Kirk-Othmer Encyclopedia of Chemical Technology.

ANALYTICAL METHODS, TRENDS

Analytical chemistry, the science of the measurement and characterization of systems, is being revolutionized by the rapid advances in computer technology (qv), microelectronics, and materials science (qv). New techniques in chemical analysis, as well as significant developments in more established instrumentation and methodology, have resulted in the production of more analytical information of higher quality in less time than ever before. These developments are being driven by five separate factors: (*1*) the quest for truly automated or intelligent instruments (see Expert systems); (*2*) the continued development of multidimensional hyphenated instruments, which involve the marriage of two instruments having complementary capabilities by way of a sampling interface and a common computer, thus facilitating the analysis of complex samples (see Analytical methods, hyphenated instruments); (*3*) continuous improvements in analyte detection limits bringing single atom analysis closer to reality; (*4*) increased capabilities in data manipulation, miniaturization of hardware, and remote *in vivo* sensing, leading to new instrumentation capable of both separation and specification; and (*5*) the development of nondestructible, stable, field-operable instrumentation utilizing fiber optic technology to serve as in-field sampling devices and process control (qv) tools (see Fiber optics).

1. Intelligent Instruments

The use of "fixed" automation, automation designed to perform a specific task, is already widespread in the analytical laboratory as exemplified by autosamplers and microprocessors for sample processing and instrument control (see also Automated instrumentation) (1). The laboratory robot originated in devices constructed to perform specific and generally repetitive mechanical tasks in the laboratory. Examples of automation employing robotics include automatic titrators, sample preparation devices, and autoanalyzers. These devices have a place within the quality control (qv) laboratory, because they can be optimized for a specific repetitive task. Application of fixed automation within the analytical research function, however, is limited. These devices can only perform the specific tasks for which they were designed (2).

Preferable in the analytical laboratory is "flexible" automation, ie, automation which can improve analysis efficiency, remotely control processes, automate analytical problem solving, and link other flexibly automated or intelligent instruments together in decision making. Instrumentation that has the ability to select an appropriate method, schedule a work program, develop a chemical method, self-optimize, do pattern recognition and decision analysis, fault alarm and self-diagnose, and repair itself is considered to be intelligent (3).

Microelectronics and microcomputers have made it possible to build flexible automated devices (4) leading to the introduction of programmable laboratory robots, which can perform a sequence of tasks within the domain of their mechanical capabilities. Developments in programmable automated instrumentation have turned into a launching platform for a fleet of new sophisticated instruments which are expected to appear in the marketplace during the 1990s (5). Changes at every stage of the analytical process are foreseen, ranging from sample preparation to analytical techniques and on into data interpretation.

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There is a trend in the marketplace toward increasing instrumental flexibility through designing multitask capability. One way this increased versatility can be implemented is through the characterization end of an instrument, ie, through the sampling system and procedures, to the signal channel, and onto the detector/transducer. Another way is through the instrument processing module, which includes the amplifier, processing functions, and readout. It is in this module that the next step in bringing the laboratory robot toward its full potential is expected to take place.

In the 1990s robotics guided by artificial intelligence are expected to play a role comparable to that of electronics in the 1940s and 1950s (6). Expert systems, which are knowledge-based systems that can effectively represent and apply factual knowledge in specific areas of human expertise, seem ideally suited to robot supervision.

Another step in laboratory automation to be achieved is the conversion of standard chemical procedures such as titrations or thermal gravimetric analysis, into unit laboratory operations. A procedure could then be selected from these laboratory operations by an expert system and translated by the system to produce a set of instructions for a robot. The robot should be able to obey specific instructions, such as taking a specified sample aliquot and titrating it using a specified reagent.

Evidence of the application of computers and expert systems to instrumental data interpretation is found in the new discipline of chemometrics (qv) where the relationship between data and information sought is explored as a problem of mathematics and statistics (7–10). One of the most useful insights provided by chemometrics is the realization that a cluster of measurements of quantities only remotely related to the actual information sought can be used in combination to determine the information desired by inference. Thus, for example, a combination of viscosity, boiling point, and specific gravity data can be used to a characterize the chemical composition of a mixture of solvents (11). The complexity of such a procedure is accommodated by performing a multivariate data analysis.

