the same archeological site can be analyzed and averaged together and the radiation of the site can be determined. But for authenticity testing, the error rate may be fairly high—plus or minus 30%, accord-

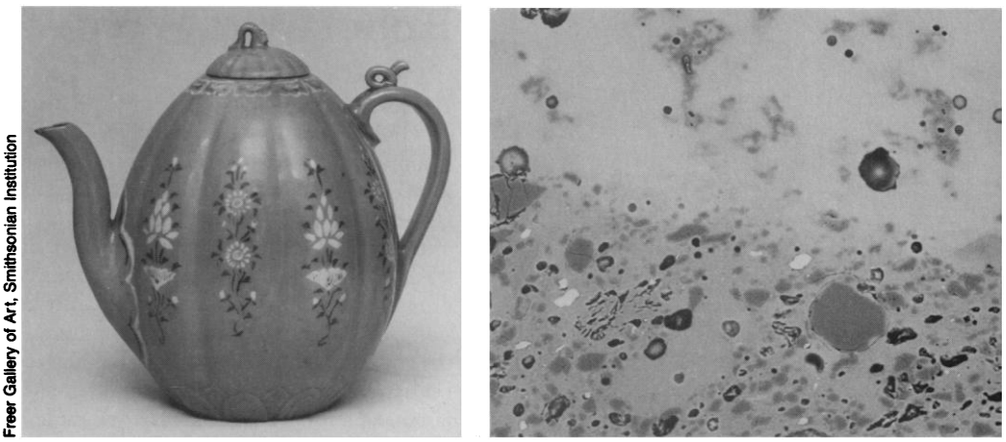
ing to Bortolot. He has used TL dating on a Peruvian urn said to be made about 900 A.D. It was thought to be a fake, but Bortolot showed that it was not. In contrast, when he analyzed tourist pottery from Mexico known to be a fake, TL dating showed that the "art" was only about 8 years old. He has also shown that three seated figures reportedly made by a Vera Cruz artist 1200 to 1300 years ago, were in fact less than 100 years old. "I want to emphasize that in all of these cases, TL dating is only one of many pieces of evidence that is taken into account," he says. "But for a suspected forgery, TL dating can put the nail in the coffin."

Pamela Vandiver of the Smithsonian Institution in Washington, D.C., studies the colorants used in ceramic glazes of pottery from the southern Song Dynasty (1127–1279 A.D.) in China and the Koryo Dynasty (918–1392 A.D.) in Korea. She analyzes an object using a variety of methods to determine both its macrostructure and microstructure. For instance, Vandiver examines the secondary and backscattered spectra from tiny particles of the glazes using scanning electron microscopy. These techniques allow her to determine what materials the glazes consist of and also to infer how they were made.

The art of glaze-making in China and Korea was quite advanced by the period of 900–1200 A.D., according to Vandiver. "All the glazes were heated for long periods of time to cause the nucleation and growth of crystals," she says. The trick was to heat the raw materials until they were almost liquid so that crystals would grow. In both China and Korea, craftsmen produced a celadon glaze that was meant to look like jade, but their approaches to this goal were quite different.

"One Chinese technique was to grow both anorthite (a calcium feldspar) crystals and wollastonite (a calcium silicate) crystals in the bulk of the glaze and to grow only anorthite crystals at the back of the glaze—at the glaze-body interface," she says. "It was an intuitively based technology to give a very special visual effect." The final result is a high-quality translucent glaze. Chinese craftsmen attained this effect by grinding their raw materials very coarsely and then mixing them prior to heating.

The Korean technique differs in two major ways—the raw materials are different and they ground them much finer. "The Koreans did not nucleate and grow crystals," says Vandiver. "Instead, they milled very fine particles of quartz and magnetite (an iron oxide) and mixed them." Then, like the Chinese, they created bubbles in the glaze that scatter the light and brighten the glaze. Korean glazes from the 10th to the 14th century contain more lime than the Chinese celadon glazes, and the layers of the Korean glazes are thinner. A remaining mystery, however, is whether the Chinese adapted their glaze-making technique from the Koreans or vice versa.



