Instrumentation in Analytical Chemistry 1982–86

Edited by
Stuart A. Borman
Associate Editor
ANALYTICAL CHEMISTRY

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Preface

This book is the third in a series on analytical instrumentation. The first volume, "Instrumentation in Analytical Chemistry", was put together in 1973 by Alan J. Senzel, then Associate Editor of ANALYTICAL CHEMISTRY. Although the second book in the series was designated "Volume 2", the term "1982–86" has been used in the present title (instead of "Volume 3") to emphasize the fact that neither of the former volumes is a prerequisite to understanding and appreciating this book.

The articles reprinted here were all published in the REPORT and INSTRUMENTATION columns of ANALYTICAL CHEMISTRY's A pages (magazine section) between April 1982 and March 1986. In soliciting and editing these papers, the goal of the editors has been to provide the readers of ANALYTICAL CHEMISTRY with articles on a broad range of topics.

In compiling the articles for this volume, I was reminded once again of this impressive breadth of coverage. From month to month, ANALYTICAL CHEMISTRY covers topics that range from chemical microsensors to fiber optics, from supercritical fluid chromatography to new electroanalytical instruments, from laboratory automation to hyphenated techniques, from atomic and molecular spectroscopy to soft-ionization mass spectrometry.

I thank Alan Kahan and Sharon H. Wolfgang for the creativity, skill, and patience that go into the art and layout of every A-page article. Thanks are also due to the indomitable Gail M. Mortenson for her continuing efforts to correct the multitude of style, grammar, and spelling errors that all too often slip past the scrutiny of authors and, yes, even editors. I also extend a personal thank you to ACS management, including Executive Editor Josephine M. Petruzzi, for suggesting this series and for giving me the opportunity to contribute to it.

Stuart A. Borman
Associate Editor
ANALYTICAL CHEMISTRY

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Chemical Sensors Based on Fiber Optics

The devices considered in this REPORT involve a reagent phase on the end of a fiber optic. In operation, interaction with analyte leads to a change in optical properties of the reagent phase, which is probed and detected through the fiber optic. Depending on the particular device, the optical property measured can be absorbance, reflectance, luminescence, or something else. An example of such a device would be a pH sensor based on an immobilized dye whose color or fluorescence properties vary with pH.

The term *optrode* is used to describe these devices (*1*). This term, formed by combining "optical" and "electrode," emphasizes that *in use* optical sensors are very similar to electrodes. However, *in operating principles* they are quite different, offering new possibilities relative to electrodes while at the same time being subject to limitations and problems that are not observed with electrodes.

Attractive features of optical sensors relative to electrodes include the following:

- Because the signal is optical, it is not subject to electrical interference.
- No "reference electrode" is required.
- Because the reagent phase does not have to physically contact the fiber optic, it is a simple matter to change reagent phase. In fact, it is possible that in some contexts it will be practical to use reagent phase on a disposable basis. Also, the reagent phase can be in a different environment from the fiber optic. For example, the reagent phase could be in a high-pressure reactor and be probed through a window in the reactor.
- Optical sensors can offer significant cost advantages over electrodes, particularly if a single spectrometer is used with several sensors.
- The most exciting possibility offered by optical sensors is the use of multiwavelength and temporal information. For example, one can envision sensors that respond simultaneously to two or more analytes distinguished by measurements at two or more probe–detection wavelength combinations. If the optical sensor involves luminescence, then time resolution also can be used. In addition, multiwavelength measurements may be used to monitor reagent phase stability or to relate analyte concentrations to intensity ratios at two wavelengths.
- Optical sensors can be developed to respond to analytes for which electrodes are not available.

Optical sensors also are subject to several limitations relative to electrodes:

- Ambient light will interfere with optical sensors. They must either be used in a dark environment, or the optical signal must be encoded (e.g., by modulation) so that it can be resolved from ambient background.
- Long-term stability is likely to be a problem for reagent systems used in optical sensors, although to some extent this can be compensated for by multiple-wavelength detection and by the ease of changing reagent phases.
- Because the reagent and the analyte are in different phases, there is necessarily a mass transfer step before constant response is reached. This, in turn, limits response times of optical sensors.
- For several types of optical measurements, observed intensities are proportional to the amount of reagent phase. If the amount of reagent phase is small, detected intensities can be increased by using more intense probe radiation. However, this will accelerate any reagent photodegradation processes. As a consequence, optical sensors involve a three-way tradeoff between amount of reagent phase, intensity of probe radiation, and stability.
- In general, optical sensors will have limited dynamic ranges compared to electrodes, for reasons that will be considered below.

Optical sensors may be classified as reversible or nonreversible. A sensor is reversible if the reagent phase is not consumed by its interaction with analyte. If the reagent phase is consumed, then the sensor is nonreversible. For such a device to be useful for sensing, the relative consumption of reagent phase must be small or there must be provisions for renewing the reagent. These two categories of devices will be considered separately. This REPORT will not consider systems in which a reagent phase is used on a one-time basis.

Instrumentation

The instrumentation for fiber-optic-based chemical sensors can be simple or complex depending on the

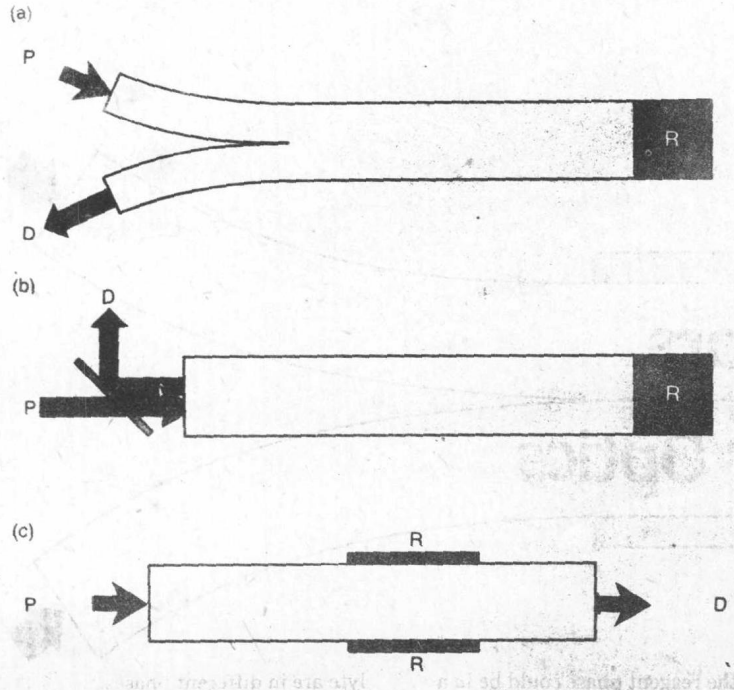


Figure 1. Fiber optic sensors

P = probe radiation, D = detected radiation, R = reagent phase. (a) Sensor based on bifurcated fiber optic in which separate fibers carry probe and detected radiation. (b) Single optic with a beam splitter to separate probe and detected radiation. (c) Single optic sensor in which the reagent phase is coated on the optic

particular application. Laser excitation may be used for long-distance transmission of excitation radiation or to get a useful signal from a small amount of reagent. However, it is also possible to construct devices with incandescent sources that use filters for wavelength selection and phototubes to measure light.