Near infrared reflectance analysis (nira) is an example of an approach to automated characterization and intelligent instrumentation (12–15). In nira, which is used to examine complex samples, the resulting spectral pattern is heavily overlapped and spectral differences resulting from composition are barely observable. However, instead of resorting to conventional data reduction procedures, a combination of spectral correlation and a "self-teaching" algorithm is used. The procedure involves first measuring a set of preanalyzed samples, cross-correlating the spectra to composition using multilinear regression analysis, and using an optimization algorithm to select a set of measurement wavelengths and calibration coefficients for performing the analysis. Nira is capable of providing approximately 0.1% reproducible analysis utilizing spectral data in which gross compositional differences are barely observable. The power of such self-optimizing procedures, using the entire data set, goes much further than merely performing difficult measurements. A number of applications of the technique, such as in the analysis of grain and food products (16), have been successful in determining sample composition for which no known spectral feature, or no spectral feature at all, existed.

The ultimate goal of intelligent instrumentation is an automated system capable of accepting any type of sample and performing any analysis and characterization, such that all desired information would be obtained. Whereas such a highly flexible instrument clearly belongs to the future, more limited systems are already being developed along these lines.

2. Hyphenated Instruments

Multidimensional or hyphenated instruments employ two or more analytical instrumental techniques, either sequentially, or in parallel. Hence, one can have multidimensional separations, eg, hplc/gc, identifications, ms/ms, or separations/identifications, such as gc/ms (see Chromatography; Mass spectrometry). The purpose of interfacing two or more analytical instruments is to increase the analytical information while reducing data acquisition time. For example, in tandem-mass spectrometry (ms/ms) (17, 18), the first mass spectrometer

applies soft ionization to separate the mixture of choice into molecular ions; the second mass spectrometer obtains the mass spectrum of each ion.

Gc/ms is an example of a hyphenated instrument consisting of two techniques that provide orthogonal (independent) information. If two independent techniques are linked in tandem, a large number of data resolution elements is obtained from each. The more dimensions present, and the higher the available resolution along each dimension, the greater is the obtainable information (19). Because hyphenated systems generate an immense amount of data, serious hardware and software constraints are imposed on the data system. In addition, data processing systems also require enormous databases (qv) for rapid automatic data interpretation. Many of these databases are commercially unavailable and there is an obvious need for improvements in this area throughout the 1990s.

The use of pattern recognition (20, 21) in automatic data interpretation provides an alternative to the development of huge databases. Most qualitative analysis procedures operate by recognition against databases, which therefore need to contain information about hundreds of thousands of compounds. Another approach involves the use of software to make molecular structure determinations from data through fundamental laws of physical chemistry and spectroscopy. This method allows qualitative data interpretation to become mostly independent of large databases. The difficulty lies in the extreme complexity of the programming, which involves the use of advanced artificial intelligence.

Hyphenated instrumental techniques that are commercially available include: gas chromatography/mass spectrometry (gc/ms) (22–25), liquid chromatography/mass spectrometry (lc/ms) (26, 27), inductively coupled plasma/mass spectrometry (icp/ms) (28, 29), and liquid chromatography/gas chromatography (lc/gc) (30). Among other techniques that are expected to become popular in the near future are capillary zone electrophoresis/mass spectrometry (cze/ms) (31, 32), which is particularly suited for biological samples; gas chromatography/atomic emission spectroscopy (gc/aes) (33), a low cost technique for trace metals analysis; electrophoresis/fluorescence (34), a combination of flow photometry and capillary electrophoresis technology; and liquid chromatography/nuclear magnetic resonance (lc/nmr) (35), a nondestructive technique for multicomponent analysis (see Nondestructive testing).

