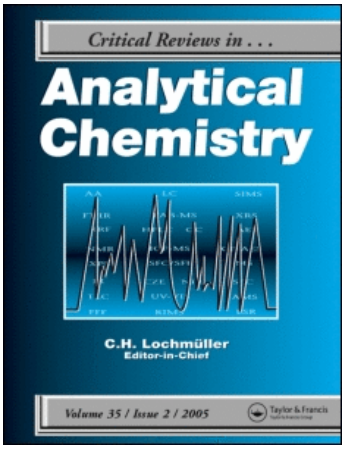

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CHEMICAL SENSORS BASED ON IMMOBILIZED INDICATORS AND FIBER OPTICS

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I. INTRODUCTION

In recent years, there has been increasing interest in the development of optically based chemical sensors. While a large variety of devices are possible, they share as a common feature an immobilized reagent phase which changes the optical properties in some way upon interaction with an analyte on either a continuous or a reusable basis. Factors stimulating this interest include the following:

1. The fiber optics revolution in communications has led to the availability of optical fibers with high transparency in the visible region, thus making it possible to transmit light to and from remote locations over considerable distances without serious intensity losses. Because some of the advantages of optical fiber for communications, such as ruggedness and immunity to electrical interference, apply equally to sensors, there has been considerable interest in the development of fiber optic-based sensors for physical parameters. Chemical sensors represent a continuation of this trend.
2. Optical sensors are thought to have the potential to improve upon the performance of currently used electrical sensors such as potentiometric and amperometric electrodes. Unlike electrodes, optical sensors do not require a separate reference sensor. In addition, they offer the possibility of improved stability with respect to calibration, particularly in devices where the measured parameter is an intensity ratio. Furthermore, since optical sensors are a very different approach to sensing, they may be applicable to problems that cannot be dealt with by present electrical sensors.
3. On a broader scale, the revolution in computers and information processing has made it possible to do sophisticated on-line data processing. The ability to apply these techniques to chemical problems is currently limited by the quality of *in situ* information that can be obtained. Consequently, there is strong interest in all types of chemical sensors. Breakthroughs in chemical sensor technology are more likely in the new field of optical sensing than in the more mature field of electrical sensing. Particularly exciting in this reviewer's opinion is the prospect of developing devices that exploit the possibility of using multiwavelength information to achieve stability and/or reliability.

A. Scope of the Review

The primary foci of this review are devices in which an immobilized reagent phase is used for chemical sensing either continuously or repeatedly after a recharging operation. The review considers systems that have not involved fiber optics. Applications of fiber optics to remote spectroscopic measurements are considered only in passing. Fiber optic sensors for physical parameters are considered only insofar as they may serve as sensing elements for chemical sensors.

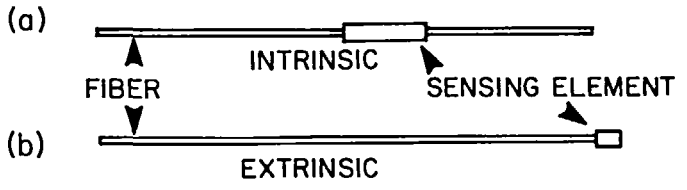


FIGURE 1. Schematic of intrinsic (a) vs. extrinsic (b) fiber optic sensors. In the intrinsic sensor, the sensing element, i.e., the chemical indicator, somehow modifies the transmission properties of the fiber, while in the extrinsic sensor optical fiber merely serves to conduct light to and from the indicator.

The first section of this review considers the characteristics of optical fibers as they relate to chemical sensing. The second section considers other instrumental components used in sensor systems. The third section deals with the chemical requirements for optical sensing. The last major section reviews applications reported to date. The review concludes with a brief look to the future. Previous reviews, while available, are shorter and have necessarily dealt with the subject in a more superficial manner.[1-6] Several scientific news articles have also described the possibilities of fiber optic sensors.[7-9]

II. FIBER OPTIC SENSING

Fiber optic sensors may be divided into two categories, illustrated schematically in Figure 1. Extrinsic sensors involve a sensing element external to the fiber itself. In the case of chemical sensors, this sensing element is an immobilized reagent phase that changes optical properties upon interaction with an analyte. The optical fiber serves merely as a conduit to transport light to and from the sensing element, much as a wire serves to conduct electrical energy to and from an electrical sensing element. For these types of sensors, the significant fiber optic parameters are the efficiency of light transport through the fiber, i.e., the transmittance, and the angle over which light is accepted into the fiber.

Intrinsic sensors involve a change in the characteristics of the optical fiber itself. To understand how these sensors operate, it is important to understand the basic principles of light transmission in fiber optics. A variety of books dealing with fiber optics are available,[10-16] however, most of them are oriented toward the application to communications and/or deal with the physics in more depth than is required by the chemist interested in sensors. This reviewer found the books by Keiser[17] and Wolf[18] to be the most digestible sources of information.

A. Fiber Optic Fundamentals

Optical fiber is based on the phenomenon of total internal reflection. Step-index fiber consists of a core of refractive index, n_1, surrounded by a cladding of a lower refractive index, n_2, as illustrated in Figure 2. Incident light is transmitted through the fiber if it strikes the cladding at an angle greater than the critical angle, so that it is totally internally reflected at the core/cladding interface. Light entering the fiber over an acceptance cone is transmitted. The acceptance cone half-angle, a, depends on the refractive indexes of the core and cladding as well as the refractive index of air, n_0:

$$\sin a = (n_1^2 - n_2^2)^{1/2}/n_0 \quad (1)$$

More commonly, the range of angles accepted by the fiber is described in terms of the numerical aperture (NA) or f/number.

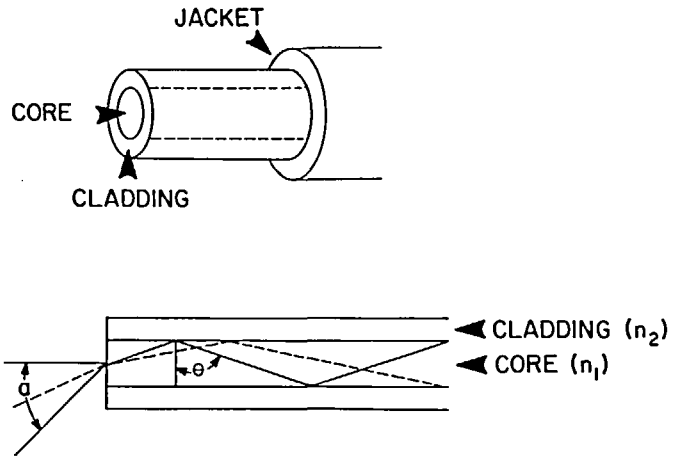


FIGURE 2. Optical fiber schematic. a = Acceptance angle; n_1 and n_2 are the refractive indices for core and cladding interface. Total internal reflection occurs when θ, the angle of incidence at the core-cladding interface, exceeds the critical angle. Note that the ray entering at a large angle relative to the fiber axis (—) has to travel further to get the same distance down the fiber as the ray entering at a small angle (---).

Table 1
FIBER LIGHT GATHERING CHARACTERISTICS (ASSUMING CORE REFRACTIVE INDEX = 1.50)

$n_{cladding}$	Critical angle (degrees)	Sin a (degrees)	NA
1.48	80.6	14.1	0.24
1.45	75.2	22.6	0.38
1.40	69.0	32.6	0.53
1.35	64.2	40.8	0.65
1.30	60.1	48.4	0.75

$$NA = n_0 \sin a \tag{2}$$

$$\text{f/number} = 1/(2 \tan a) \tag{3}$$

Some typical refractive index values and the corresponding NA are listed in Table 1.

B. Modes and Fiber Types

The ray optic representation of light propagation through optical fiber (Figure 2) is an approximation that does not account for interference effects. A more rigorous description requires solution of Maxwell's equations for light propagation with the cylindrical boundary conditions imposed by the fiber. This leads to the conclusion that light propagates in discrete modes through the fiber. Each mode corresponds to a unique incidence angle and has a distribution of electromagnetic and magnetic field intensities such that a given ray of light does not interfere with itself.

Not all modes propagate through the fiber at the same rate. Instead, modes corresponding to a larger angle between the entering ray and the fiber axis propagate more slowly because

they have to travel a greater distance to get to the end of the fiber. This effect is called modal dispersion and is illustrated in terms of ray optics in Figure 2.

Modal dispersion is undesirable for long-distance communications applications because it causes input pulses to spread as they propagate through optical fiber and limits the rate at which digital information can be transmitted over the fiber. As a consequence, special measures are taken to reduce the degree of modal dispersion in fibers designed for communications. In a step-index fiber, the number of possible modes is related to the NA and diameter of the fiber:

$$N_m = 0.5(\pi\, d\, NA/\lambda)^2 \qquad (4)$$

where N_m is the number of modes and d is the diameter of the fiber. The NA can be reduced by fabricating the fiber to minimize the difference between the refractive indexes of the core and the cladding. The other measure that can be taken is to reduce the fiber diameter. "Single-mode fiber" has a core diameter so small (typically 1 to 5 μm) that only a single mode can propagate.

An alternative way to reduce modal dispersion is to use "graded index fiber" in which there is a continuous decrease in refractive index with distance from the center of the core. The more off-axis a particular ray is, the further it penetrates into the region of lower refractive index. This accelerates the ray and compensates for the longer distance that off-axis rays have to travel to propagate through the fiber.

Multimode step-index fiber is most practical for chemical sensors based on changes in intensity because it is less expensive and transmits more light. Interferometric sensors, however, require single mode fiber.