Freer Gallery of Art, Smithsonian Institution

Korean pottery and microscale view of the glaze. *Pottery (left) from the 10th to 13th century is covered with a celadon glaze. Backscattered scanning electron micrograph (right) of celadon glaze shows quartz and magnetite inclusions (light, upper layer) covering glass stoneware body (below). Magnification, ×140.*

Robert Muggli of McCrone Associates, Inc., in Westmont, Illinois, specializes in analyzing micrometer-sized particles from art objects and other materials. He has developed a technique for manipulating picogram (a million times smaller than a millionth of a gram) quantities of a sample. "We handle the samples for scanning electron microscopy (SEM) on fine tungsten needles," he says. "Then we use a drop of nitrocellulose dissolved in amyl acetate to adhere the particle to the substrate, so when the electron beam hits this very small particle it doesn't jump off the glass slide."

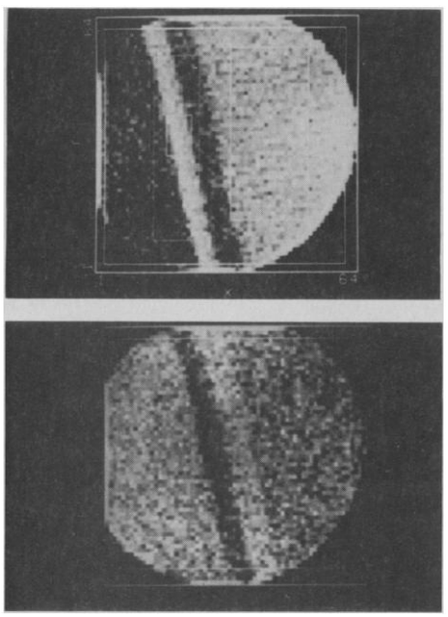
These manipulative techniques allow Muggli to use SEM for analyzing substances present in the wood pulp of paper for antique maps, for instance. "The ancillary materials present in wood pulp help to date the paper," says Muggli. For example, the presence of titanium dioxide pigments would signal a recent rather than an old map, because this pigment was not used until after 1925, he says.

Muggli can also determine the molecular composition of a sample from tiny particles of it. For example, when the authenticity of an Indian doll was questioned, Muggli analyzed the composition of particles of pigment by Raman spectroscopy and found that it was made of materials that were available to craftsmen at that time. He stresses that these methods do not date a sample per se, but that like the optical analysis of paper, molecular analyses make it possible to determine if the composition of the sample is appropriate for the period in question.

Surfaces: Up Close and Personal

In keeping with a major trend evident at PitCon '88, chemists who analyze the surfaces of biomaterials can now assay smaller and smaller areas. "MicroESCA™ [electron spectroscopy for chemical analysis] gives the elemental composition of the top few monolayers and the chemistry of a surface," says Donna Bakale, a consultant to Kevex Corporation, whose division of Surface Science Instruments in Mountain View, California, makes MicroESCA™. As yet MicroESCA™ is only being used for experimental purposes, but the technique can determine the kinds of elements and the chemical bonds they form—carbon-carbon versus carbon-oxygen bonds, for example—in a surface area of a few microns diameter. (One micrometer is one-millionth of a meter.)

Until very recently, the smallest area that surface chemists could analyze was about 150 micrometers in diameter, approximately 5 to 2000 times larger than the area that can now be evaluated by MicroESCA™. "Standard ESCA is done by putting an x-ray beam on a sample and looking at the energy of the emitted electrons," says Bakale. "ESCA measures the total number of electrons of a given energy. The primary difference with MicroESCA™ is in the way the electrons are detected. In this new technolo-



Images from MicroESCA™. *Images generated from aluminum (top) and silicon (bottom) show a field of view 300 micrometers in diameter and resolve metallization lines 20 micrometers wide. The Stanford Synchrotron Radiation Laboratory used Surface Science Instruments' MicroESCA™ to generate these images.*

gy, we now use a superconducting magnet to maintain the relative positions of the emitted electrons. The electrons are then detected in an image. As yet, the process requires synchrotron radiation as the energy source."