The characteristics of the fiber optic itself are critical in determining the device capabilities. The material of the fiber optic determines the usable range of wavelengths. Fused-silica optics permit measurements in the ultraviolet down to 220 nm, but are relatively expensive. Glass is less expensive and is suitable for measurements in the visible. Plastic fibers are less expensive still, but are restricted to wavelengths above 450 nm.

Devices may involve either bifurcated or single fiber optic bundles as illustrated in Figure 1. A single fiber optic or fiber optic bundle, as shown in Figure 1b, requires that the detected radiation be distinguished from probe radiation either temporally or by wavelength to avoid serious background problems due to scattered probe radiation. Alternatively, the reagent phase can be coated on the outside of a single fiber to modify its transmission characteristics. In bifurcated devices, separate optics transmit probe and detected radiation. These devices observe only the zones of reagent phase that fall within both the cone of emitted probe radiation and the cone of accepted detected radiation.

The diameter of the fiber optic can be on the order of the wavelength of transmitted light. In practice, sensors in the submillimeter size range have already been developed.

Reversible Sensors

Theory of direct sensors. Most reversible sensors involve an equilibrium between analyte and immobilized reagent. If the stoichiometry of this reaction is 1:1 then the reaction may be represented

$$A + R \rightleftarrows AR \qquad (1)$$

where A is analyte, R is reagent, and AR is the combined analyte–reagent. The equilibrium constant, K_e, may be represented

$$K_e = \frac{\overline{AR}}{\overline{R}\,[A]} \qquad (2)$$

where [A] is analyte concentration and $\overline{R}$ and $\overline{AR}$ are the number of free and combined reagent molecules in the immobilized phase, respectively. It is assumed that activity effects in the immobilized phase are equivalent for $\overline{R}$ and $\overline{AR}$ and thus cancel.

$\overline{AR}$ and $\overline{R}$ vary with analyte concentration as follows:

$$\overline{AR} = \frac{K_e[A]}{1 + K_e\,[A]}\,C_R \qquad (3)$$

$$\overline{R} = \frac{1}{1 + K_e\,[A]}\,C_R \qquad (4)$$

where C_R is the sum of free and combined reagent molecules.

Figure 2a shows response vs. analyte concentration for the case in which the measured optical parameter is proportional to $\overline{AR}$. At low concentrations ($[A] \ll 1/K_e$), response is proportional to [A]. As concentrations increase, response is curved, reaching a limiting value when $[A] \gg 1/K_e$. This corresponds to saturation of the reagent with analyte.

Figure 2b shows the case in which the optical measurement is proportional to $\overline{R}$. In this case, increasing analyte concentration leads to a decrease in the measured parameter. Rather than working with a curve as shown in Figure 2b, it is possible to rearrange Equation 4 to

$$\frac{C_R}{\overline{R}} = 1 + K_e\,[A] \qquad (5)$$

A plot of $C_R/\overline{R}$ vs. [A] will be linear with an intercept of one.

The preferred situation in practice is likely to be the case where the ratio of $\overline{AR}$ to $\overline{R}$ is measured. From Equations 3 and 4

$$\frac{\overline{AR}}{\overline{R}} = K_e\,[A] \qquad (6)$$

The ratio is directly proportional to analyte concentration as well as being independent of C_R and thus insensitive to slow loss of reagent. Ratio measurements frequently have the additional advantage of being less sensitive to instrumental fluctuations as well. The limitation of the ratio approach is that both $\overline{AR}$ and $\overline{R}$ must be present in sufficient amounts to be measured with adequate precision. As a consequence, dynamic range is limited.

It is assumed above that the amount of analyte combining with reagent is small relative to the amount of analyte in the sample, a condition that can be achieved by keeping the amount of reagent small. If this condition is not achieved, the analyte will be depleted in the sample. The response function has to be revised to account for this, and the device is not a true sensor since it is perturbing the sample.

The above discussion emphasizes the importance of the equilibrium constant in determining sensor response. The equilibrium constant is involved in the response function whether one measures $\overline{R}$, $\overline{AR}$, or the $\overline{AR}/\overline{R}$ ratio. In each case, the equilib-

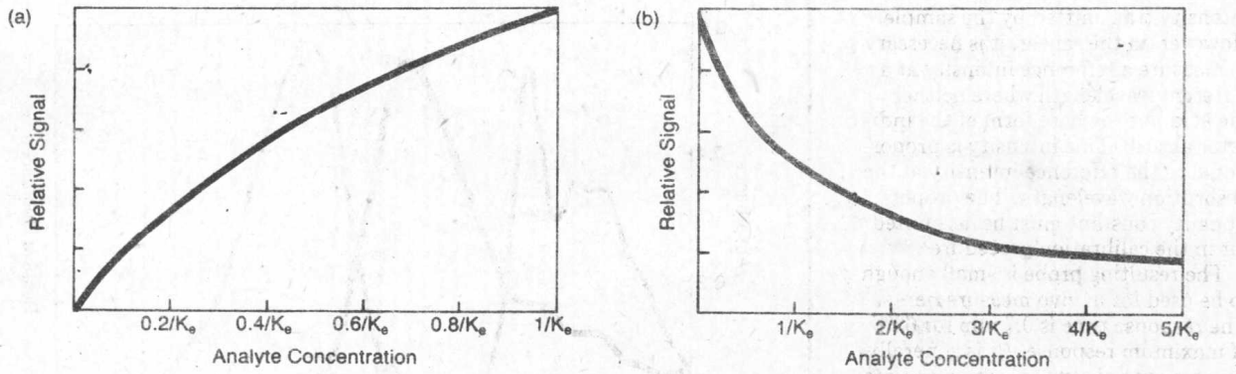


Figure 2. Theoretical response curves for reversible sensors
Analyte concentration is expressed in terms of K_e. (a) Case where $\overline{AR}$ is measured. (b) Case where $\overline{R}$ is measured

rium constant defines the range of analyte concentrations that can be measured with a given reagent phase.