C. Evanescent Wave

Although light that strikes the core-cladding interface of an optical fiber at angles greater than the critical angle is totally internally reflected, there is an electromagnetic field, called an "evanescent wave", that penetrates a small distance into the cladding. The evanescent wave propagates parallel to the core-cladding interface and can interact with molecules in the cladding near the interface. The use of evanescent wave interactions at interfaces to get chemical information in both the UV-visible and infrared (IR) regions of the electromagnetic spectrum is the basis of internal reflection spectroscopy, an established technique in both the visible and IR. The standard text in this field is by Harrick.[19] The evanescent wave may also be used to selectively excite fluorescence at surfaces.[20]

The evanescent field intensity I(z) decays exponentially with perpendicular distance z from the interface:

$$I(z) = I_o \exp(-z/d_p) \qquad (5)$$

where I_o is the initensity at $z = 0$ and

$$d_p = \frac{\lambda_o}{4\pi}(n_1^2 \sin^2\theta - n_2^2)^{-1/2} \qquad (6)$$

for angles of incidence, θ, greater than the critical angle, where λ_o is the wavelength in vacuum and d_p is the penetration depth. Figure 3 shows the penetration depth for a series of incidence angles. Typically, penetration depths are on the order of a wavelength or so, although the value goes to infinity as the angle of incidence approaches the critical angle. I_o, the intensity at $z = 0$, depends on both the angle of incidence and the incident beam polarization. For incident field intensities, $I^{\parallel}$ and $I^{\perp}$, the equations that relate the evanescent

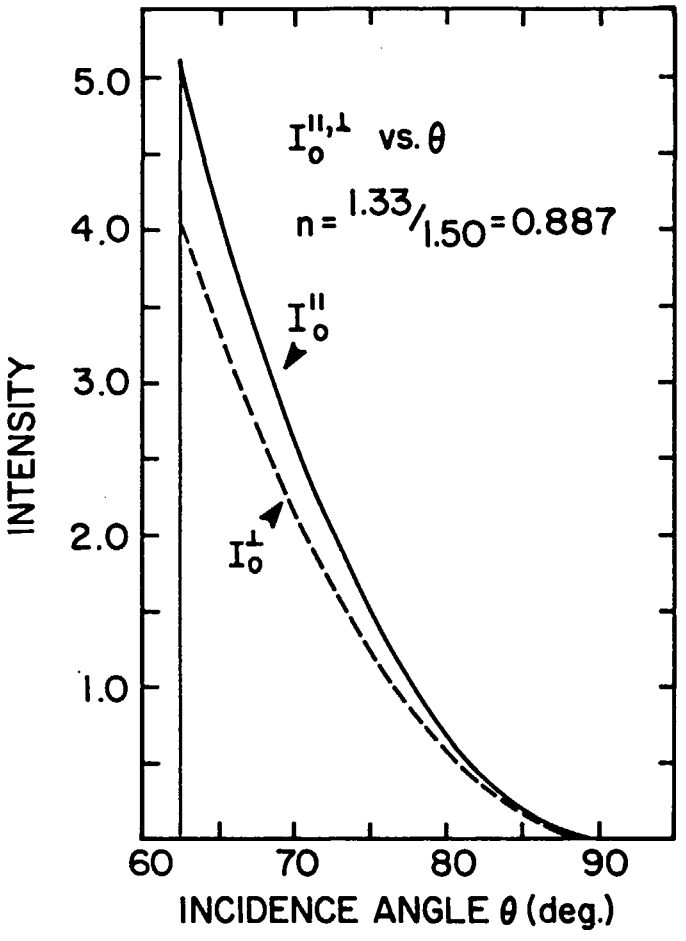


FIGURE 3. Effect of incidence angle on the penetration depth. Curves 1 through 7 are for critical angles, 10, 15, 20, 25, 30, 35, and 40°, respectively.

field intensities to the angle of incidence are as follows for light that is polarized, parallel, and perpendicular to the plane of incidence:

$$I_o^{\parallel} = d^{\parallel} \frac{4 \cos^2 \theta (2 \sin^2 \theta - n^2)}{n^4 \cos^2 \theta + \sin^2 \theta - n^2} \tag{7}$$

and

$$I_o^{\perp} = d^{\perp} \frac{4 \cos^2 \theta}{1 - n^2} \tag{8}$$

where the superscripts $\parallel$ and $\perp$ designate parallel and perpendicular, respectively, d is the incident intensity, and

$$n = n_2/n_1 \tag{9}$$

Figure 4 shows calculated values for $I^{\parallel}$ and $I^{\perp}$, assuming $n_1 = 1.50$ and $n_2 = 1.33$. Note that at angles of incidence approaching the critical angle, the intensities are considerably

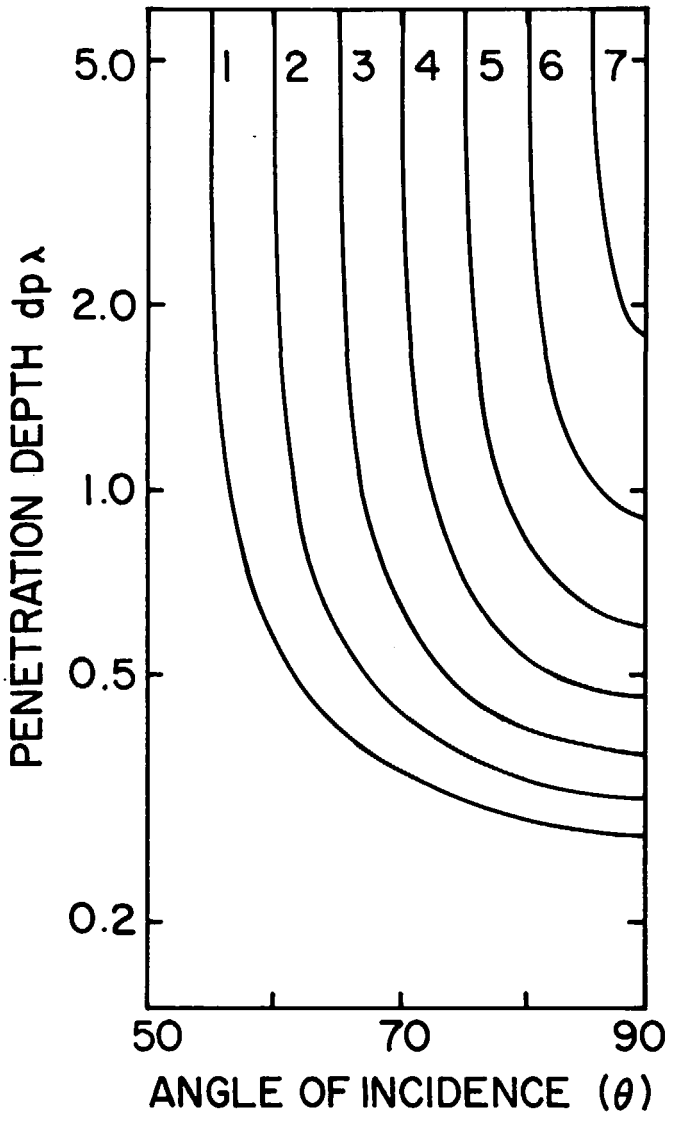


FIGURE 4. Light intensity at the core-cladding interface as a function of incidence angle.

greater than the incident intensity, indicating that the cladding-core interface is located near or at an intensity maximum.

In an optical fiber, a fraction of the light is transmitted through the cladding in the evanescent wave rather than the core. This fraction depends on both the optical parameters of the fiber itself and the distribution of light in the fiber among various modes. An approximate equation for the ratio of intensities in the core, I1, and cladding, I2, is

$$I1/I2 = \frac{3}{4} N_m^{1/2} - 1 \quad (10)$$

Substituting in for N_m using Equation 4 relates I1/I2 directly to fiber parameters:

$$I1/I2 = \sqrt{3/8}\, \pi\, d\, NA/\lambda - 1 \quad (11)$$

Table 2
CORE-CLADDING POWER RATIO FOR TYPICAL FIBER CHARACTERISTICS

Core diameter (μm)	NA	Wavelength (nm) 500	1000
200	0.20	153	76
200	0.40	307	153
100	0.20	76	37.5
100	0.40	153	76
50	0.20	37.5	18
50	0.40	76	37.5

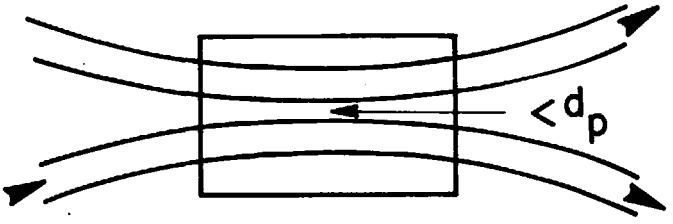


FIGURE 5. Schematic of fiber optic coupler. When the distance between the fibers is less than the penetration depth, light couples from one fiber to the other.

Table 2 lists some calculated values for I1/I2 for typical fiber conditions.

The evanescent wave is important for chemical sensors in several ways. As is discussed further in Section II.F, devices can be designed such that cladding is the immobilized reagent phase that changes optical properties upon interaction with an analyte. An important advantage of this approach is that the evanescent wave interaction occurs within a thin layer of reagent. Thus, it is possible to configure sensors so that the reagent layer is thin enough to allow rapid equilibration with a sample, yet still thick enough to prevent any interaction between the evanescent wave and the sample which might lead to errors and artifacts associated with optical variations from sample to sample.

The existence of the evanescent wave makes it possible to couple light from one fiber to another by bringing the fiber cores close together, such that the evanescent wave from one fiber extends into the core of the other fiber (Figure 5). Fiber optic couplers function as beam splitters and are used in sensors based on interferometry. They can also potentially be used in instrumentation for sensors based on intensity changes.

D. Fiber Types

Materials used in optical fibers must be both flexible and transparent. Furthermore, it must be possible to draw fibers such that a core is surrounded by a physically compatible cladding with a lower refractive index. Both glasses and plastics can be used. Low-melting silicate glasses are convenient for fiber fabrication but are limited to transmission in the visible. Because it is difficult to eliminate impurities, these fibers are not suitable for long-range transmission of light. However, they are inexpensive, and it is possible to prepare fibers with relatively large differences in core and cladding refractive indexes and, thus, a high NA. Figure 6A shows a typical transmission spectrum for this type of fiber. These fibers typically have core diameters of 100 to 200 μm. Larger devices are made by combining

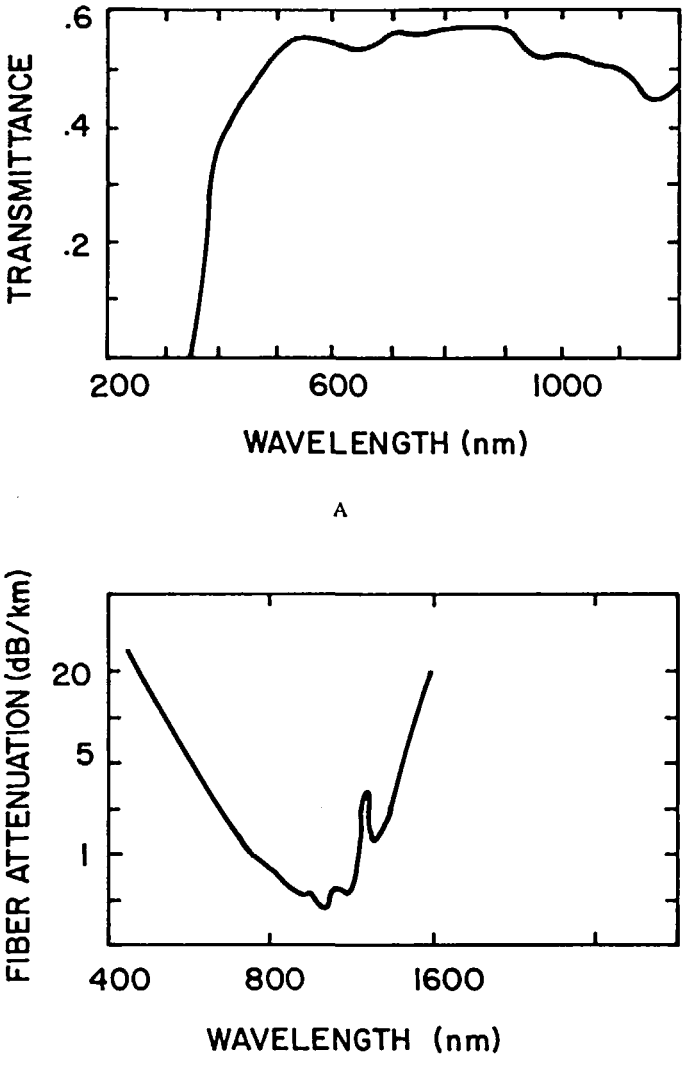


FIGURE 6. Fiber transmission spectra for (A) 0.91-m-long glass-on-glass fiber bundle; (B) typical communications grade fiber; (C) a 0.50-m bundle of plastic clad-fused silica fiber. Note the different wavelength and transmission scale in B. (Spectra A and C are taken from Oriel Catalog.)

fibers into bundles. Bifurcated bundles are prepared by combining two bundles of fiber such that they are randomly mixed at one end but separate at the other end.