In general, ESCA is a tool for analyzing the chemistry of surfaces of biomaterials such as a vascular prosthesis or a plastic tissue culture dish. For the former, the goal is not to have blood components stick, because clotting would result. For the latter, the objective is to get cultured cells to adhere to the substrate. By analyzing the polymer surface with ESCA, a researcher can judge whether the chemical properties are appropriate, and then modify the surface if necessary. ESCA can be used before and after the modification to see if they were successful, which in the examples given above, should predict the appropriate stickiness or nonstickiness of the surface on a microscopic scale.

The new MicroESCA™ greatly increases the spatial resolution of the company's previous ESCA technology. Kevex projects that MicroESCA™ will become a laboratory instrument for studying certain basic phenomena in physics and materials science. To date, the only installed MicroESCA™ is at the Stanford Synchrotron Radiation Laboratory in California. The current estimated price of the total system is $650,000 to $750,000.

Robot War Weeds Out Competitors

While lacking the personality of R2-D2 or C3PO, the second generation Zymate laboratory robot is much more practical. Zymark, of Hopkinton, Massachusetts, fashions this year's robot as the center of an integrated pie (or "PY," as the company calls it) of instruments that separate and analyze drugs in the blood, measure drug dissolution in the stomach, or pesticide residues in the environment. Meanwhile, remaining robot rival Perkin-Elmer has a model that is faster and stronger, but their robot's application to laboratory operations is less well defined.

Zymark has elaborated its new entry from last year—"PyTechnology," which allows a single robot arm to perform different tasks from its central position in a circular array of instruments. This year, the company is selling the total systems approach to robotized automation. For example, one system can be used "to look at the amount of drug and drug metabolites in blood or urine," says Zymark's director of marketing, Richard Brown. The system is targeted toward pharmaceutical companies for the early stages of clinical testing of a new drug.

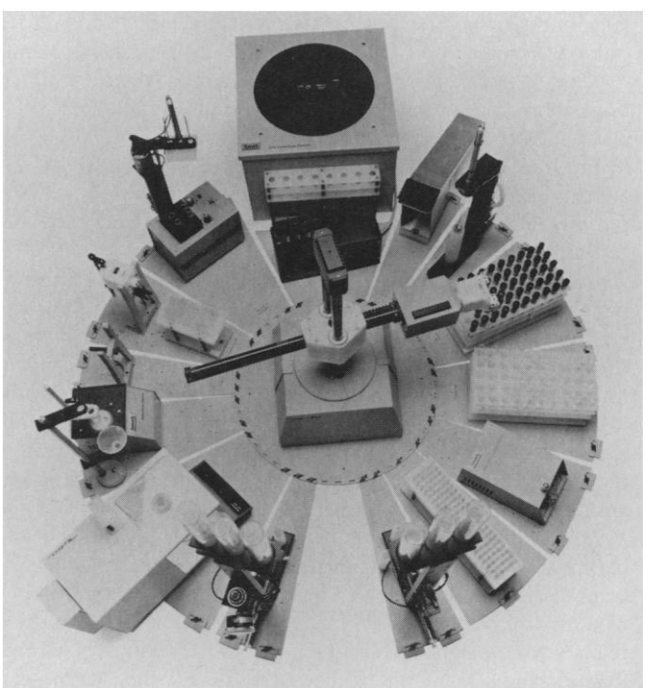
The new devices on Zymark's automated system for extracting drugs and metabolites from biological fluids include a cooled input rack for chilling and storing samples, a solid-phase extraction station where the drug is extracted from the serum sample, and a filtering process for the sample prior to its analysis by high-performance liquid chromatography (HPLC). Other companies collaborate with Zymark to supply components of the system—Mettler contributes an analytical balance; Analytichem International makes the extraction column; and Gelman Sciences has developed the stackable, disposable filters.