The conditions for optical sensing thus differ from the common analytical situation where large equilibrium constants and reagent excesses are desired to drive an analytical reaction to completion. If that were the case with a sensor, essentially all the analyte would be extracted into the reagent until the point where the reagent phase were saturated. The device would not function as a reversible sensor.

Because the response depends on the equilibrium constant, optical sensors will measure only the concentration of analyte in a form available to interact with reagent. For example, an optical metal ion sensor based on immobilized ligand will measure free metal rather than total metal (just like a potentiometric electrode).

Theory of competitive-binding sensors. A competitive-binding-based sensor includes an immobilized reagent, R, that specifically binds the analyte, A. The reagent phase also includes a ligand, L, which competes with A for binding sites on R. The reactions and associated equilibria are

$$A + R \rightleftarrows AR \quad K_A = \frac{[AR]}{[R][A]} \qquad (7)$$

$$L + R \rightleftarrows LR \quad K_L = \frac{[LR]}{[R][L]} \qquad (8)$$

assuming both R and L are in solution. This can be achieved if L and R are larger molecules than A such that R and L can be confined by a dialysis membrane that allows analyte A to transfer freely between phases. An essential feature of L is that its optical properties change in a measurable way upon binding to R. The detected parameter is based on L, either [L] or [LR].

From mass balance considerations the total concentrations of L and R in the reagent phase, C_L and C_R respectively, are given by

$$C_L = [L] + [LR] \qquad (9)$$

$$C_R = [LR] + [AR] + [R] \qquad (10)$$

Eliminating [R] from Equations 7 and 8 and solving for [A] yields

$$[A] = \frac{[AR]\,K_L\,[L]}{K_A\,[LR]} \qquad (11)$$

If the equilibrium constants K_A and K_L are large enough, then $[R] \ll [LR]$ and [AR], and can be dropped from Equation 10. Substituting for [AR] in Equation 11 yields

$$[A] = \frac{(C_R - [LR])\,[L]}{[LR]} \times \frac{K_L}{K_A} \qquad (12)$$

Using Equation 9 to substitute for either [L] or [LR] yields an expression with a single variable. The K_L/K_A term reflects the fact that response depends on the relative affinity of L and A for reagent R.

Competitive-binding-based sensors make it possible to use analytical reactions that don't directly produce an optical change. The use of antibodies as the specific reagent would allow many possible sensors. However, since more processes and large molecules are involved, response times may be relatively slow. Only one such sensor has been developed to date.

Absorbance-based sensors. The first reversible optical sensor described in the literature is a pH sensor based on the absorbance of phenol red covalently bound to polyacrylamide microspheres (*2*). Figure 3 shows how the probe is constructed. Two 0.15-mm-diameter plastic fiber optics are inserted into a cellulose hollow fiber that contains the polyacrylamide microspheres plus additional polystyrene microspheres included to scatter light. Radiation from a tungsten source is directed onto the reagent through one fiber optic. Radiation scattered back is detected through the other optic using a filter for wavelength selection. The plug at the end of the fiber keeps incident radiation from interacting with the sample. The wavelength was chosen to measure the basic form of the phenol red.

In the usual absorbance measurement, the reference intensity is mea-

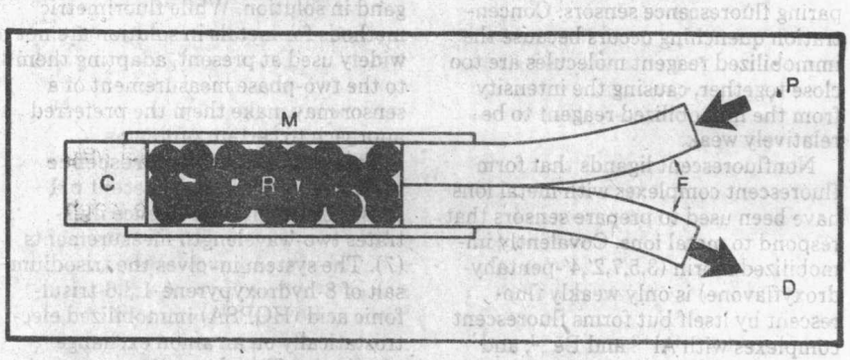


Figure 3. pH sensor based on absorbance
P = probe radiation, F = optical fibers, R = reagent phase immobilized on polyacrylamide spheres, M = cellulose membrane, and D = detected radiation. The reagent phase includes polystyrene spheres to redirect incident probe radiation. A filter wheel is used to sequentially determine transmitted light at two wavelengths, one where the base form of reagent absorbs and the other where no absorption occurs. The cap, C, serves to confine the reagent and prevent incident radiation from entering the sample. The same arrangement is also used for an oxygen sensor based on fluorescence quenching

sured at the same wavelength as the intensity transmitted by the sample. However, in the sensor, it is necessary to measure a reference intensity at a different wavelength where neither the acid nor the base form of the indicator absorb. This intensity is proportional to the reference intensity at the absorption wavelength. The proportionality constant must be accounted for in the calibration procedure.

The resulting probe is small enough to be used for in vivo measurements. The response time is 0.7 min for 63% of maximum response. As is generally true for optical sensors, response time is limited by diffusion of analyte, H^+, into the reagent phase.

Fluorescence sensors. Fluorescence is particularly well suited for optical sensing. It is compatible with a single optic measurement because the detected radiation can be distinguished from probe radiation by wavelength. In addition, fluorescence is an inherently sensitive technique capable of measuring low analyte concentrations. At low fluorophor levels, response to analyte is linear. However, in sensors it may often prove more practical to work at reagent levels where probe radiation is absorbed to a significant extent in the reagent phase. If the absorbance changes with analyte concentration, the response may be affected by the inner filter effect. The geometry of a fluorescence-based sensor corresponds to front-surface detection, a well-characterized situation.

The simplest type of fluorescence sensor involves measuring fluorescence at a single wavelength. An example of this is a pH sensor based on fluoresceinamine covalently coupled to cellulose (*3*). In this sensor, pH is related to an increase in intensity as the acid form of immobilized dye is converted to base. This particular device also illustrates one of the difficulties that can be encountered in preparing fluorescence sensors. Concentration quenching occurs because the immobilized reagent molecules are too close together, causing the intensity from the immobilized reagent to be relatively weak.