High-transmission fibers for communications applications are prepared using silica with a dopant such as B_2O_3, GeO_2, or P_2O_5 to modify the refractive index. To get the required purity, the oxides are prepared by oxidation of highly pure metal halide vapor prior to drawing the fiber. Figure 6B shows a typical transmission spectrum for a communication-grade fiber. In the visible and UV, Rayleigh scattering is the primary mechanism of light attenuation. The inverse fourth-power dependence of scattering on wavelength accounts for the sharp decrease in transmission at shorter wavelengths. Highest transmission is in the near IR, the region of the spectrum used for communications.

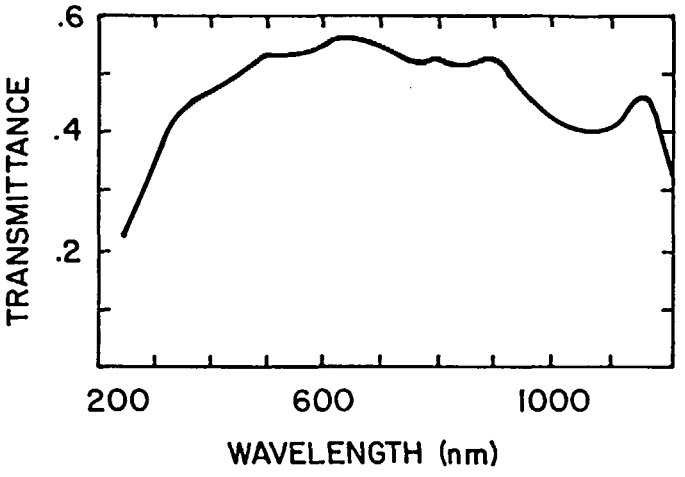


FIGURE 6C.

It should be noted that the fiber attenuation in Figure 6B is expressed in terms of decibels (dB) per kilometer, a unit favored by communication engineers but not particularly familiar to analytical spectroscopists. Attenuation in terms of dB is defined:

$$\text{Attenuation (dB)} = 10 \log I_2/I_1 \tag{12}$$

where I_2 is the power entering the fiber and I_1 is the power leaving the fiber.

Silica fiber can also be coated with a transparent plastic such as a silicone resin or FEP Teflon®. Plastic clad silica, often referred to as PCS, is of interest to analytical spectroscopists because it has a higher NA than communications fibers and is transparent in the UV. Because fused silica is relatively flexible, PCS is commonly available with core diameters of 0.6 and 1 mm as well as smaller sizes. Figure 6C shows a typical transmission spectrum for a PCS fiber.

All-plastic fibers have also been developed. Although they do not transmit light as efficiently as glass and silica fibers, they are rugged and inexpensive and can be fabricated with large core diameters. Transmission characteristics depend on the particular plastics used in the fiber.

Development of new fiber materials remains an active research area. Zirconium fluoride fibers appear to offer promise for further improving transmission characteristics of optical fiber in the near IR as well as extending the available wavelength range to longer wavelengths.

In addition to fiber itself, the needs of the communications industry have led to the development of a variety of accessories including (1) couplers which act as beam splitters, (2) connectors for splicing together two pieces of fiber with minimal light loss, (3) connectors for interfacing fiber to LED sources and semiconductor photodiode detectors, (4) tools for cutting and polishing fiber and removing sections of cladding, and (5) wavelength demultiplexers which resolve multiwavelength light from a fiber into component wavelengths (more commonly known to analytical chemists as monochromators). Because these accessories are designed for use in the near IR, it remains to be established how useful they will be for chemical sensors. One of the goals of the research to develop reagents for sensors can be to find systems that operate at long-enough wavelengths to exploit technologies developed for communications.

It should be realized that commercial fiber is not required for sensors. Total internal reflection will occur at the interface between any transparent solid or liquid and air (since

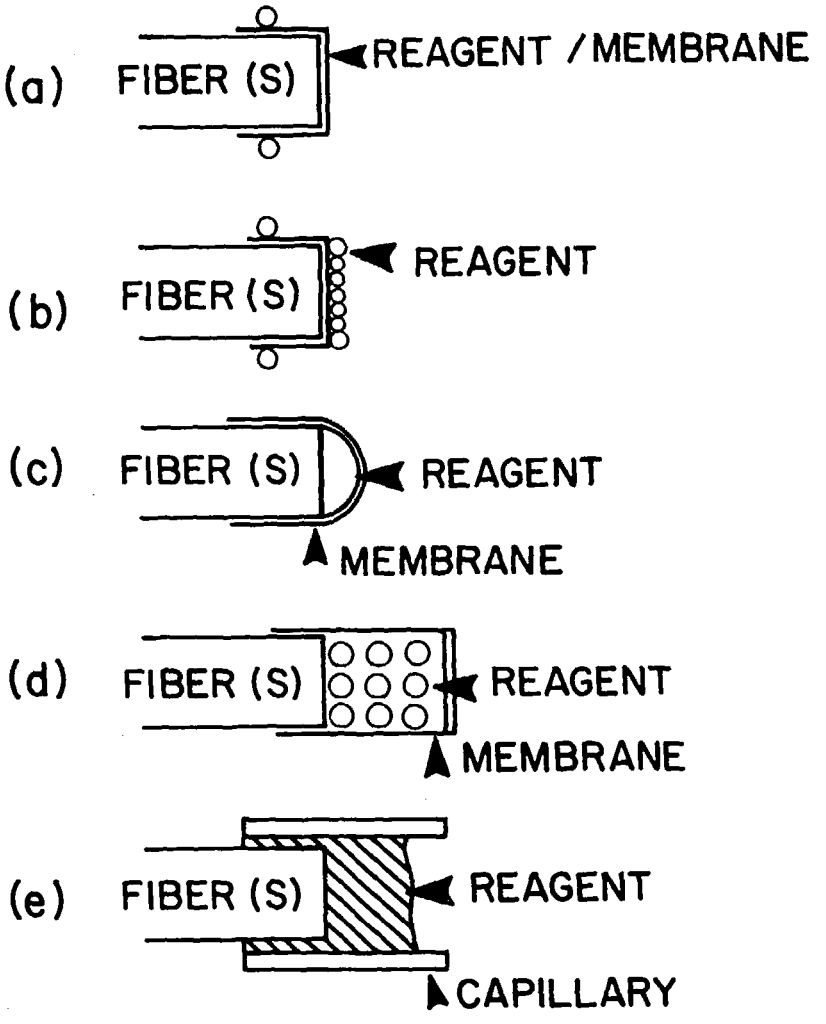


FIGURE 7. Typical extrinsic sensor configurations.

the refractive index of air is essentially equivalent to a vacuum). Glass capillary tubes have been successfully used for chemical sensors.[21] Furthermore, there is no requirement that the "fiber" be round. Rather, there are several advantages to using flat glass slides as the medium for light propagation. A flat surface is easier to coat with a thin layer of reagent. Also, the angle of incidence of source radiation is more easily controlled. (Note that internal reflection spectroscopy as a technique for obtaining spectral information is normally applied to flat surfaces.)

E. Extrinsic Sensors

In extrinsic sensors, fiber optics serve only to conduct light to and from an immobilized reagent phase. There is no need for direct physical contact between the reagent and the fiber. This can be important in applications where frequent changes in the reagent phase may be required or where the actual measurement was to be made in an environment unsuitable for the rest of the optical system. (An example would be high-pressure samples where the indicator would be in the sample and would be viewed optically through a window.)

Typical extrinsic sensor configurations are illustrated in Figure 7. Indicator can be im-

mobilized directly on a membrane which is held against the end of a single fiber or fiber bundle as shown in (a). Alternatively, the indicator can be a powder which is somehow attached to a membrane mounted over the end of a fiber as shown in (b). The arrangement in (b) allows for more efficient mass transfer of analyte into the reagent phase, provided a satisfactory way of gluing the indicator to the membrane can be found. The author has found that Scotch® tape is a convenient substrate for mounting indicator powders reproducibly. However, because it gradually swells in water, changing the optical properties, it can only be used for a few hours.

The indicator may also be confined on the end of a fiber by a membrane as shown in (c). In addition to holding the indicator in place, the membrane can serve other purposes. The permeability characteristics of the membrane can exclude other interfering substances. For example, hydrophobic membranes are used to exclude nonvolatile substances in sensors for measuring gas concentrations in water. The membrane can also help to isolate optically the indicator from the sample so that variations in the optical characteristics of the sample do not affect response. Finally, the membrane can be designed to be compatible with the sample such that fouling does not occur. This is a particularly important issue for sensors designed for in vivo biomedical measurements.

Figure 7d shows another arrangement which has been used successfully for sensors. The indicator phase is confined within a tubular membrane which is permeable to the analyte. The tubular membrane fits over the end of a fiber or fiber bundle. The tubular membrane is capped so that incident radiation is blocked from the sample. When this arrangement is used with single fibers, the diameter of the tube is small enough so that the time required for complete mass transfer of the analyte into the reagent phase is short and the response times are satisfactory. The pH sensor developed by Peterson et al. is a good example of a sensor using this arrangement.[22]

In Figure 7e, the indicator reagent is confined in a capillary tube which fits over the end of the fiber. This can be used when the indicator phase is prepared directly by polymerization.

In most sensors reported to date, the reagent phase is somehow physically confined to the end of the optical fiber(s). It is also possible to directly bond reagent to the fiber itself. Because the end of the fiber is flat, the amount of reagent that can be directly bonded to the surface is limited. A more promising alternative is to bond a thin layer of reagent-containing polymer onto the fiber.[23]

It is important to recognize that most reagent phases are poorly defined media for optical measurements. Most, particularly those involving particles, scatter light to a significant extent. This problem has been dealt with by calibrating reagent phases with solutions of known analyte concentration. However, in the absence of theory to characterize the response of typical reagent phases, it is not known how variations in the refractive index of the medium may influence response.