The possibilities for multidimensional instrumental techniques are endless, and many other candidate components for inclusion as hyphenated methods are expected to surface as the technology of interfacing is resolved. In addition, ternary systems, such as gas chromatography-mass spectrometry-infrared spectrometry (gc/ms/ir), are also commercially available.

Hyphenated analytical methods usually give rise to increased confidence in results, enable the handling of more complex samples, improve detection limits, and minimize method development time. This approach normally results in increased instrumental complexity and cost, increased user sophistication, and the need to handle enormous amounts of data. The analytical chemist must, however, remain cognizant of the need to use proper analytical procedures in sample preparations to aid in improved sensitivity and not rely solely on additional instrumentation to increase detection levels.

3. Extended Limits of Detection

Ultrasensitive detection capability is becoming more important in high purity materials development, geological and geochemical studies, and biological and environmental areas (see Trace and residue analysis). As a result, an ongoing task for the analytical chemist is to establish the smallest concentration at which a given substance can be detected. Previously, the more sensitive spectroscopic techniques enabled scientists to detect specific atomic and molecular ingredients of a sample at nanomolar (10^{-9} molar) levels. Improvements in sample inlet systems and in spectroscopic detection enabled picomolar (10^{-2} molar) quantities to be detected. The implementation of techniques such as radioimmunoassay and potentiometry allows for femtomolar (10^{-15} molar) detection, which represents millions of molecules (see Immunoassay).

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As femtomolar detection of analytes become more routine, the goal is to achieve attomolar (10^{-18} molar) analyte detection, corresponding to the detection of thousands of molecules. Detection sensitivity is enhanced if the noise in the analytical system can be reduced. System noise consists of two types, extrinsic and intrinsic. Intrinsic noise, which represents a fundamental limitation linked to the probability of finding the analyte species within the excitation and observation regions of the instrument, cannot be eliminated. However, extrinsic noise, which stems from light scattering and/or transient electronic sources, can be alleviated.

The use of laser (qv) radiation as an excitation source has had a major impact on analytical chemistry (36–39). A powerful, coherent, monochromatic laser source can serve a variety of applications, including sample-induced fluorescence and chemiluminescence (qv). Because ionization efficiency is high within the laser probe zone and because all ions can be collected and efficiently measured, the sensitivity of the method is high. Also, because ionization depends on resonant transitions of the analytes, a relatively high degree of selectivity is obtained.

An example of the use of lasers in optimizing analyte detection is provided by the technique of laser excited atomic fluorescence spectroscopy (leafs). The detection of subfemtogram amounts of cadmium, thallium, and lead has been reported (40). In this experiment, the sample of interest is first volatilized in a plasma (see Plasma technology) and then tuned photons from one or two dye lasers excite the analyte. When these atoms or ions relax, the resulting fluorescence signal is shunted into a photomultiplier for detection. Attomole detection levels are achievable using this technique. Continued advances in complex, multilaser spectroscopic determinations are expected to result in even lower levels of detection.

It is well known that fluorescence is one of the most sensitive techniques for chemical analysis (see Spectroscopy). A molecule can undergo many excitation-emission cycles before it photochemically degrades. An application using laser fluorimetry with capillary zone electrophoresis has been reported to analyze amino acids at the attomolar range (41). Fluorescence analysis is made possible by employing the fluorescein [2321-07-5] isothiocyanate (fitc) derivative of a target amino acid. In this study, the analysis of a synthetic mixture of some 18 amino acids in the form of their fitc derivatives was performed. Detection limits ranged from 1.5×10^{-19} mol for lysine, to 9×10^{-21} mol for arginine. The extreme sensitivity and high resolving power of the method apparently led to the amino acid sequence determination of even minute quantities of proteins.