Nonfluorescent ligands that form fluorescent complexes with metal ions have been used to prepare sensors that respond to metal ions. Covalently immobilized morin (3,5,7,2′,4′-pentahydroxyflavone) is only weakly fluorescent by itself but forms fluorescent complexes with Al^{+3} and Be^{+2}, and may be used to sense these ions (*4*, *5*).

Another metal-ion-sensing system is based on the sodium salt of 8-hydroxyquinoline-5-sulfonic acid immobilized by electrostatic attraction on an anion exchanger (*6*). This form of immobilization is not only convenient but also allows for easy control of the coverage of immobilized reagent on the reagent phase. Leaching of ligand from the anion exchanger does not occur at an observable rate even at high ionic strength when the anion exchanger is undersaturated with respect to ligand. The immobilized salt of 8-hydroxyquinoline-5-sulfonic acid fluoresces only very weakly but forms strongly fluorescent complexes with Mg(II), Zn(II), Cd(II), and Al(III).

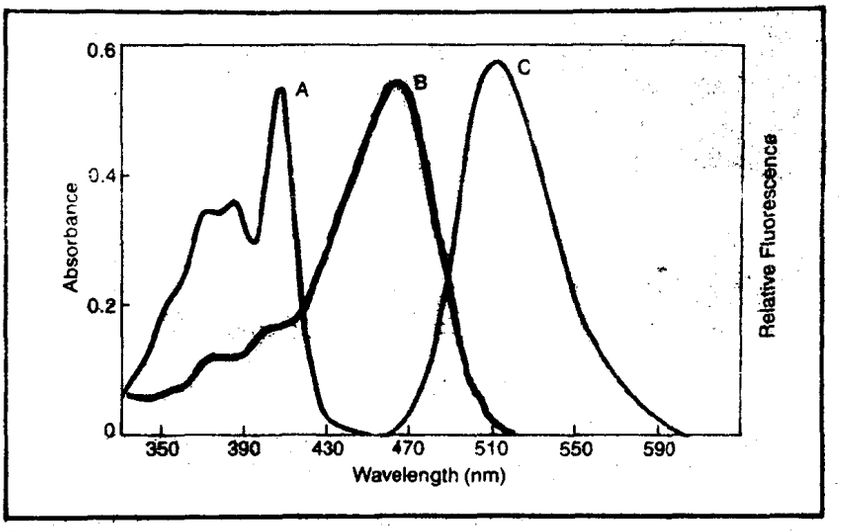
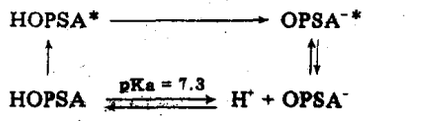


Figure 4. Spectral characteristics of 8-hydroxypyrene-1,3,6-trisulfonate (HOPSA) immobilized on anion exchange membrane

A and B are absorption spectra for acid and base forms of HOPSA, respectively. C is the fluorescence spectrum of the base form. Because HOPSA undergoes rapid excited-state deprotonation at pH >1, the fluorescence of the base is observed even when the ground state is in the acid form

In general, metal ions that can be determined fluorimetrically in solution can potentially be sensed optically providing that the appropriate fluorigenic reagent can be immobilized without adversely affecting its fluorescence properties and that the equilibrium constant for complex formation is suitable for sensing (i.e., not too large). The selectivity of sensors based on immobilized fluorigenic ligands will be similar to the selectivity of the ligand in solution. While fluorimetric methods for metals in solution are not widely used at present, adapting them to the two-phase measurement of a sensor may make them the preferred approach to certain problems.

Two-wavelength fluorescence measurements. A more recent pH sensor based on fluorescence illustrates two-wavelength measurements (*7*). The system involves the trisodium salt of 8-hydroxypyrene-1,3,6-trisulfonic acid (HOPSA) immobilized electrostatically on an anion exchange membrane. The absorption spectra for acid and base forms of HOPSA—as well as fluorescence emission of the base form—are shown in Figure 4. As shown below, electronically excited HOPSA undergoes rapid excited-state deprotonation so that $OPSA^-$ fluorescence is observed between pH 1 and 7 even though HOPSA is the dominant ground state species.



The measured parameter in this sensor is the ratio of fluorescence intensity emitted at 510 nm and excited at 470 nm (specific for base) to intensity excited at 405 nm (selective for acid). The ratio measurement is insensitive to source fluctuations, drift, temperature, quenching, ionic strength, and slow loss of reagent, all of which can affect the single intensity measurement. As a consequence, this type of measurement is likely to be more practical.

Other approaches to ratio measurements are used in pH sensors based on fluorescence from immobilized β-methylumbelliferone (*8*). One approach is to excite at an isosbestic point in the fluorescence excitation spectrum to measure the total amount of indicator present, independent of its form. A second excitation wavelength permits the specific measurement of the base form of the indicator. The ratio of the intensities excited at these two wavelengths is directly proportional to the fraction of β-methylumbelliferone in the base form, which is related to pH and independent of the total amount of indicator.

The second approach is to measure the ratio of fluorescence intensities at two wavelengths, one where the acid form fluoresces and the other where the base form fluoresces. This is possible with fluorescent indicators that do

not undergo excited-state proton transfer like HOPSA. However, it is subject to error if either the acid form or the base form of the indicator is selectively quenched.

The pH sensor based on immobilized β-methylumbelliferone is formulated as a thin layer on a quartz window. The layer is separated from sample by an ion-permeable membrane. The pH sensor also has been used as the internal element of a CO_2 sensor by covering the sensor with a CO_2-permeable membrane and bringing it in contact with HCO_3^-. When the sensing element is a thin layer, it is not optically shielded from the sample. To avoid interferences due to sample fluorescence, it may be necessary to include a layer of a black or reflecting membrane between the sample and the reagent phase. It should be noted that this particular reagent phase was not used with a fiber optic although it easily could be.

Fluorescence quenching. Sensors also can be based on a decrease in reagent phase fluorescence upon association with analyte. Although this is inherently less desirable than systems involving increases in intensity with analyte concentration, it allows access to analytes that could not otherwise be sensed by fluorescence.