In sensors based on color changes, the measured parameter is the change in the intensity of light reflected back to a detector. The variation in this intensity as a function of the amount of indicator in a particular form has not been studied beyond the preparation of calibration curves. Sensors based on fluorescence have involved reagent phases that significantly absorb excitation radiation, causing inner filter effects which influence response curves.[24] Modeling of the optical processes occurring in various reagent phase geometries has yet to be reported. As the field matures, one may expect such studies to be undertaken and to provide a basis for optimizing sensor configurations.

Another important issue for extrinsic sensors based on fluorescence is the efficiency of light collection. Much of the emitted radiation is not directly viewed, particularly in sensors involving separate fibers for excitation and emission. The use of a small sapphire ball at the end of a single fiber has been reported to enhance light collection efficiencies by focusing both the excitation and emission radiation.[25,26] The development of higher NA fibers will

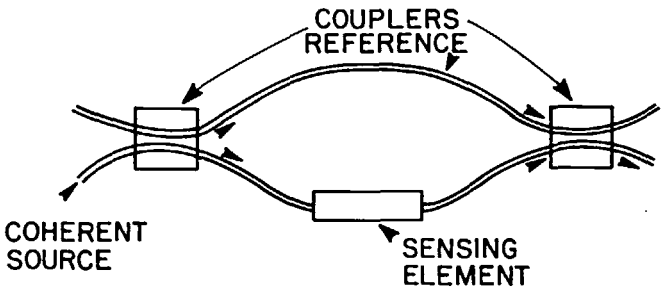


FIGURE 8. Schematic of Mach-Zehnder interferometer.

help to improve light collection. This is particularly important in applications requiring sensor miniaturization since signal levels decrease as the sensor is made smaller.

The response times of extrinsic sensors depend on both the time required for mass transfer of the analyte into the indicator phase and the kinetics of the interaction between the analyte and the indicator. Most devices developed to date have involved rapid reactions, so that mass transfer has been the dominant factor establishing response time. It has been generally recognized that the smaller the distance over which mass transfer must occur, the faster the response. However, this consideration has yet to be dealt with in a quantitative way.

F. Intrinsic Sensors

1. Interferometric Sensors

Interferometric sensors are based on changes in the phase of light transmitted through single-mode optical fiber. Figure 8 shows a schematic of a sensor based on a Mach-Zehnder interferometer. Coherent monochromatic light is introduced to one branch of the interferometer. The first coupler (or beamsplitter to a spectroscopist) allows half of the light to enter the other branch. At the second coupler, the light in the two branches of the fiber is combined. Constructive interference is observed if the length of both fibers between the couplers is equal or differs by a multiple of the source wavelength. The parameter to be sensed interacts with one branch of the interferometer, causing its optical properties to change so that there is a shift in the phase of the transmitted light. The other branch serves as a reference. As the value of the measured parameter changes, the detector records and counts a series of maxima and minima, as light from the two branches of the interferometer goes alternately in and out of phase. The use of interferometric fiber optic sensors to detect physical parameters, including pressure fluctuations, temperature, and magnetic field changes, has been reviewed.[27]

The first reported interferometric chemical sensor responds to the partial pressure of hydrogen.[28] The sensing element in this device is a coating of palladium on the outside of the fiber. The higher the partial pressure of hydrogen, the more that is adsorbed in the palladium. This constricts the fiber and modifies the phase of light transmitted through the fiber. The sensor is reversible and responds to a wide range of partial pressures.

An interferometric fiber optic sensor has also been coupled to an enzyme-catalyzed process leading to a temperature change.[29]

Interferometric fiber optic sensors have several attractive features. The instrumentation is simple and inexpensive. In addition to single-mode fiber and the couplers, it includes a semiconductor photodiode as the detector and a helium-neon laser or cw diode laser as the source. These sensors have been demonstrated to be extremely sensitive. Unfortunately, this is also a source of difficulty. Chemical sensors will also respond to changes in temperature and pressure (even though the reference arm of the interferometer should compensate for these changes). At this time, it is difficult to predict how useful interferometric fiber optic chemical sensors will prove to be.

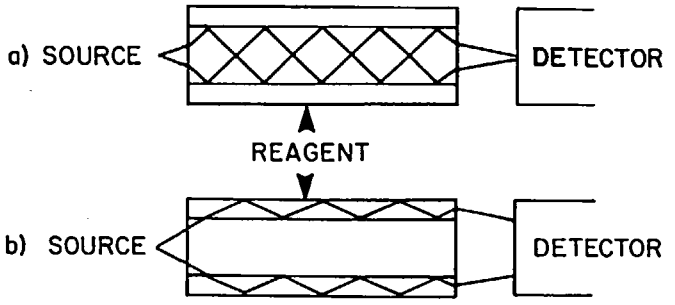


FIGURE 9. Schematic of chemical sensor in which the reagent is a thin coating on an optically transparent core. In (a), the refractive index of the cladding is less than that of the core and the reagent is probed by internal reflection spectroscopy. In (b), the refractive index of the reagent is greater than the core, so the reagent layer acts as the waveguide.

2. Reagent as Cladding

Figure 9a shows schematically a sensor in which the normal cladding has been removed and replaced by a thin layer of reagent phase. The possibility of using reagent-coated fibers was first proposed by Hardy et al.[30] and has subsequently been used in devices based both on color and fluorescence changes and on changes in refractive index. In this arrangement, the fiber is serving as an internal reflection element for internal reflection spectroscopy. Because penetration depths do not exceed a few wavelengths (Figure 3), except right at the critical angle, reagent layers a few micrometers thick are sufficient to isolate incident radiation from the sample, while still being thin enough to allow for efficient mass transfer and rapid response times.

While reagent-coated fibers have attractive properties as sensors, it is important to be aware that an optical fiber is far from ideal as an internal reflection element. As shown in Figures 3 and 4, both penetration depths and evanescent wave intensities increase as the angle of incidence approaches the critical angle. Accordingly, internal reflection spectroscopy normally employs incidence angles close to the critical angle in order to maximize sensitivity.[19] However, with a cylindrical internal reflection element, it is not possible to maintain a single incidence angle. The problems of controlling the incidence angle become even more severe when the cylinder is a fiber with a very small diameter. Furthermore, even if light is successfully directed into a fiber at an angle, processes can occur within the fiber to redistribute the light energy among various modes and to reduce effectively the angle of incidence. Bends in the fiber facilitate this process. The practical consequence is that the coated area of the fiber has to be relatively long for adequate sensitivity (10 cm is typical in published reports of chemical sensors). In devices based on fluorescence, the use of evanescent wave excitation results in inefficient excitation and relatively weak fluorescence.

Devices in which the reagent phase has served as the cladding include sensors for gas phase ammonia, water based on color changes,[21,31] and immunosensors based on fluorescence.[32,33]

One can also base devices on changes in the refractive index of the cladding. The requirement is that the analyte cause an increase in the refractive index of the cladding, but not to the point where it exceeds the refractive index of the core. Examples of this approach include an aromatic hydrocarbon sensor[34] for aqueous samples and a gas phase alkane sensor.[35]

3. Reagent as Core

It is also possible to prepare sensors in which a thin layer of reagent phase is coated on a substrate with a lower refractive index as illustrated in Figure 9b. In this case, light can

be propagated directly through the reagent film, which is effectively serving as the "core" of an optical fiber. For this to work, the refractive index of the sample must also be lower than the refractive of the reagent layer. This is a far more efficient way of coupling light into the reagent phase, leading to improved sensitivity.

The idea of propagating light through a reagent layer was first proposed by Hardy et al.[30]

III. INSTRUMENTATION AND MEASUREMENT CONSIDERATIONS

Although the field of chemical sensors based on fiber optics is still quite young, a great variety of instrumental arrangements have already been used. This reflects the fact that investigators in this area have had a variety of objectives in developing sensors.

It is important to recognize that instrumentation used for optical sensors and the chemistry are intimately related. Specifically, the wavelength and intensity requirements of a particular reagent phase determine what kinds of instrumental arrangements are possible, which in turn determine the cost of a sensor. Conversely, if a specific application requires certain instrumentation (e.g., long-distance transmission of light through optical fiber requires an extremely intense source such as an argon ion laser), then this places constraints on the available chemistries for developing immobilized reagent phases.

A. Sensors Based on Intensity

The NA and transmission properties of a fiber determine its ability to accept light and transmit it to another location. The major instrumental problem to be confronted in making intensity measurements is dealing with scattered and reflected radiation. Some of the incident light is reflected at the interface where it enters the fiber as well as the interface, where it leaves the fiber and enters the reagent phase. The percentage of reflected light depends on the difference in refractive indexes on either side of interfaces. In addition, many reagent phases involve media such as particles which efficiently reflect and scatter light.

The problem of reflected light at the interfaces where source radiation enters and exits the excitation fiber can be avoided by using the separate fibers to conduct light to and from the reagent phase. In this context, reagent phase scattering and reflection are helpful in redirecting part of the incident light back into the fiber used to transport light to the detection system. However, since this increases the size of the sensor, considerable effort has been devoted to developing single-fiber devices using luminescence-based reagent phases and appropriate optics to distinguish scattered and reflected excitation radiation from fluorescence on the basis of wavelength.

1. Systems Involving Bifurcated Fiber Optics

Several investigators have used separate fibers or fiber bundles to transmit light to and from an immobilized reagent phase as shown in Figure 10. In this arrangement, the detection system is not exposed to light reflected at the interfaces where the source radiation enters and exits the excitation fibers. This is particularly important for sensors based on changes in reagent phase absorption since in these devices measured light cannot be distinguished from incident light on the basis of wavelength. While single fiber absorbance measurements are possible, they require a correction for reflected and scattered source radiation.[36] The disadvantages of using separate fibers for transmitting light to and from a reagent phase are that (1) the sensor is necessarily larger and requires twice as much fiber and (2) there is a volume at the surface of the sensor which is not within the acceptance angle of both excitation and emission fibers and thus is effectively not viewed by the detection system.

Most bifurcated fiber optic sensors reported in the literature have used incandescent sources with filters for wavelength selection and glass or plastic fiber,[3,22,37,38] although monochromators can also be used.[39] Reagent phases that change optical properties in the visible have

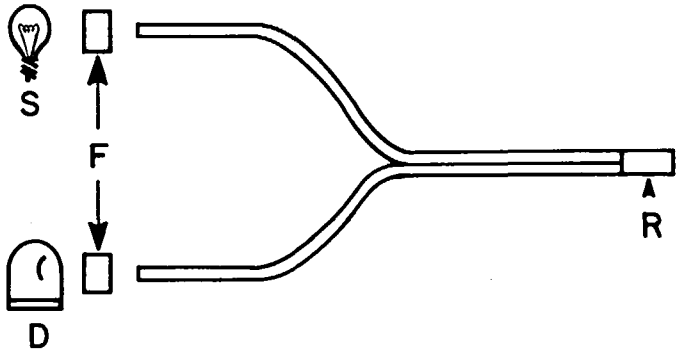


FIGURE 10. Schematic of bifurcated fiber optic chemical sensor. S = source, F = filters, R = immobilized indicator reagent, D = detector.