Another example of reducing the measurement volume to enhance detection sensitivity involves the observation of a single solution microdroplet (42). Micrometer-sized droplets can be generated on demand. These droplets have a high surface charge and thus can be trapped in an electrodynamic trap which holds the droplet stationary in space to within a fraction of its diameter. A laser beam is then employed to excite fluorescent molecules inside the droplet. The resulting fluorescence is measured by a photomultiplier assisted by supporting optics and electronics (see Photodetectors). The microdroplet approach offers such significant detection advantages as: independent control of the time of measurement, less diffusion of analyte from the analysis volume than in a flowing stream, and a possibly shortened fluorescence lifetime for a molecule confined to a droplet. The droplet may also act as an optical cavity, influencing the dynamics of photon emission and absorption. Reducing fluorescence lifetime can result in more signal photons per molecule and thus increased detection. Results indicated a detection limit in the range of 10^2 to 10^3 molecules assuming a signal to noise ratio of 2. Further improvements in detection may even be attained by measuring smaller droplets which would further reduce the background signal.

An alternative approach to trace analyte detection results from the measurement of chemiluminescence in a laser-generated plume of plasma, formed when the laser beam evaporates a small amount of sample (43). In these experiments, a pulsed excimer laser-induced-plasma, formed by laser vaporization and ionization, is probed directly to measure ion intensity. Ground state sodium atoms, excited state copper atoms, and sodium dimer molecules have all been monitored using this technique. This laser enhanced ionization may well be one of a very few techniques which can be used to probe extremely dense plasmas with good spatial and temporal resolution.

In addition to direct ion-measurement studies, the use of chemiluminescence as a detection method has also been investigated for elemental analyses using the laser microprobe (44). The chemiluminescence is produced by the reactions of analyte atoms formed in the laser plume with an ambient gas reagent. The chemiluminescence can then be measured directly, without either additional excitation or sample transfer, thus providing a simple and potentially quite sensitive method. This technique has been applied to studies of silicon, germanium, aluminum, and copper atoms in reactions with fluorine or fluorine-containing compounds. In the case of reaction between silicon and sulfur hexafluoride [2551-62-4], the limit of detection for silicon was approximately 10 picograms. Whereas this level of sensitivity is not as good as that which can be obtained by atomic emission techniques, continued reductions in background noise levels permit lower detection levels to be obtained.

Still another ultrasensitive detection method is provided by indirect detection of analytes (45, 46). In indirect analysis an instrument does not respond to the presence of the species of interest, but rather monitors a decrease in the larger background signal as a means of detecting the presence of the analyte. Hence, one is actually measuring a negative response, rather than a positive one as in an emission peak. An example of this technique was demonstrated using microbore high pressure liquid chromatography, utilizing a polarimeter as a detector in the separation of octyl, allyl, and nonylphthalate plasticizers (qv). Using an optically active mobile phase containing limonene [138-86-3], $C_{10}H_{16}$, the three phthalates can be separated and quantitated down to part per trillion levels by observing the large shift in optical rotation exhibited by the optically active mobile phase as the background signal. The extension of this technique to more sensitive forms of detection, such as fluorescence, is expected to result in improvements in detection sensitivity (to attogram levels) as well as dynamic range.

The extreme sensitivity and high resolving power of trace analysis techniques encourage the quest for single atom detection.

4. New Instrumental Techniques

Continued advances in analytical instrumentation have resulted in improvements in characterization and quantification of chemical species. Many of these advances have resulted from the incorporation of computer technology to provide increased capabilities in data manipulation and allow for more sophisticated control of instrumental components and experimentation. The development of miniaturized electronic components built from nondestructible materials has also played a role as has the advent of new detection devices such as sensors (qv). Analytical instrumentation capabilities, especially within complex mixtures, are expected to continue to grow into the twenty-first century.