An important example of a sensor based on quenching is the O_2 probe developed by Peterson et al. (*9*). The O_2 sensor is similar to the pH probe shown in Figure 3, except that the reagent phase is a dye, perylene dibutyrate, adsorbed on a polymeric support, and the membrane containing the immobilized reagent is porous polypropylene, which is hydrophobic and highly permeable to O_2. Fluorescence is excited through one fiber and observed through the other. Filters are used to measure separately the green fluorescence of perylene dibutyrate and scattered blue excitation radiation. The intensity of fluorescence is ratioed to the intensity of scattered radiation to compensate for changes in source intensity. This sensor is particularly attractive because it is reversible. Unlike the widely used oxygen electrode, it does not require continued mass transfer of oxygen to the sensor surface to maintain a constant signal.

The O_2 sensor is based on dynamic quenching, i.e., an excited-state interaction with the fluorophor. The response follows the form of Equation 5 (Figure 2b) where "free" immobilized reagent is measured as a function of analyte concentration. However, the equilibrium constant is replaced by a quenching constant that depends on the relative rates of fluorescence and nonradiative return to the ground state through interaction with the quencher. One of the reasons perylene dibutyrate was selected as the dye for the O_2 sensor was that its quenching constant was the right magnitude to yield sensitive response to the range of oxygen partial pressures of physiological interest.

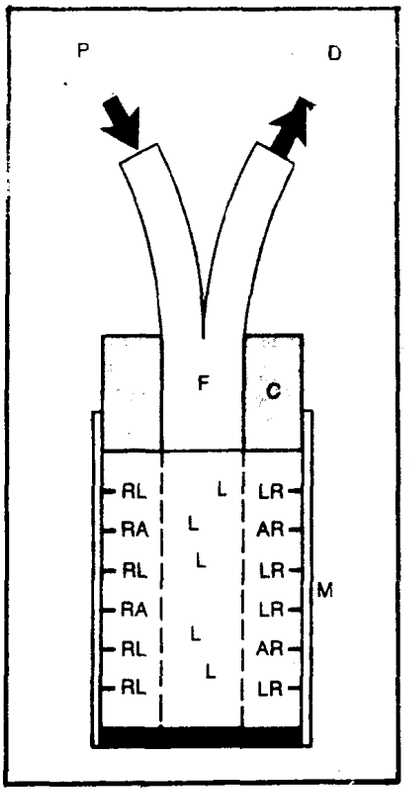


Figure 5. Competitive-binding fluorescence sensor for glucose

F is the bifurcated fiber optic; C is the cladding around the fiber bundle; R is the reagent, concanavalin A immobilized on sepharose and coated on the walls of the hollow fiber, M; L is the competing ligand, dextran labeled with fluorescein; and A is the analyte, glucose

A similar device has been based on fluorescence quenching of pyrenebutyric acid by O_2 (*8*). This study demonstrated how the medium of the pyrenebutyric acid affects the rate of quenching by O_2 and how this influences the slope of the response curve. These media effects can serve as a means of tailoring the O_2 sensor to optimally measure a particular O_2 partial pressure range.

A range of quenching-based sensors is possible. In addition to the O_2 sensor, sensors based on transition metal quenching of ligand fluorescence and iodine quenching of rubrene fluorescence have been proposed (*1*).

Competitive-binding sensor based on fluorescence. A fluorescence-based, competitive-binding sensor for glucose has been described (*10*). The specific glucose-binding reagent is concanavalin A immobilized on sepharose. The competing ligand is dextran labeled with fluorescein. A schematic of the device is shown in Figure 5. The fiber optic fits in a hollow fiber with a plug on the end. Glucose can diffuse freely through the hollow fiber but dextran cannot. The immobilized concanavalin A is on the walls of the fiber and thus out of the volume illuminated through the fiber optic. Increasing glucose concentration displaces the labeled dextran from the concanavalin A, causing it to be free to diffuse into the illuminated solution volume. This in turn leads to an increase in fluorescence, which is related to glucose concentration.

Reflectance-based sensors. When dealing with solid phases, it becomes difficult to measure transmitted light satisfactorily. In these cases the intensity of reflected light may be used as a measure of the color of an immobilized reagent phase. In the context of a sensor, a true reference intensity cannot be measured at the analytical wavelength. Instead, an intensity proportional to the reference intensity must be measured at a wavelength other than the analytical wavelength. This wavelength should be chosen so that it is not absorbed either by reagent alone or by reagent combined with analyte.

Because it is difficult to prepare a sensor that truly measures absorbance, reflectance is likely to be the more widely used technique for sensors based on color changes. In fact, it is not clear that the pH sensor developed by Peterson et al. (*2*) truly measures absorbance rather than reflectance, since adherence to Beer's law was not verified. An attractive feature of reflectance is that it can be used to measure the ratio of free and combined reagent if the analytical reaction is accompanied by a change in color. The functional relationship between reflectance and concentration will depend on the nature of the reagent phase and the optical arrangement.

An example of a reflectance-based sensor is an ammonia detector (*11*). In this particular device a layer of oxazine dye is coated on the outside of a fiber optic (see Figure 1c). Interaction with ammonia changes the color of the dye, changing its reflectance and modifying the intensity of light transmitted to the detector. Another example involves a method for monitoring oil in water (*12*).

Nonreversible Sensors

It is possible to design sensors based on analytical reactions that consume reagent. Although such sensors necessarily have finite lifetimes, these lifetimes can be quite long if the rate of reagent consumption is small relative to the total amount of reagent available. Another disadvantage of nonreversible sensors is that they require

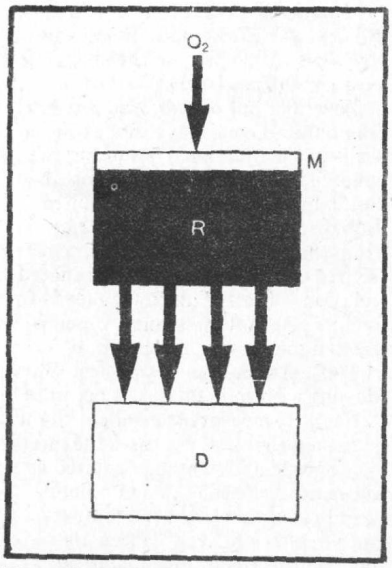


Figure 6. Nonreversible O_2 sensor
O_2 diffuses through Teflon membrane, M, into reagent phase, R, where it reacts to yield chemiluminescence measured by detection system, D

steady-state mass transfer to get a constant signal. Any processes that perturb mass transfer are potential sources of error.

The difference between reversible and nonreversible optical sensors is similar to the difference between potentiometric and amperometric electrodes. Amperometric electrodes, like nonreversible optical sensors, involve a reaction and require mass transfer to an electrode surface.