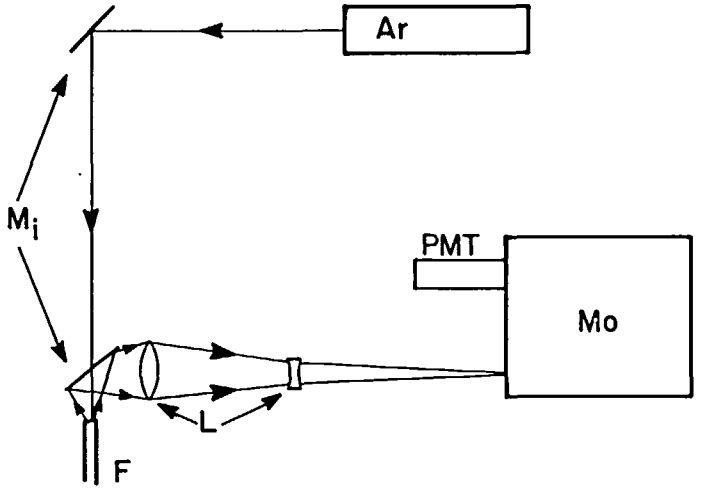


FIGURE 11. Diagram of single-fiber remote fiber spectrofluorometer. AR = argon ion laser, Mi = mirrors, L = lens, Mo = monochromator, PMT = photomultiplier tube, and F = single optical fiber.

been used to avoid the additional expense that would be required with a xenon or mercury arc lamp as the source and fused silica fiber. Sensor instrumentation designed for measurements at more than one wavelength have included either a filter wheel or some other provision for changing filters[3,22,40] or have used a dichroic filter to split the emission beam into two wavelength ranges for simultaneous measurement by two detectors.[41] The dichroic filter approach is preferred because it provides simultaneous detection of two wavelengths and does not require moving parts. However, it requires that the sensor be based on emission from two different wavelengths. It is not applicable if illumination at two different wavelengths is required.

2. Systems Involving Single Fibers

Extrinsic single fiber sensors based on intensity changes have employed luminescent reagent phases because this allows the luminescence signal to be resolved from reflected and scattered excitation radiation on the basis of wavelength. Various arrangements have been used. Figure 11 diagrams instrumentation developed at the Lawrence Livermore Na-

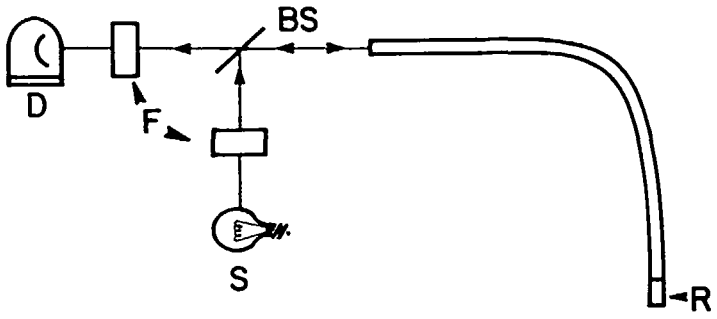


FIGURE 12. Spectrometer for single-fiber measurements using a beamsplitter (BS). R = indicator reagent, S = source, F = filter, and D = detector.

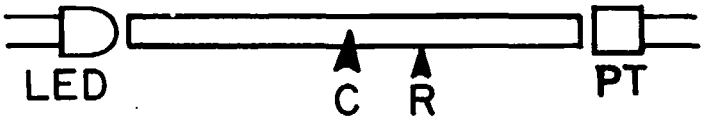


FIGURE 13. Schematic of LED-based chemical sensor. C = capillary tube acting as a waveguide, R = thin coating of reagent on the surface on the tube acting as the cladding, and PT = phototransistor serving as the detector.

tional Laboratory for remote fluorescence measurements over distances as great as several hundred meters.[26,42] An argon ion laser is used as the source because of the need for high intensity to overcome transmission losses in the fiber. Fluorescence returning through the fiber is focused into a double monochromator which resolves fluorescence from scattered excitation radiation. Rather than using a beam splitter, this instrumentation exploits the highly collimated character of the laser beam. Incident light goes through a small hole in the mirror which reflects light emerging from the fiber. This enhances collection efficiency and discriminates against light reflected at the interface where radiation enters the fiber. Even with this arrangement, however, a double monochromator is required to resolve fluorescence from the more intense reflected and scattered excitation radiation. Another feature of this instrument is the use of Raman scatter from the fiber itself as a reference signal to compensate for instrumental fluctuations.

Others have used a more conventional beam splitter arrangement, as shown in Figure 12.[3,43-45] The use of a dichroic beamsplitter which has a high reflectivity for the excitation beam and high transmittance for the emission beam helps in discriminating against excitation radiation. The use of fiber optic beamsplitters based on evanescent wave couplers would simplify alignment. However, they are less efficient than dichroic beam splitters at discriminating against excitation radiation.

Intrinsic fiber optic sensors are necessarily single-fiber devices. The source is usually at one end of the fiber and the detection system at the other. Of particular interest are the devices developed at the Naval Research Laboratory for vapor detection, shown schematically in Figure 13.[21] The source is a pulsed LED and the detector is a phototransistor. No other optical components are required. Because the complete sensor system is highly compact and very inexpensive, it can be used for field measurements. Another feature of this system is the use of a coated glass capillary tube as the "optical fiber". Because the tube is rigid, light injected at angles near the critical angle is more likely to stay at the same angle after multiple reflections, thus enhancing the sensitivity of the internal reflection measurement. In curved fibers, light tends to couple to other modes corresponding to angles further from the critical angle.

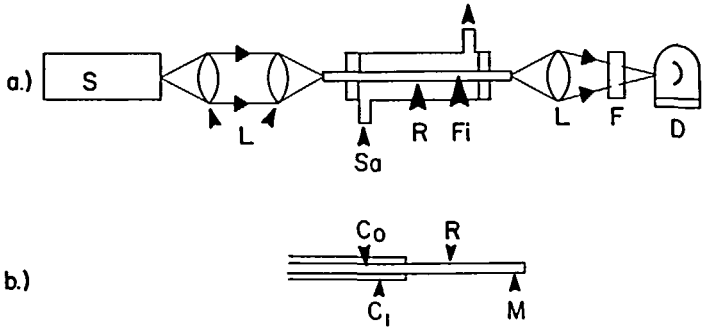


FIGURE 14. Instrumentation for single-fiber-based internal reflection measurements. In (a), S = source, L = lens, Sa = sample, R = indicator reagent coated on fiber (Fi), F = filter, and D = detector. In (b), Co = core, Cl = cladding, and M = mirror on the end of the fiber to reflect light back through the fiber.

Single-fiber total internal reflection fluorescence measurements have been made using optical arrangements shown in Figure 14. In the arrangement of Figure 14a, excitation and detection systems are at opposite ends of the fiber.[33] A cutoff filter is used to block excitation radiation transmitted through the fiber.

In the arrangement of Figure 14b, excitation and detection systems are positioned on the same side of the fiber.[45] The distal end of the fiber is coated with a mirror so that it reflects light back along the fiber to a beamsplitter. The cladding at the distal end of the fiber is removed and can be coated with a luminescent reagent phase. This arrangement makes it easier to develop an intrinsic sensor that can be inserted into a sample.

In devices with source and detection systems on opposite ends of the fiber, an insertable sensor can be made by coiling the section of fiber with the active coating. In addition to making the sensor element more compact, coiling enhances the interaction of the incident light with the reagent phase by changing the angle of incidence at which the light strikes the core/cladding interface, so that it is closer to the critical angle. Some of the radiation, however, is lost into the cladding because the angle of incidence will be less than the critical angle.

B. Sensors Based on Interferometry

While most chemical sensors reported to date have been based on intensity changes, there has been considerable interest in the development of interferometric sensors for physical parameters.[27] Most sensors are based on the Mach-Zehnder interferometer shown in Figure 8. Coherent light from either a helium-neon laser or a cw diode laser is injected into one arm of the interferometer. In the coupler, this light distributes equally among the two branches of the interferometer. In the Mach-Zehnder interferometer, the beams from the two arms recombine in the second coupler. They interfere constructively if the difference in the length of the two branches of the interferometer is equal to a multiple of the wavelength, and destructively if it is not. Because interferometry requires that the beam remain coherent through a considerable length of fiber, it can only be implemented with a single-mode fiber.

Sensing is based on changes in the pathlength of one branch of the interferometer which affects the kind of interference observed where the beams recombine. The other branch serves as a reference. Small changes in the pressure experienced by the core of the fiber are sufficient to cause observable changes. This pressure can be transmitted through the cladding and the protective coating that surrounds the cladding. While pressure is the parameter that is actually sensed, a variety of other parameters can be sensed based on their influence on pressure. For example, expansion accompanying increases in temperature causes pressure changes.

The sensing branch can be coated with a layer of material that interacts with the measured parameter to produce a detectable pressure change. This material can be an immobilized reagent phase that interacts with an analyte. Two examples have been reported. The first is a sensor for hydrogen using palladium as the reagent phase,[28] with adsorption of hydrogen causing swelling of the palladium which exerts a pressure change. The second involves a fiber coated with immobilized enzyme.[29] The increase in temperature due to the reaction leads to a detectable signal.

Sensors for physical parameters based on interferometry have attracted considerable attention because they combine simplicity with extreme sensitivity. This reviewer, however, finds it difficult to confidently predict their potential for chemical sensing. Because they involve the measurement of a single parameter, they have an inherently lower information content than devices based on the measurement of intensities at two or more different wavelengths. There is no way to confirm the performance of the sensor other than recalibration. Thus, they are more likely to be useful with reagent phases that can be relied upon to be highly stable. Another issue involves the influence of pressure and temperature fluctuations on response. The detection limit in a practical context would be dependent on the ability of the reference branch of the interferometer to null out these fluctuations.

C. Measurement Considerations

1. Luminescence vs. Absorption

Indicators for optical sensors have involved both changes in luminescence properties and changes in absorption. The two offer different advantages and limitations, so the choice of which type of indicator to use may depend upon the specifics of a particular application.

In sensors based on absorption, the measured parameter is the attenuation of source radiation by the reagent phase as a function of analyte concentration. Because the measurement is made at the same wavelength as the incident radiation, the actual intensities observed are generally considerably larger than is the case with luminescence-based sensors (although the extent to which this is true depends on the specifics of the optical arrangement). This can be exploited to analytical advantage in several ways. The sensor can be miniaturized more readily. Alternatively, for a given sensor size, it is easier to implement sensors using inexpensive LED sources, and the rate of any photodegradation processes will be slower.