Marching to a different drummer, Perkin-Elmer introduces MasterLab II, a demonstration robot arm that articulates more like the human version. It twists, turns, and tracks back and forth on its own runway, ringing a bell, catching a ball as it rolls down a ramp, and tracing its own outline of a house on a piece of paper. These tricks show off the robot's precision and speed, say company spokesmen. The robot is designed to work alone in a room by itself and do repetitive boring jobs. The workhorse model may be suited for industrial tasks, because it can lift up to 6 pounds. The robot can also be programmed to place and remove objects along an angular path as well as position them along a horizontal or vertical axis. But the robot's ability to perform specific laboratory tasks was unclear at the meeting.

Zymark's price-tag for a core robot and its controller is $29,000 and the entire automated system (not including the HPLC) is approximately $70,000. The robotized system will process 100 samples in less than 24 hours. Perkin-Elmer's unit costs $29,000 for the robot arm alone. Zymark and Perkin-Elmer were the only two companies showing laboratory robots at PitCon '88.

Laboratory robot

Zymark's robot arm (center) is surrounded by work stations for extracting drugs and metabolites from biological fluids.



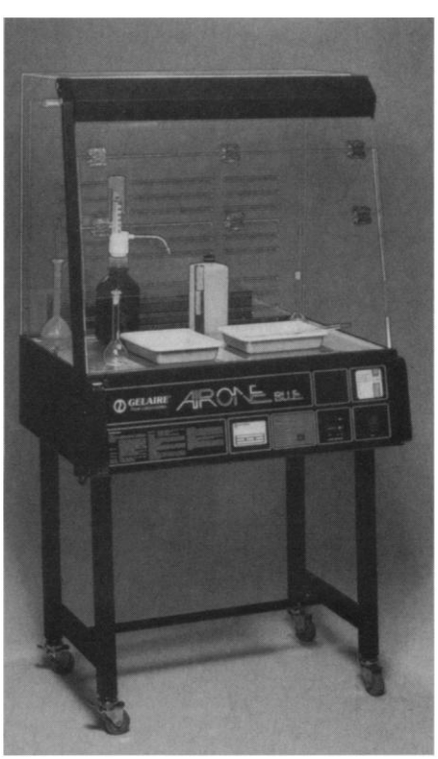
Ductless Fume Hood Reaches the U.S.

An ever-present concern in chemistry laboratories is protecting workers from toxic fumes. The usual solution is to handle all volatile liquids inside fume hoods, which are typically equipped with a ventilation system that draws the fumes toward the back of the hood and out of the room through air ducts. But installing ducts is not always a trivial task, especially if the laboratory is at the bottom of an old or especially tall building. Now, chemists can celebrate the advent of the ductless fume hood—it is designed in Italy and imported this year for the first time to the United States.

Flow Laboratories, an internationally based marketer of fume hoods *with* ducts, is now offering the ductless AIRONE work station, a fume hood made by Gelaire in Milan, Italy. "With our system, we can dispose of volatile toxins such as formaldehyde at the workplace," says George Sarossy, a consultant to Flow. The key component is a Gelaire filter that contains activated carbon from ground shells of coconuts that grow only in Sri Lanka. "This material is far better than any other mineral carbon available," says Sarossy. It is also used in the masks worn by the military to protect them from nerve gases, he says.

In the new fume hoods, air flows horizontally over the work surface. Vapors, fumes, and particles are trapped in the filter, which is located under the work surface. "Although this is a ductless system, it can be used in a variety of ways," says Sarossy. "It can be hooked up to an existing air duct. However, it pays to listen to our advice and use the filters."

Flow is offering 14 different kinds of filters for the new hoods. Each is specifically designed to capture and retain a particular toxin (the Cl-200 filter is for formaldehyde), or combinations of toxins (the Cl-400 filter is for sulfur dioxide, sulfuric acid, hydrochloric acid, and other inorganic acids). Many of the filters are impregnated with substances to make them maximally absorbent or to convert absorbed toxins to nontoxic, stable compounds. But laboratory workers must monitor the filter and change it when it becomes saturated. Flow is selling three models of the ductless fume hood, one of which is mobile and targeted at high school or university chemistry laboratories. The hoods cost from $2500 to $4500.