4.1. Multidimensional Nmr Spectroscopy

An example of increased experimental capability through advances in data manipulation procedures is demonstrated with the advent of multidimensional nuclear magnetic resonance (nmr) techniques (see Magnetic spin resonance) (47–50). By means of clever, sophisticated pulsed radio frequency excitation techniques, and advances in both computer hardware and software technology, it is possible to excite normally weak, multiple quantum transitions and to record nmr spectra in more than one dimension. The primary benefit derived from two-dimensional (2-D) nmr is confirmational information from spectral data which is lost in a conventional proton noise decoupled carbon-13 nmr experiment. In the 2-D case, spectra appear as contour maps where different types of interactions are spread out as resonances along two axes. In addition to the characteristic frequency shifts caused by nearest neighbors, the new dimension reveals more distant interactions. Thus conformational information can be determined for complex molecules without obtaining single crystals and x-ray

structures. Multidimensional nmr is especially useful for biological molecules because it can give access to conformational information under conditions comparable to the *in vivo* environment.

The application of two-dimensional nmr techniques, the availability of high field (600 MHz) spectrometers, and the utilization of high speed mathematical and computational algorithms have enabled the advent of three-dimensional (3-D) nmr techniques for protein structure determination in solution. The resulting 3-D nmr spectrum is increased in complexity by another order of magnitude and structural analysis is extremely tedious and time consuming. Traditionally, nmr parameters for structural analysis involve the determination of chemical shifts (or ring current shift calculations), paramagnetic relaxation effects, vicinal coupling constants, and nuclear overhauser enhancement (NOE) effects. NOE arises from the fact that if two protons are separated by less than 0.5 nm, they can exchange magnetization with each other. This cross relaxation rate is proportional to $1/r^6$, where r is the internuclear distance. Hence, by perturbing the magnetization of one nucleus and watching its effect on the other, approximate internuclear distances can be obtained.

For a macromolecule such as a large protein, the steps in characterization involve, first, identification of the spin systems present, using correlated spectroscopy, and identification of neighboring amino acids. The long range noes are then assigned, and three bond coupling constants are determined. The secondary structure elements are then identified, and finally, the three-dimensional protein structure is obtained from the measured interproton distances and torsion angle parameters. This procedure requires a minimum of two days of nmr instrument time per sample, because two pulse delays are required in the 3-D experiment. In addition, approximately 20 hours of computing time, using a supercomputer, is necessary for the calculations. Nevertheless, protein structure can be assigned using 3-D nmr and a resolution of 0.2 nanometers is achievable. The largest protein characterized by nmr at this writing contained 43 amino acid units (51). However, attempts are underway to characterize the structure of interleukin #2 [85898-30-2], which has over 150 amino acid units.

4.2. Chemical Microsensors

Microsensor technology holds the promise of developing highly sophisticated chemical detectors which can both qualitatively and quantitatively quickly analyze the immediate environment for a variety of chemicals in spite of potential interferences at extremely low levels. Microsensor technology has already started to move away from the use of simple probes or electrodes coupled with chemically sensitive membranes, toward fully integrated solid-state sensing devices which have all of the electronic components etched on a single integrated chip.

A chemical microsensor can be defined as an extremely small device that detects components in gases or liquids (52–55). Ideally, such a sensor generates a response which either varies with the nature or concentration of the material or is reversible for repeated cycles of exposure. Of the many types of microsensors that have been described (56), three are the most prominent: the chemiresistor, the bulk-wave piezoelectric quartz crystal sensor, and the surface acoustic wave (saw) device (57).

Chemiresistors, as the name implies, are chemically sensitive devices that detect a change in the electrical resistance of a coating on the surface of a microsensor. The change occurs when the microsensor is exposed to a vapor-borne challenge. Solid-state chemiresistors are enjoying widespread applications in analytical laboratories. Some employ enzymes; others use ion-conducting polymers. Some chemiresistors are nonintegrated devices, ie, only the integrating device is on the chip; other chemiresistors are integrated sensors that consist of the detecting device plus various electronic components on the chip. Research in the area of chemiresistors is expected to aid any process requiring rapid, *in situ* identification and monitoring of chemical or biological species.

Bulk-wave piezoelectric quartz crystal sensors indirectly measure mass changes of the coating on the surface of the sensing device. This change in mass causes changes in the resonant frequency of the device, and measurements are based on frequency differences.