In most other respects, luminescence offers advantages relative to color changes. Luminescence measurements are more readily implemented with single fibers. Luminescence measurements offer an inherently greater dynamic range than reflectance measurements. Furthermore, the functional dependence of luminescence intensity on analyte concentration is more easily defined than is the case with reflectance. This is important in knowing how small changes in indicator concentration will affect measured intensities and how sensitive the ratios of intensities at two wavelengths will be to changes in the amount of indicator.

It should be noted that indicators based on luminescence are likely to be used under conditions where there are "inner filter" effects. The excitation, and possibly also the emission, radiation is attenuated by absorption as it passes through the indicator phase. If the color of an indicator phase can be observed visually, then significant absorption of the excitation radiation is probably taking place. Inner filter effects help to shield the excitation radiation from the sample and decrease the likelihood of error due to variations in sample optical properties. They also affect the magnitude and shape of response curves, necessitating a separate calibration for every sensor unless the amount of indicator can be reproduced from device to device. Even in sensors based on intensity ratio measurements, changes in the amount of indicator may have a differential effect on the magnitude of the inner filter effect at the two wavelengths. This is illustrated by calculated response curves for a ratio-based luminescence pH indicator shown in Figure 15. It should also be noted that the extent of inner filter effects on fluorescence will depend on the geometry of the reagent phase. Longer pathlength arrangements are more likely to be subject to inner filter effects.

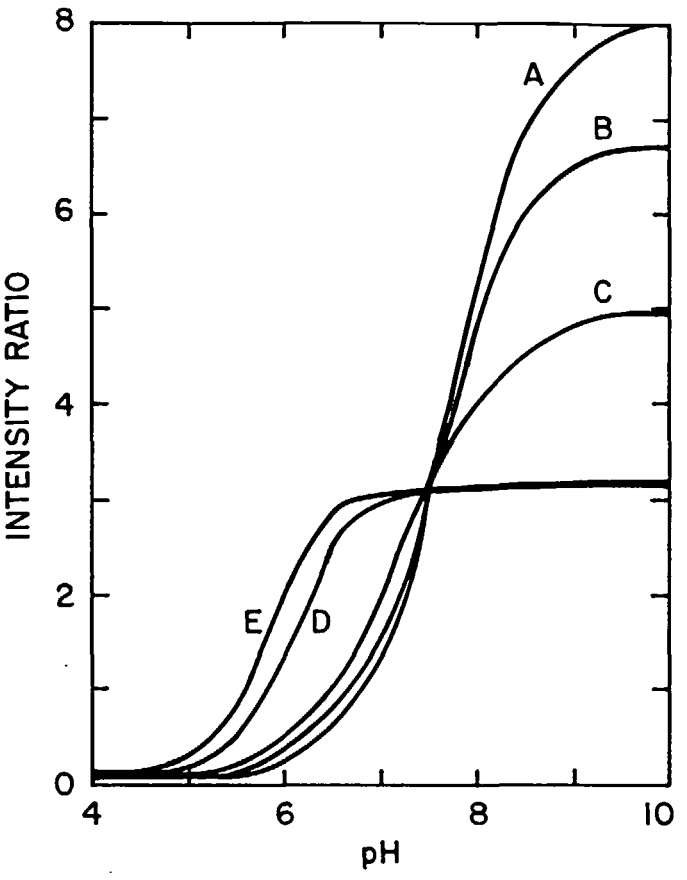


FIGURE 15. Calculated response vs. pH for varying amounts of fluorigenic pH indicator on a membrane. "A" corresponds to a low concentration of indicator which does not significantly absorb excitation radiation. "B" through "E" correspond to increasing levels of indicator. (See Reference 24 for Equation and indicator used to calculate these curves.)

Inner filter effects can be exploited to make indicator phases that are based on absorption but involve the measurement of fluorescence. This is accomplished by adding a fluorophor to the reagent phase. The fluorophor is chosen so that its emission properties are not influenced by sample composition and its emission and/or excitation spectra overlap with the absorption spectrum of the indicator. Under these conditions, the intensity of fluorescence varies with changes in the absorbance of the indicator at the excitation/emission wavelengths. This approach has been demonstrated in a single fiber pH sensor using phenol red as the indicator and eosin as the fluorophor.[46] The advantage of this approach is that it greatly simplifies the construction of single fiber sensors based on absorption changes.

If the absorption spectrum of an indicator overlaps the emission spectrum of a fluorophor in the indicator phase, one can also get attenuation of fluorescence due to Forster energy transfer, provided the average distance between absorber and fluorophor is short enough for significant energy transfer to occur. This appears to be the dominant effect in the phenol red/eosin pH sensor.[46]

2. "Reference" Intensity

As emphasized elsewhere in this review, one of the advantages of the optical approach to sensing is the possibility of relating analyte concentration to the ratio of intensities at two

different wavelengths. One of these intensities must change with analyte concentration and can be thought of as the "analytical" intensity. The other can either be constant or vary with analyte concentration in a different way than the analytical intensity. This will be referred to as the "reference" intensity. The primary purpose of the reference intensity is to compensate for changes in any of the variables other than analyte concentration that affect the value of the analytical intensity. These include fluctuations in source intensity, electronic drift in the detection system, changes in either the amount of indicator or the optical properties of the indicator phase, and changes in "bending loss" of intensity due to bends in the fiber which change the angle at which the transmitted intensity strikes the core/cladding interface.

The simplest reference is to directly measure source intensity at the analytical wavelength to compensate for source fluctuations. A more attractive approach is to use a reference intensity which follows the same optical path as the analytical intensity since this will compensate for changes in bending losses and for variations in the optical properties of the indicator phase. Back-scattered excitation radiation has been used as a reference in an oxygen sensor based on fluorescence.[41] Alternatively, a fluorophor that is insensitive to analyte concentration can be incorporated into the indicator phase to provide the reference signal.[3] In sensors based on absorption, the reference signal can be transmitted intensity at a wavelength where no absorption occurs.[22] Although these types of reference signal significantly improve the stability of sensors with respect to calibration, they have some limitations. They do not account for loss of indicator due to either leaching from the indicator phase or decomposition. Furthermore, they are subject to error associated with differences in the wavelength dependence of any variable that changes with time.

Where feasible, the best approach is to incorporate the reference signal into the immobilized reagent itself. For example, pH sensors have been based on the ratio of luminescence intensities for two different excitation wavelengths[24] and on the ratio of luminescence intensities at two different emission wavelengths.[47] This type of reference signal compensates not only for instrumental fluctuations and variations in indicator phase optical properties, but also for any loss of indicator. It is subject to error, however, if loss of indicator leads to inner filter effect changes that are different at the two measurement wavelengths. In sensors based on absorption, it is possible to measure the ratio of intensities transmitted or reflected at two different wavelengths.[48] However, this does not perfectly compensate for indicator loss because the relationship between transmitted/reflected intensity and chromophore concentration is nonlinear.

The availability of indicators with appropriate reference intensities creates the possibility of sensors which are "precalibrated" in the factory rather than those which have to be calibrated by the user. This is effectively what is done with pH indicator papers where the "spectroscopic" measurement is the visual perception of color which depends on the ratios of intensities reflected by the acid and base forms of the indicator, and the "precalibration" is the color chart on the indicator container. Because of the convenience they offer, there would undoubtedly be many applications for suitably precalibrated sensors. However, the development of precalibrated sensors will require that the amount of indicator be at least fairly reproducible from device to device since most reference intensities do not completely compensate for variations in indicator amount, as noted in the following section.

3. Indicator Amount

An important variable that has not received much attention is the amount of indicator in the reagent phase. Indicator amount can affect response in several ways. The amount of indicator must be significantly less than the amount of analyte in the sample. Otherwise, when a sensor is exposed to a changed concentration, the amount of analyte combining with or dissociating from the indicator may be large enough to alter significantly the amount of analyte in the sample. This will lead to error since the amount of analyte in the sample at

equilibrium will differ from the initial amount of analyte. For example, an optical pH sensor with a large amount of indicator will be subject to error when measuring the pH of a sample with low buffer capacity.

The amount of indicator will also affect the magnitude of the observed optical signals. In the case of indicators that change colors, the relative change in reflected or transmitted intensity per unit change in analyte concentration will depend on the amount of indicator. The relative change will tend to be greater if the absorbance is relatively low, i.e., in the range of 0.1 to 0.5. The actual optimum will depend on the functional relationship between reflected/transmitted intensity and the amount of indicator chromophore.

In the case of luminescence-based indicators, luminescence increases with the amount of indicator up to a point. In the absence of concentration quenching, luminescence intensity is governed by the inner filter effect. No further increase in luminescence is observed once the amount of indicator is sufficient to absorb essentially all the excitation radiation. This situation has been illustrated experimentally[24] using a pH indicator electrostatically bound to an ion exchange membrane. If the indicator is subject to concentration quenching, then the average distance between immobilized indicator molecules becomes an important parameter. Decreases in luminescence with increasing indicator concentration may be observed even at relatively low amounts of indicator.

4. Luminescence Lifetime Measurements

An important class of indicators is based on luminescence quenching by an analyte. Oxygen, in particular, is an important analyte that quenches luminescence efficiently. Static quenching involves a ground-state interaction between quencher and analyte, while dynamic quenching involves an excited-state interaction. Dynamic quenching is accompanied by a shortening of the luminescence lifetime.

In sensors based on dynamic quenching of indicator luminescence, analyte concentration can be related to either intensity or lifetime. Lifetime measurements have yet to be exploited because the required instrumentation is complex and costly. This is particularly true for fluorescence lifetimes which typically fall between 1 and 10 nsec. However, lifetime measurements offer important advantages relative to intensity measurements. They are not affected by changes in the optical properties of the indicator phase or by loss of indicator due to leaching or degradation. Furthermore, since time bases are very stable, lifetime measurements are not subject to instrumental drift even over extended intervals.

Given the advantages of lifetime measurements, there is considerable incentive to develop indicators with relatively long luminescence lifetimes, i.e., microseconds or longer, such that the above advantages can be realized at modest costs.

While lifetime measurements through short lengths of fiber do not pose any special problems, lifetime measurements through extended lengths of multimode fiber are distorted both by modal dispersion (see Section II.A) and chromatic dispersion. The latter is due to the decrease in refractive index with increasing wavelength, which causes longer wavelengths to travel through fibers slightly faster than shorter wavelengths.