Ductless fume hood. *Gelaire's AIRONE fume hood has filters designed for specific toxins.*

Capillary Electrophoresis: Anticipating the State of the Art at an Early Stage

Emerging from the collection of current techniques used to separate macromolecules from a mixture lies the utility of capillary electrophoresis (CE). Excitement about CE and use of the technique—to identify the active compound used in an experimental cancer therapy, for instance—have grown exponentially this year. But as yet the method lacks a marketed instrument, leaving researchers who do CE to fabricate their own equipment. Several companies, including Beckman Instruments in Palo Alto, California, Microphoretics in Sunnyvale, California, and Applied Biosystems in Foster City, California, are likely to change that within the next few months, as their first CE products reach the marketplace.

"The methodology for capillary electrophoresis is still in a very developmental stage," says James Jorgenson of the University of North Carolina at Chapel Hill. "It has become an instrumental approach to electrophoresis in the same sense that HPLC [high-performance liquid chromatography] is an instrumental approach to chromatography." Like HPLC, but unlike standard electrophoresis technology, the capillary electrophoresis system can easily be automated for the delivery of samples and also for the collection of fractions after the compounds in the sample have been separated. In addition, CE can separate very small quantities of sample from a tiny volume—currently the system resolves a billionth of a gram of sample from a billionth of a liter of fluid.

Another major advantage of CE is its speed, according to Victoria McGuffin of Michigan State University in East Lansing. The speed with which a sample moves through the capillary depends on how much voltage is applied, which in CE is 20,000 volts or more. Very high voltages would be a problem in standard electrophoresis because a great deal of heat is generated, but in CE the thin capillary tubing quickly dissipates the heat away from the sample. Thus, sample migration and separation can take anywhere from seconds to 30 minutes with CE. A typical run, says McGuffin, usually takes about 10 minutes, which is much faster than standard gel electrophoresis.

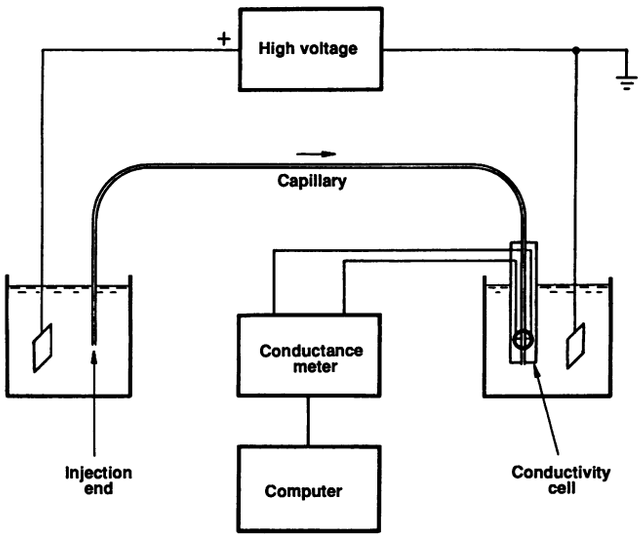
"Capillary electrophoresis is an incredibly simple technique," says Richard Zare of Stanford University in California. Typically, the system consists of a thin, open, fused-silica capillary tube (10 to 100 centimeters long with an inner diameter of 10 to 100 micrometers) that runs between two reservoirs, each of which contains an aqueous buffer solution or organic solvent. The setup also includes a power source, a capillary electrophoresis "cell," a detector, and a computerized system to store and analyze data.

The researcher introduces a sample (containing a mixture of proteins or peptides, for example) into the reservoir that also contains the anode from the power source. Thirty thousand or so volts are applied, which drives different compounds through the capillary at different speeds. Each compound passes as a narrow zone or band through a detector, which is positioned near the opposite end of the capillary. "You are detecting the sample as the ions go across this part of the column," says Barry Karger of Northeastern University in Boston. The resolution of each zone is directly proportional to the amount of voltage applied.