Surface acoustic wave (saw) devices are finding increasing use as chemical sensors and in a variety of applications. A saw device is a quartz plate having electrodes attached to its surface. Although they are similar to bulk-wave piezoelectric devices in that the mode of detection is based on a mass change of the coating on the surface of the device, the saw sensor operates using waves of higher frequency and, consequently, having greater sensitivity. Saw devices also possess several other advantages over bulk-wave piezoelectric sensors; including a greater ease of coating, uniform surface mass sensitivity, and improved ruggedness. Traditionally, the saw device has been used as a chemical vapor sensor responding to changes in mass, or in the conductivity of a thin film on its surface, caused by reaction with the impinging vapors. More recent experiments (58) indicate that the saw also responds to changes in the elastic properties of surface films. Thus these sensors can also be used to characterize solids such as polymeric materials.

In addition to chemical microsensors, studies indicate that sensors for biological and biomedical species, as well as other complex molecules that cannot be easily detected, should be commercialized in the 1990s (59). Biosensors (qv) utilize natural products, such as enzymes, cells, microorganisms, or plant tissue. Biosensors basically come in three types: immunological, biocatalytic, and sensory. Immunological biosensors take advantage of immunoreactions between immobilized antibodies and an antigen, bringing about cell lysis and allowing a detectable marker to escape from the antigen. Biocatalytic and sensory biosensors, however, provide measurement capability for a wider variety of complex molecules.

4.3. *In Vivo* Biosensing

In vivo biosensing involves the use of a sensitive probe to make chemical and physical measurements in living, functioning systems (60–62). Thus it is no longer necessary to decapitate an animal in order to study its brain. Rather, an electrochemical biosensor is employed to monitor intercellular or intracellular events. These probes must be small, fast, sensitive, selective, stable, rugged, and have a linear response. The microdialysis sampling process which allows the monitoring of small molecules in circulation within an animal, is an example. An artificial capillary is placed in the tissue region of interest, and a sample is collected via dialysis. In the case of a laboratory animal such as a rat, a probe is placed in the jugular vein under anesthesia. Flow rates are of the order of 1 μL/min.

The advantages of *in vivo* biosensing are that the monitoring occurs inside a living species. Sampling rates are high, multiple analytes can be measured in the same location at the same time, and a wide range of analytical techniques can be employed for monitoring purposes. Concerns resulting from using this approach are that the living system is being perturbed for the measurement process, ie, removing chemicals from a living system disturbs its biology. Additionally, the region being sampled is not clearly defined, and very small volume samples are recovered. Nevertheless, the advantage of not having to sacrifice laboratory animals and the ability to make measurements on living systems make *in vivo* monitoring a potentially attractive assay technique.

4.4. Biomolecule Separations

Advances in chemical separation techniques such as capillary zone electrophoresis (cze) and sedimentation field flow fractionation (sfff) allow for the isolation of nanogram quantities of amino acids and proteins, as well as the characterization of large biomolecules (63–68) (see Biopolymers, analytical techniques). The two aforementioned techniques, as well as chromatography and centrifugation, are all based upon the differential migration of materials. Trends in the area of separations are toward the manipulation of smaller sample volumes, more rapid purification and analysis of materials, higher resolution of complex mixtures, milder conditions, and higher recovery (69).

The mode of operation of cze, a technique which is commercially available, is similar to conventional rod or slab electrophoresis, except that the sample is introduced into an open tubular, 75–100 micrometer, fused-silica

capillary. Use of the capillary enables on-column detection using one or more of a variety of on-line detectors, such as uv/vis, fluorescence, electrochemical, and conductivity. In addition, this technique allows for the usage of higher electric fields than conventional slab gels. Higher applied voltage results in less diffusion and better resolution. A 10^6 theoretical plate efficiency is attainable.