The use of tetrakis(dimethylaminoethylene) (TMAE) to measure oxygen serves as an example of a nonreversible sensor (*13*). The device is illustrated in Figure 6. Oxygen in the sample diffuses through a hydrophobic O_2-permeable membrane into a reservoir of TMAE. There it reacts with TMAE yielding chemiluminescence, which is measured by a light detection system. (No probe radiation is required for this device.) Steady-state chemiluminescence is proportional to the partial pressure of oxygen. Both the flow rate of O_2 past the membrane and the temperature affect response because they affect the steady-state supply of O_2 into the reagent phase. The lifetime of the sensor is determined by the rate of TMAE consumption relative to the total TMAE available. The lifetime can be quite long—months and even years—if a large TMAE reservoir is used with a low rate of oxygen permeation. Again, it should be noted that this particular reagent was not combined with a fiber optic but readily could be.

Other nonreversible sensors include UO^{2+} and halide sensors (*1*). These devices differ from the O_2 sensor in that they require that reagent be diffused into the sample. In the UO^{2+} sensor, phosphate and nitric acid are diffused into the sample to generate a medium in which UO^{2+} becomes fluorescent. In the halide sensor, Ag–fluorescein is diffused into the sample. Halides combine with Ag^+, rendering fluorescein fluorescent.

The Future

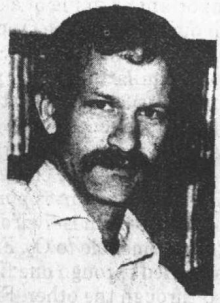
Chemical sensors based on fiber optics are clearly attractive in concept. Success in practice requires the development of appropriate reagent phases. This process is only beginning. However, the devices developed to date already illustrate the wide variety of approaches and systems that are possible.

Interest in optical sensors is certain to intensify in the next few years, and there are some trends that are likely to emerge. Because the dynamic range of sensors is limited and depends on equilibrium constants, a series of reagent phases will be developed for a single analyte in order to respond to different levels of analyte concentration. Other probable trends will be the increased use of measurements at two or more wavelengths and the increased use of reflectance to exploit reactions causing color changes.

Successful development of optical sensors that respond directly to common analytes will inevitably lead to coupled sensors in which a reagent phase "transduces" the analyte of interest to a species measured by the optical sensor. As already mentioned, optical pH sensors in contact with bicarbonate have already served as the internal sensing elements of CO_2 sensors (*8*, *14*, *15*).

References

(1) Borman, S. A. *Anal. Chem.* **1981**, *53*, 1616–18 A.
(2) Peterson, J. I.; Goldstein, S. R.; Fitzgerald, R. V.; Buckhold, D. K. *Anal. Chem.* **1980**, *52*, 864–69.
(3) Saari, L. A.; Seitz, W. R. *Anal. Chem.* **1982**, *54*, 821–23.
(4) Saari, L. A.; Seitz, W. R. *Anal. Chem.* **1983**, *55*, 667–70.
(5) Saari, L. A.; Seitz, W. R., submitted for publication in *Analyst*.
(6) Zhujun, Z., unpublished work.
(7) Zhujun, Z.; Seitz, W. R., submitted for publication in *Anal. Chim. Acta*.
(8) Lübbers, D. W.; Opitz, N. In "Proceedings of the International Meeting on Chemical Sensors"; Fukuoka, Japan; Elsevier: Amsterdam, 1983; pp. 609–19.
(9) Peterson, J. I.; Fitzgerald, R. V.; Buckhold, D. K. *Anal. Chem.*, **1984**, *56*, 62–67.
(10) Schultz, J. S. U.S. Patent 43 444 38, 1982.
(11) Giuliani, J. F.; Wohltjen, H.; Jarvis, N. L. *Opt. Lett.* **1983**, *8*, 54–56.
(12) Kawahara, F. K.; Fiuten, R. A.; Silvus, H. S.; Newman, F. N.; Frazar, J. H. *Anal. Chim. Acta* **1983**, *151*, 315.
(13) Freeman, T. M.; Seitz, W. R. *Anal. Chem.* **1981**, *53*, 98–102.
(14) Zhujun, Z.; Seitz, W. R., submitted for publication in *Anal. Chim. Acta*.
(15) Vurek, G.; Peterson, John; Goldstein, Seth; Severinghaus, John. *Federation Proceedings* **1982**, *41*, 1484.



Rudolf Seitz received his PhD from MIT in 1970. After working at the EPA's Southeast Environmental Research Laboratory and at the University of Georgia, he joined the faculty of the University of New Hampshire in 1976. Although his earlier research dealt with chemiluminescence, he is now primarily interested in fluorescence and the development of optical sensors.

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Chemical Microsensors and Microinstrumentation

Progress in analytical chemistry has often been led by progress in instrumentation. In recent times analytical instrumentation has undergone a metamorphosis as a result of extraordinary advances in microelectronics. The advent of inexpensive computing power has dramatically changed the way experiments are controlled and data are acquired, manipulated, displayed, and stored. In many respects a tremendous gap has developed between the size, cost, and performance of modern microcomputers and the corresponding size, cost, and performance of the instruments they control. The size and cost of microcomputers have dropped precipitously while performance has soared. The same cannot be said for the measurement instrumentation. This article will describe several approaches aimed at closing the technology gap between instruments and computers. Perhaps it is not surprising that the most promising approach for reducing the gap derives from the same technology used in the microelectronics industry, namely, microfabrication and microlithography.

Microsensors and microinstrumentation are relative newcomers to the analytical scene. Virtually unheard of a decade ago, microsensor research has grown steadily, driven perhaps by the tantalizing array of opportunities afforded by small low-cost sensors. Such areas as clinical chemistry, environmental monitoring, process control, and automobile emission control are all heavily dependent on sensor technology. If pocket calculators can be made small and inexpensive, it is exciting to think of the possibilities of a comparably sized sensor system. Suddenly it becomes feasible to measure things that have not previously been routinely measured. Thus, the cost of information can be reduced, and knowledge can increase at a quicker pace.

All of the devices described in this article have one thing in common—they are fabricated, at least partially, using microlithographic techniques. Thus, a chemical microsensor is broadly defined as a microfabricated device that provides a reproducible electronic output signal when exposed to a chemical stimulus. Work being conducted on small electrodes (e.g., coated wires [*1*] or carbon fibers [*2*]) or optical fiber sensors will not be considered here since they are not microfabricated. A chemical microinstrument is defined in a similarly broad fashion, as a small, chemically sensitive system whose components can be microfabricated.