IV. REAGENT PHASES FOR OPTICAL SENSORS

The function of the reagent phase is to interact with the analyte so as to render it detectable through optical fiber. Specific roles that the reagent phase can fill include the following:

1. *Reactant* to directly or indirectly react with analyte to form an optically detectable product
2. *Adsorbent/extractant* to preconcentrate an optically detectable analyte in the field of view of an optical fiber (the adsorbate may be directly detectable optically or it may modify optical properties of the adsorbent such as its refractive index)

Table 3
ANALYTICAL REACTIONS FOR OPTICAL SENSORS

Stoichiometric	Indicator
$A + R \rightarrow AR$	$A + R \leftrightarrow AR$
Complete	Reversible
Keq independent	Keq dependent
Measures amount	Measures concentration
For sensor	For sensor
Integrating	Reversible
Mass transfer dependent	Equilibrium

3. *Catalyst* to accelerate the rate at which analyte is converted to an optically detectable product
4. *Substrate* for the detection of catalysts. Each of these is considered separately in the following subsections.

A. Reactant

Reactions used for analytical spectroscopy may be classified as "stoichiometric" or "indicating". Properties of these two classes are summarized in Table 3.

1. Stoichiometric Reactions

In practice, most quantitative spectrophotometric and fluorometric methods involve "stoichiometric" reactions. The analyte is reacted with an excess of reagent and is completely converted to an optically detectable product. The volume of sample needs to be known in order to determine analyte concentration because the optical measurement is related to the amount of product formed which equals the amount of analyte initially present (multiplied by an appropriate mole ratio factor).

Stoichiometric reactions can and have been adapted to sensors. The reagent phase contains all the components required for product formation. However, it is important to recognize that this type of reaction involves serious disadvantages. Since the reaction goes to completion, all analyte that comes in contact with the reagent phase reacts to form product. The amount of product continually increases with time until the supply of reagent is exhausted. The measured signal is related to the integrated amount of analyte that has contacted the reagent since the sensor was first contacted with sample. This limits the lifetime of the sensor and greatly complicates calibration. The situation is particularly difficult if the measured optical parameter does not vary in a linear, or at least a predictable, way with the amount of product formed. It is naive and incorrect to assume that all chemistries used for laboratory photometric analysis can be adapted for continuous *in situ* measurements using fiber optics.

Sensors based on stoichiometric reactions also require controlled conditions with respect to mass transfer of analyte to the surface of the reagent phase. (The situation is similar to amperometric electrochemical measurements where analyte is completely reacted at the surface of an electrode.)

In practice, most "sensors" based on stoichiometric reactions are effectively "one-shot" devices that can only be used on a throwaway basis. The opinion of this reviewer is that there is limited merit in using fiber optics with reagent phases used on a one-shot basis. While they can be used for spectroscopic measurements *in situ,* they have to be prepared with sufficient reproducibility so that response is the same from sensor to sensor, otherwise, the sensor cannot be satisfactorily calibrated. The necessary reproducibility is more readily

achieved if the "one-shot" reagent phases are formulated as flat strips or slides. There are exceptions to these generalizations. In some situations, the reagent phase can be "reset" to zero. For example, it may be possible to "recharge" the reagent phase, by removing it from the sample and exposing it to different conditions where the analytical reaction is reversed, renewing the reagent phase.[33,49,50] Another way to reset the reagent phase has been proposed by Tomas Hirschfeld of the Lawrence Livermore National Laboratory. This involves irradiating the reagent with a pulse of light of sufficient intensity to bleach the optically detectable product. Note, however, that this approach does not renew the reagent, so the lifetime of the sensor will be limited by the quantity of available reagent.

Another important exception to the aforementioned generalization involves sensors based on reagents that react with an analyte, producing chemiluminescence. The measured intensity of light depends on the number of photons emitted per unit time, which in turn depends on the number of molecule reactions per time or the rate of the reaction. At steady state, the rate at which analyte enters the reagent phase equals the rate at which it is reacted to produce light. If reagent is in sufficient excess, the steady-state intensity is a measure of analyte concentration. The build-up of product does not directly affect the measured intensity, although it may indirectly influence intensity by affecting either the rate or the efficiency of the chemiluminescence reaction. Chemiluminescence sensors responding to peroxide,[51] oxygen,[52,53] and chlorine dioxide[54] have been reported.

Devices based on stoichiometric reactions have been proposed in which excess reagent reservoir diffuses out at a constant rate, meeting analyte in a zone where product formation can be observed optically through fiber optics.[26] If the reagent reservoir is large enough relative to the rate of consumption, such a device could function for a considerable length of time. However, sensors based on this approach would require careful control of mass transfer of both analyte and reagent, not to mention transport of product away from the sensor surface. The reviewer regards this approach as a form of flow analysis and believes it would be more effectively and reliably implemented using conventional flow instrumentation.

2. Indicator Reactions

Indicator reactions are most familiar to the analytical chemist in the context of titrations and indicator strips commonly used for semiquantitive estimates of pH. The concentration of indicator is much lower than that of the analyte so that analyte concentration is not significantly affected. The range of concentrations detected by the indicator depends on the equilibrium constant for the reaction of the indicator with the analyte. (For example, the pH range sensed by an acid-base indicator depends on the pKa of the indicator.)

Indicators have not generally been used for quantitative laboratory measurements because they do not offer the same sensitivity as stoichiometric reactions and they are subject to error if there are sample-to-sample variations which affect the equilibrium constant of the indicator reaction. However, in the context of sensors, indicators are strongly preferred because they provide continuous, reversible response without perturbing the sample. The equilibrium response of indicators does not depend on mass transfer (although the time required to reach equilibrium, i.e., the response time, is mass transfer dependent).

Most indicators developed for applications involving visual detection have the property that they change color upon interacting with the analyte. What is actually observed is the change in color rather than a change in the "intensity" of a single color. The primary source of information is the spectral shift accompanying the reaction between indicator and analyte. The color perceived by the analyst depends on the relative amounts of "free" vs. "combined" indicator. The same principle applies to sensors. If there is a spectral shift accompanying the indicator reaction, then there is potentially sufficient spectral information to determine the relative amounts of "free" and "combined" indicator by measuring the ratio of intensities at two wavelengths. As is evident from examining a rearranged form of the

Table 4
EXAMPLES OF DIRECT INDICATOR SYSTEMS USED FOR OPTICAL SENSING

Reaction type	Analyte	Ref.
Acid-base	pH	22, 24
	Carbon dioxide	3, 55
	Ammonia	21, 56
Complexation	Al (III)	57
Ligand exchange	Water vapor	31, 58
	Oxygen	48
Luminescence quenching	Oxygen	3, 22
	Halides	59

equilibrium expression for the reaction of the indicator with analyte, this ratio is directly related to analyte concentration:

$$[A] = Kin[InA]/[In] \quad (13)$$

where A = analyte, In = indicator, and Kin is the equilibrium constant for the reaction. Since this is a ratio measurement, it is inherently more stable with respect to drift. One of the primary advantages of the optical approach is the possibility of developing sensors which have long-term stability with respect to calibration because they are based on ratio measurements.

It should also be noted that the ratio of InA to A is independent of the amount of indicator. Because of this, it should be possible to design "precalibrated" sensors based on intensity ratio measurements. The analyst can proceed directly to the analysis and use a calibration provided by the manufacturer. This is not practical with currently available electrical chemical sensors.

A majority of chemical sensors reported to date involve indicators. Table 4 lists some examples organized according to the type of reaction involved. Most often, the indicator reaction involves a ground-state interaction. However, in the case of dynamic quenching of luminescence, e.g., by molecular oxygen, an excited state interaction occurs.

3. Indirect Indicators

It is possible to classify indicators as "direct" and "indirect". A "direct" indicator combines with an analyte as shown in Table 3 to form a product in a reaction that causes a change in the optical properties of the indicator. An "indirect" indicator, as defined here, includes two or more components whose interaction varies with the concentration of the analyte.

Optical sensors based on competitive binding, first proposed and demonstrated for glucose sensing by Schultz and co-workers,[44,60] provide an example of indirect indicators. The components of the glucose-sensing indicator include dextran and concanavalin A, a carbohydrate-binding protein. The reaction involves the competitive displacement of dextran from a limiting amount of concanavalin A by glucose:

$$\text{glu} + \text{dex-con A} \leftrightarrow \text{dex} + \text{glu-con A} \quad (14)$$

An important feature of this approach is the ability to vary the tendency for glucose binding by varying the excess concentration of dextran. In general, indirect indicators offer the

potential of varying the effective equilibrium constant which, in turn, controls the range of concentrations to which the sensor responds. This is not possible with direct indicators.

To date, relatively little has been done with indirect indicators. In addition to sensors based on competitive binding, an indicator system responding to alkali metal ions based on competitive ion pair extraction has been reported.[61] However, because of the ability to vary the effective equilibrium constant and because of limitations in the number of possible direct indicators, the reviewer feels that the development of indirect indicators will be an important growth area. It should be noted, however, that because the chemistry involves more than a single one-step reaction, the kinetics of indicator response may be slow, leading to unacceptably slow sensor response times. This is a particularly difficult problem for sensors based on competitive binding, where the rate of dissociation of analyte or labeled analyte analog from the selective reagent is often slow.

B. Adsorbent/Extractant

The reagent phase can adsorb or extract analyte, bringing it in the field of view of an optical fiber where it can be detected directly. The adsorption process may be required to increase the effective analyte concentration so that it is detectable optically. The extent to which analyte is preconcentrated depends on the partition coefficient. Adsorption/extraction may also serve to separate analyte from an optically variable background which would otherwise interfere with its measurement.

Since partitioning between phases is inherently a reversible process, sensors based on adsorption/extraction can respond on a continuous equilibrium basis. Examples reported to date include a sensor for alkanes in the gas phase based on extraction in a thin polymer layer[34] and a sensor for oil in water based on adsorption onto the surface of a fiber core chemically derivatized with octadecyl groups to enhance hydrocarbon adsorption.[35] In both cases, the readout was based on changes in refractive index.

Sensing based on adsorption/extraction is straightforward in concept and execution and potentially useful in a variety of analytical contexts. However, unless such devices uniquely exploit optical properties of the analyte, they are in competition with other readouts that can also be coupled to adsorption, such as piezoelectric and surface acoustic wave detectors.

C. Catalyst

The reagent phase can catalyze the conversion of analyte to a product that differs in optical properties. Such a device can be operated continuously on a steady-state basis if the rate at which analyte comes in contact with the catalyst is balanced by the rate of product diffusion away from the catalyst. This principle has been demonstrated using alkaline phosphatase as the catalyst and *p*-nitrophenylphosphate as the substrate.[38] It is potentially applicable using other enzymes as catalysts. However, it is inherently a steady state rather than an equilibrium approach and thus requires controlled conditions with respect to mass transfer.

D. Substrate

Enzyme activities are most frequently determined by measuring the rates of optical changes accompanying the conversion of substrate to product. Enzyme activities can be measured through optical fiber using an appropriate immobilized substrate as the reagent phase. However, such devices suffer from the same disadvantages as sensors based on stoichiometric reactions. Because the analytical process is based on reagent consumption, sensors for enzyme activities have limited lifetimes, are difficult to calibrate, and require controlled conditions with respect to mass transfer. Nevertheless, the feasibility of such a sensor has been demonstrated.[62] Such devices may be useful where *in situ* measurement of enzyme activity is critical.