Applications of cze include the detection of trace amounts of DNA and the separation of peptide fragments. Furthermore, this technique is beneficial to forensic scientists because restriction mapping can be performed, allowing assays for DNA to be carried out at the scene of a crime (see Forensic testing). It is also possible to interface capillary electrophoresis on-line with a mass spectrometer as a sample introduction technique in the analysis of amino acids and proteins (70). Further improvements in capillary electrophoresis include the need to increase column capacity. Most reported separations involve the resolution of only 20–30 components, whereas high resolution hplc is capable of resolving several hundred components in a mixture (see Chromatography).

Sedimentation field flow fractionation (sfff) techniques are being implemented for molecular weight characterization of macromolecules (71, 72). Sfff is capable of separations up to 10^{12} daltons and can also accommodate solid particulates. In contrast, gel permeation chromatography (gpc) has a separation range of up to approximately 10^7 amu, and electrophoresis to 10^9 amu. In sfff, an external centrifugal force field is applied to a sample pushing the material of interest against the wall of an empty channel. A laminar flow of solvent, which has a faster rate in the center than closer to the wall, is introduced. Thus, sfff is a separation mechanism which is independent of the shape of the molecule: small solutes elute first, larger ones later. This technique is really a combination of hplc and ultracentrifugation (see Separation, centrifugal). A knowledge of the material's density allows calculation of both mass and particle size distribution.

The sfff technique is versatile: it can be used to study both solids and solutions, no standards are required for calibration, and it is a nondestructive technique. Low shear forces are employed and minimal absorption occurs. Hence, sample degradation does not take place. The technique cannot, however, be routinely used for molecular weight characterization of small molecules. Newer variations of sfff include the development of thermal fff (73), whereby a temperature gradient is placed between two parallel metal blocks to allow the particles to migrate toward the wall. This approach is said to allow for the molecular weight characterization of polymeric materials which are much smaller in molecular size. Advantages of this technique over gpc lie in the absence of conventional band broadening effects.

4.5. Surface Characterization

Most modern techniques for the characterization of surfaces have been developed since 1970 (74, 75). Surface techniques allow for both qualitative and quantitative characterization of trace levels of molecular species (see Surface and interface analysis). Most recently an extension of surface analysis utilizing laser ionization has been introduced (76). In surface analysis by laser ionization (sali), a probe beam, composed of ions, electrons, or laser light, is directed to the surface under examination to remove a sample of material. An untuned, high intensity laser passes close to, but parallel and above the surface. The laser has sufficient intensity to induce a high degree of nonresonant, and hence nonselective, photoionization of the vaporized sample of material within the laser beam. The nonselectively ionized sample is then subjected to mass spectral analysis to determine the nature of the unknown species. A highlight of this technique is the use of efficient, nonresonant, and therefore nonselective photoionization by pulsed untuned laser radiation. The commercial availability of intense laser radiation makes this technique viable. The mass spectrometer, not the laser, performs the chemical differentiation.

An advantage of sali over a conventional surface analysis technique, such as secondary ion mass spectrometry (sims), is the feasibility of simple quantitative measurements. In addition, sali provides high sensitivity, mass resolution, and mass range, as well as a means of low damage analysis of organic surfaces. Sali is a versatile surface tool which can be applied to three-dimensional mapping of integrated circuits (qv), the chemical

analysis of bulk synthetic polymer blends (qv), analysis of near-surface regions in passive films for corrosion prevention, and all kinds of biomedical materials analyses.

5. Analytical Instrumentation for Process Control

It is becoming more and more desirable for the analytical chemist to move away from the laboratory and into the field via in-field instruments and remote, point of use, measurements. As a result, process analytical chemistry has undergone an offensive thrust in regard to problem solving capability (77–79). *In situ* analysis enables the study of key process parameters for the purpose of definition and subsequent optimization. On-line analysis capability has already been extended to gc, lc, ms, and ftir techniques as well as to icp-emission spectroscopy, flow injection analysis, and near infrared spectrophotometry (80). In addition, new technology developments, such as on-line Raman spectroscopy, x-ray diffraction and gc/lc are under commercialization for the 1990s.