Since microlithography is the key to understanding present and future microsensor approaches, it is appropriate to quickly review some of the fundamental concepts in microfabrication technology.

Lithography

The technique of lithography (which means, literally, writing on stone) was first developed over 100 years ago by the graphic arts industry. In recent times, the microelectronics industry has refined the technology nearly to the physical limits of perfection. Transistors and integrated circuits consist of layers of patterned thin films of metals, dielectrics, and semiconductors on a substrate, usually silicon (*3*). The patterns are defined in the thin film by a process shown in Figure 1. A radiation-sensitive *resist* layer (usually a sensitized organic polymer) is coated on top of the thin film to be patterned. Radiation, in the form of visible, UV, or X-ray photons, electron beams (E-beams), or ion beams, is permitted to strike the resist wherever a thin-film pattern is (or is not) desired. After this "mask" expo-

sure, the resist is developed in an appropriate solvent that leaves some areas of the thin film still protected with resist and other areas bare. A positive resist becomes more soluble after irradiation, and exposed areas are rinsed away in the developing step. Negative resists become insoluble in the areas of exposure. The areas of the thin film that are not protected by resist can then be etched away (e.g., in acid solution or a reactive plasma) to yield the desired pattern. Processing of additional levels by evaporation of new thin films etc. can be conducted after the resist layer is stripped away with a solvent.

The sizes of patterns that can be defined using lithographic techniques are breathtakingly small. Optical mask exposure can mass produce devices having 2-μm geometries. Today's X-ray and E-beam lithography systems routinely produce patterns with 0.5-μm geometries. Laboratory researchers have successfully patterned structures (using E-beam techniques) having 0.005-μm (50 Å!) feature sizes. It is important to note that at these size scales many macroscopically important physical phenomena (e.g., gravity) become irrelevant and other phenomena (e.g., surface diffusion) become increasingly significant (*4*). Besides small feature sizes, microlithography also yields exquisite precision and dimensional control. This permits enormous numbers of patterns to be replicated with incredibly small batch-to-batch variations in pattern size.

The microlithographic method is applicable to a very wide range of situations and materials. Several very general observations can be made about the present technology. Most microlithography is conducted on optically smooth substrate surfaces, deposited films of metals, or insulators with thicknesses less than about 1 μm. Etched grooves most often have aspect ratios less than 2:1 (i.e., the depth of the groove is seldom greater than two times as large as the width of the groove). Thus, the overwhelming feature of microfabricated structures is that they are planar since the lithographic images are two-dimensional, and vertical topography is only achieved slowly as more and more layers of thin films are added. Higher aspect ratio structures are rapidly emerging as progress is made in X-ray lithography, multilayer resists, and anisotropic etching techniques, but for the near future, the landscape of most conventionally microfabricated structures will have a rather flat appearance. These realities inspire microsensor enthusiasts to think small and to think in two dimensions rather than three.

Clearly there is enormous potential for using microlithography to produce structures and devices that are useful for many applications beyond microelectronics (*5*). For analytical chemistry, microlithography has been employed to make not only sensors and detectors but also other common instrument components such as valves and capillary tubes. It is obvious that this is only the beginning. As chemists become more aware of microfabrication methods, it is likely that many other parts of common instruments will be microfabricated and new techniques, not practical with macroscopic systems, will appear.

Present Microsensor Technologies

Clearly, microfabrication offers some very attractive advantages for making small, rugged, and inexpensive sensors. The primary obstacle to using it more extensively is the constraint that the devices be planar. This forces designers to think about analytical problems from a smaller and flatter perspective and to consider physical–chemical interactions that might be considered unimportant in the macroscopic world. Chemical microsensor research is therefore a multidisciplinary endeavor requiring a synergistic combination of chemistry, physics, materials science, and electrical engineering.

All chemical microsensor devices fall into two categories. The first category consists of devices that sense chemical species whose presence modulates the transport of electronic charge in the device. Examples of microsensors in this category include CHEMFETs, ion-controlled diodes, Schottky diodes, thin-film tin oxide gas sensors, chemiresistors and microdielectrometers. The second category consists of all microsensor devices that do not fit into the first category. These devices, which are based on a number of unique techniques, include surface acoustic wave devices, potentiometric gas sensors, and pyroelectric enthalpimetric sensors.

In their most general form, all chemical microsensors consist of at least two elements: a microfabricated

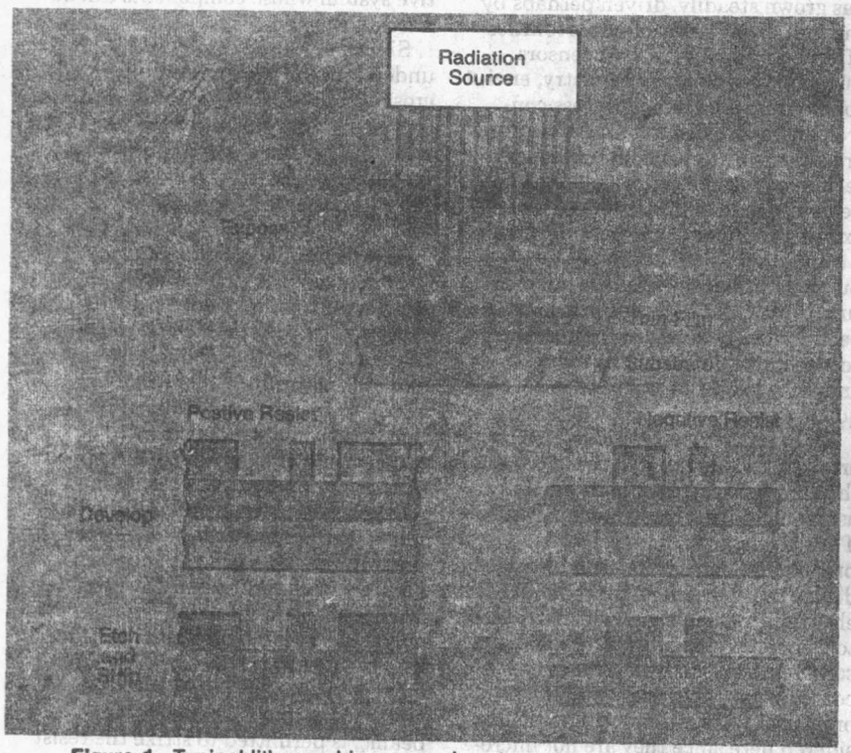

Figure 1. Typical lithographic processing steps
Positive resists become more soluble upon irradiation. Negative resists become less soluble