There are two major disadvantages of capillary electrophoresis, according to Jorgenson. One is the problem of sample detection. Current detection methods include ultraviolet absorption—tagging the sample with a fluorescent marker as it migrates out

A capillary electrophoresis system

A sample is injected into one reservoir (left). During separation, it moves through the capillary at a speed that is proportional to the amount of voltage applied (top). In this sample system, the detector (right) is a conductivity cell. [Adapted from X. Huang, M. Gordon, R. Zare, J. Chromatogr., in press]



of the capillary and then detecting the fluorescence—and conductance detection—which is good for detecting anything that carries a net charge.

The second problem is that proteins moving through the glass capillary tube tend to stick to it. This retards their flow and creates a problem called zone broadening, meaning that the compound migrates and is detected as a broad rather than a narrow band. "One approach to this problem is to run proteins at a *p*H that is above their isoelectric point," says Jorgenson. But this limits the *p*H range over which researchers can work. Another solution, which Jorgenson thinks is probably the best so far, is to coat the inner surface of the capillary tube with very thin layers of polymer. "Then the migration of the proteins depends only on their size and charge," he says.

Capillary electrophoresis has diverse applications and it can be used to separate tiny ions, large proteins, subcellular components, or even whole cells. For example, Zare and his colleagues use the technique to monitor levels of lithium, a small metal ion, in the serum of a patient receiving it for manic depressive illness. It is important to monitor lithium levels closely, because a serum concentration that is three to four times over the therapeutic range can be dangerous. McGuffin and her co-workers use CE to separate a hematoporphyrin derivative used to treat cancers that have spread throughout the body. Although the Michigan State researchers themselves do not treat cancer patients, they are trying to identify which compound is active in this experimental therapy.

In patients, the derivative of hematoporphyrin (which essentially is a red dye) preferentially binds to rapidly dividing tumor cells and a laser beam can then be used to destroy the labeled cells. Karger and his collaborators use a slightly different approach to separate proteins such as myoglobin, peptides, and oligonucleotides. They load the capillary tubing with SDS-polyacrylamide gel instead of buffer. As it does in standard electrophoresis, this material acts as a molecular sieve and aids in the separation process.

Since the late 1970s and early 1980s, when researchers described electrophoresis in very thin capillary tubes, a small group of them has continued to explore the capabilities of the technique. But this year, because enthusiasm about and use of CE has increased dramatically, private industry has an added incentive to develop a marketable instrument. According to Robert Brownlee of Microphoretic Systems, the first commercial instruments for capillary electrophoresis should be available in May of this year.

Automated Analyzer Seeks Out Sulfur

Instruments at this year's PitCon are not only designed to analyze smaller samples, they are also designed for more automation. An example is MCTS-130/120, the sulfur and chlorine analyzer from the Dohrmann® Division of Rosemount® Analytical in Santa Clara, California. Too much sulfur is bad when it comes to refining petroleum, and many steps of the process require analyzing both the sulfur and chlorine content of oil. This year's version of the company's sulfur/chlorine analyzer features increased automation of the sampling process.

Precious metal catalysts are needed in the "reforming" process of a petroleum product—to increase the octane of a gasoline, for example. But oil contains sulfur. "Sulfur actually embeds onto and degrades the platinum so it will no longer reform hydrocarbons," says John Martin of Dohrmann®. So a major concern of petroleum refiners is to remove as much of the sulfur as possible. And doing that means that they must constantly analyze the levels of sulfur.

The MCTS-130/120 sulfur and chlorine analyzer has an autosampler that holds up to 42 samples. These are fed, one at a time, into a small boat that carries them to the instrument's furnace where they are heated to over 700°C. There, organic sulfur is converted to sulfur dioxide and chlorine compounds are converted to chloride ions. The two elements are analyzed separately by a coulometric titration cell, and an automatic integration system determines the concentration of one of them within 3 to 5 minutes per sample. The system is run by the operator via a control module. The basic analyzer is $18,400 and the complete system is approximately $25,000. ■

DEBORAH M. BARNES

Sulfur analyzer

Dohrmann's® MCTS-130/120 system quantitates sulfur and chlorine in petroleum products. The system vaporizes the sample and measures sulfur and chlorine separately in a coulometric titration cell.