One revolutionary *in situ* chemical monitoring method involves the use of a spectrophotomer coupled with fiber optics (qv) that transmit the analyzing light from the analyzer to the in-line sampling point, and back to the analyzer. In short, the fiber optics take the analyzer to the sample rather than the sample to the analyzer (81). No sample pretreatment or modification is required and measurements can be made in real time, under real process conditions of temperature, pressure, and environment. In addition, this technique helps to reduce the common analyzer problems related to sampling systems, eg, maintenance, transport, response times, and modified measurement conditions. Furthermore, the use of fiber optic signal paths, which are intrinsically safe and radio frequency interference (rfi) immune, enables analyte detection at distances as much as several hundred meters away from the sampling point. Detection is also possible at different locations simultaneously. Fiber optic probes have been employed for partice size analysis, uv/vis/nir spectrophotometers, and other photochemical processes, and applications of fiber optics in light scattering, absorption, and fluorescence experiments are in progress (82).

The advantage gained by using fiber optics for spectrophotometry are accompanied by certain limitations. Practical fibers are limited to a spectral range of approximately 200–2500 nm. This range is determined by the absorption characteristics of the silica used in the fiber's core. Although a number of materials have been developed for use further into the infrared, none provides the high transparency, low cost, chemical inertness, and physical toughness required for routine chemical applications.

The development of fiber optics technology, user-friendly displays, and enhanced data presentation capabilities have made on-line analysis acceptable within the plant manufacturing environment. However, it is apparent that a barrier still exists to some extent within many organizations between the process control engineers, the plant operations department, and the analytical function, and proper sampling is still the key to successful process analytical chemistry. The ultimate goal is not to handle the sample at all.

6. Analytical Capabilities

The increased demand for analyses in applications, such as industrial processes, the environment, and health, pushed worldwide sales of analytical instruments from $300,000 to over $3,000,000 during the 1980s (83). U.S. instrument manufacturers have also proliferated.

The analytical chemistry laboratory has successfully completed the transition from wet analysis to powerful and expensive instrumentation, but all problems cannot yet be solved using existing technology. For example, there is and will be an increasing need for more rapid and more definitive methods of isolation, purification, and analysis of proteins and other macromolecules, including advances in nucleic acid sequencing methods. The human genome project is expected to involve the investment of hundreds of millions of

dollars. The development of instrumentation to expedite isolating and mapping the approximately one hundred thousand genes that comprise this gigantic project is a large analytical challenge.

Another vast field for the future is the analysis of organic molecules on surfaces. Whereas surfaces can be examined under high vacuum, in the real world surfaces are in contact with liquids and gases and these cannot be routinely explored using today's technology. Surface enhanced Raman spectroscopy (sers) can provide some of this type of information, but new techniques are needed to ascertain the nature of a species on the surface, eg, what form of arsenic is present and to what organic molecule does it belong. Quantitation of surfaces could result in higher spatial resolution, as well as the ability to map a surface microscopically as a function of both depth and breadth.

The biological and medical sciences are ripe for instrumentation advances. Whereas most immunoassays (qv) use radioactive materials, the implementation of chemiluminescent methods, enzyme techniques, and electrochemical methods is expected to become more important. New and better noninvasive methods of investigation are expected to become more routine. In addition, real-time measurements, whereby analyses of a number of components in a biological system or the body are performed instantly, should also become available.

Refinement of existing techniques and instrumentation, rather than introduction of a whole new host of methods and instruments, is expected for the 1990s. One technology challenge is clearly the migration of analytical techniques out of the area of research and development and into the quality control and process control environments. Another challenge involves continued advances in smaller sample size, higher sensitivity and specificity, and lowered costs per sample analysis. Instruments should also become more user-friendly and more integrated within the total laboratory environment. Expert systems are expected to expand to the point where usage for both identification and quantification is routine in the analytical laboratory. Essential to this usage is the proper application of sampling (qv) and the understanding of chemistry.

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