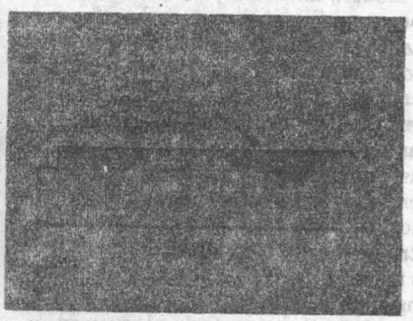

Figure 2. Chemical microsensors typically involve a microfabricated electronic probe device in contact with a chemically selective coating whose properties are altered by the species to be detected

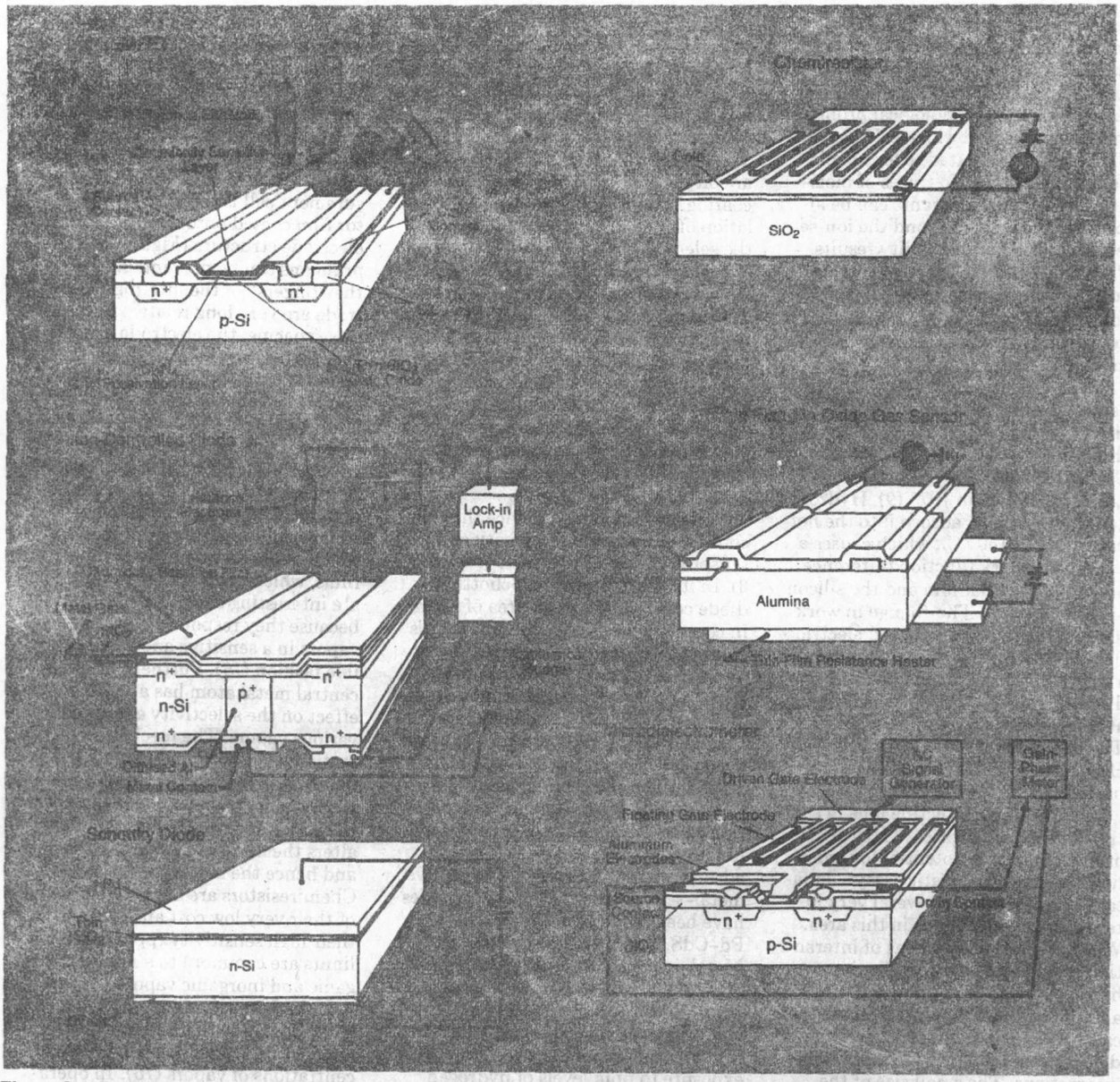

Figure 3. Microsensors based on modulation of electron transport through the device by chemical interactions
Conventional silicon electronic device technology is often employed. The p-Si regions consist of Si doped with an electron acceptor. The n-Si regions are doped with an electron donor

physical probe device and a chemically selective coating (Figure 2). The probe device contacts the coating and provides an electrical signal whose characteristics reflect the state of the coating. The coating is in physical contact with the medium in which chemical species are to be detected. Thus, the nature of the coating interaction with the chemical species determines the type of physical transduction mechanism required of the probe. For example, if the dielectric properties of a coating respond strongly to exposure to a particular vapor, then the microdielectrometer is the probe of choice. It is safe to predict that the ultimate performance of chemical microsensors will be determined by the coating chemistry.

CHEMFETs. Probably the best-known chemical microsensor is the CHEMFET illustrated in Figure 3. First reported by Bergveld in 1970 (*6*), the CHEMFET is essentially a conventional insulated-gate field effect transistor that has its metallic gate contact replaced by a chemically sensitive coating and a reference electrode. Excellent reviews of CHEMFET technology can be found in References 7 and 8. In normal operation a current is made to flow by the application of a voltage across the source and drain contacts. Variations in the electric field in the gate region, located between the source and drain, produce corresponding variations in the observed drain current. The CHEMFET can be used in a variety of ways. In solution, the gate region can be coated with an ion-sensitive membrane. Interaction of ions in solution with the membrane results in a change of the interfacial potential and a correspond ing alteration of drain current. Numerous cations and anions have been sensed (e.g., H^+, K^+, Ca^{2+}, Cl^-, I^-, and CN^-), and near Nernstian responses are commonly observed. The ion-sensing CHEMFET (or ISFET as it is often called) has advantages in its small size (e.g., less than 1 mm^2 area) and low output impedance, which