V. APPLICATIONS

The application that has attracted by far the most attention is the development of sensors for continuous in vivo measurements of pH, pCO_2, and pO_2. Not only is there a substantial potential market for these sensors for monitoring the status of critical care patients, but also this is a context where the advantages of the optical approach are particularly significant. Stability with respect to calibration is required over periods lasting hours. However, since a new reagent phase can be used for each application, long-term stability for weeks or months is not essential. Fiber optic sensors are also considered safer for in vivo measurements than electrical devices. A commercial system for monitoring pH, pCO_2, and pO_2 in blood has been described in the literature.[3]

There are a considerable number of reports demonstrating the feasibility of getting a reversible or rechargeable response to analytes in addition to pH, pCO_2, and pO_2. This section is structured by analyte and comments critically on the practical potential for each application.

A general requirement for all systems is that the reagent be immobilized. Often this is accomplished by covalently bonding indicator reagents to a solid substrate. However, adsorption also has been used. While adsorbed reagents may be more likely to leach from the substrate with use, they offer the advantage that they can be prepared easily with a known coverage of reagent on the substrate. Dialysis membrane or gels with suitably small pores have been used to immobilized macromolecular reagents based on size while still allowing access to small analytes. Confinement behind gas-permeable membranes has been successfully used to immobilize reagents for measuring partial pressures of gas in liquids.

It is important to recognize that immobilization can have significant effects on indicator chemistry. For example, when acid-base indicators are immobilized, pKa shifts of a few tenths of a unit are commonly observed.[22,24,63] The sensitivity of indicators to changes in environmental factors can also change. For example, the variation in indicator pKa with ionic strength is typically affected by immobilization. In fact, an appropriate choice of immobilization substrate has been successfully used as a means of manipulating the dependence of pKa on ionic strength.[64]

In addition to changes in the equilibrium constant, immobilization can alter indicator spectral characteristics. It is quite common to observe bathochromic shifts of a few nanometers upon immobilization. Also, indicators that do not fluoresce in solution may become fluorescent when bound to a solid substrate.[65]

A. pH

Efforts have centered on finding the most appropriate indicator for physiological pH measurements. The indicators that have received the most attention are phenol red[22,40,66] and the trisodium salt of 8-hydroxy-1,3,6-pyrenetrisulfonic acid (HPTS).[3,24,63] Structures and spectral properties are shown in Figure 16. Covalently immobilized phenol red has been used in a sensor based on the ratio of reflected intensity at 558 nm where the base form of the indicator absorbs, to reflected intensity at 600 nm where neither form of the indicator absorbs.[22,24] The indicator part of the sensor has the configuration shown in Figure 7d with two separate 0.15-mm-diameter fibers used to conduct light to and from the indicator. The suitability of this device for in vivo measurements has been demonstrated. Because the optical measurements are made at long wavelengths, plastic fiber could be used in the sensor. A single-fiber pH sensor has been developed based on the effect of phenol red on the fluorescence of eosin.[46]

Several fluorescent indicators have been used to sense pH in the physiological range. Of these, HPTS seems to offer the best combination of characteristics for physiological pH sensing.[67] Its ground-state pKa in solution is 7.3, ideal for physiological measurements.[68]

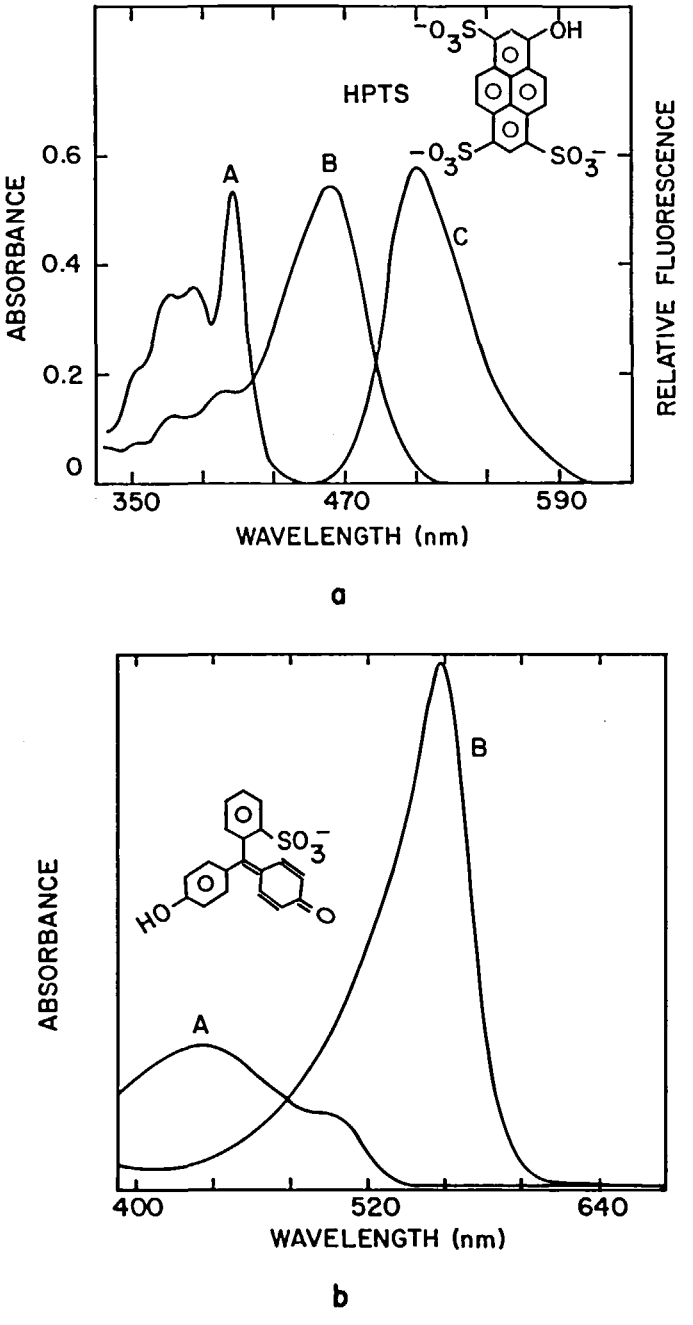


FIGURE 16. Structure and spectral properties of HPTS (a) and phenol red (b). In both (a) and (b), curves A and B represent the absorption spectra of the acid and base forms of the indicator, respectively. Curve C in (a) is the fluorescence emission spectrum of the base form of HPTS.

In the excited state, HPTS is a stronger acid than in the ground state.[68] Because in buffered media excited state deprotonation of HPTS is faster than fluorescence, fluorescence from the base form of the indicator is observed even when the indicator is initially in the ground state. For sensing applications, the measured parameter is the ratio of fluorescence intensity excited at 405 nm where the acid form absorbs selectively, to the fluorescence intensity

excited at 470 nm where only the base form absorbs. As noted in Section III.C.2, this stabilizes response with respect to drift. In addition to being amenable to two-wavelength intensity measurements in the visible region of the spectrum, HPTS has the advantage of being highly stable with respect to photodegradation. Sensors have been described using both ionically and covalently immobilized HPTS.[3,24,63]

HPTS has one serious limitation as a pH indicator. Because it is a tetraanion, its pKa is highly sensitive to ionic strength. In fact, a fluorescence method for measuring ionic strength has been proposed based on the difference in pHs measured with HPTS, a highly ionic-strength-sensitive indicator, vs. 2-methylumbelliferone which is much less sensitive to ionic strength.[69] Immobilization modifies and can even reverse the ionic strength dependence of the pKa. These effects reflect not only the changes in the indicator structure and environment accompanying immobilization, but also the changes in the immobilization substrate itself with ionic strength.

Several other pH indicators have been studied. 7-Hydroxycoumarin-3-carboxylic acid has been immobilized using a carbodiimide-coupling agent.[63] Like HPTS, it is amenable to two wavelength ratio measurements. It offers the advantage that it less sensitive to ionic strength, but the acid form of the indicator requires UV excitation and its photostability is less than HPTS. Fluoresceinamine is readily coupled to solid surfaces and responds to pH from 4 to 7.[23,37,70] Fluorescein derivatives offer the advantage that they are readily excited by the 488-nm line of an argon ion laser. However, they are relatively sensitive to photodegradation.

Relatively little work has been done outside the physiological range. The most significant accomplishment is the work of Kirkbright et al.,[39,71] who have implemented reflectance-based pH sensing with a bifurcated fiber optic system using a series of indicators immobilized by adsorption on XAD-2.

B. Carbon Dioxide

CO_2 sensors consist of a pH sensor in contact with a reservoir of bicarbonate solution which is isolated from the sample by a CO_2-permeable membrane. At equilibrium, the pH sensed in the internal solution depends on the concentration of carbonic acid in the internal bicarbonate solution, which in turn is proportional to the partial pressure of CO_2 in the sample. Both phenol red and HPTS have been incorporated into CO_2 sensors.[3,55,72] The earliest CO_2 sensors were based on the fluorescence of 4-methylumbelliferone,[73-76] but this indicator requires excitation in the UV. The internal bicarbonate concentration determines the actual pH values corresponding to various CO_2 partial pressures. In optical CO_2 sensors, the internal bicarbonate concentration should be chosen so that the range of CO_2 partial pressures to be measured yields pH values centered around the pKa of the indicator.

The fact that a gas-permeable membrane separates the sensor from the sample simplifies the development of successful CO_2 sensors. Variations in ionic strength do not directly affect response (although water vapor will tend to cross the membrane in the direction required to equalize ionic strength on either side of the membrane). Also, the indicator can be in solution in the internal reservoir of the sensor rather than being immobilized on a solid substrate. The major problem in developing a CO_2 sensor is engineering the device to minimize the time required to reach equilibrium response by reducing the distance over which mass transfer of CO_2 is required.

C. Oxygen

Most optical oxygen sensors have been based on fluorescence quenching. Because these sensors respond on an equilibrium basis, they are less sensitive to temperature than the Clark oxygen electrode and do not require controlled mass transfer of oxygen to the electrode surface. Pyrenebutyric acid has been the preferred indicator for oxygen in aqueous environments.[77] Because the electronic transition responsible for pyrene fluorescence is symmetry

forbidden, pyrene and its derivatives have relatively long fluorescence lifetimes, allowing more time for quenching by oxygen. In water, fluorescence lifetimes are typically too short to allow for significant oxygen quenching. Oxygen sensors have been developed using pyrenebutyric acid covalently immobilized to controlled pore glass[78] or dissolved in a solvent such as dioctyl phthalate and separated from the sample by an oxygen-permeable membrane.[47,76,79] While these sensors have generally favorable response characteristics, pyrenebutyric acid has the limitation that UV excitation is required.

An alternative approach has been to prepare indicators in which a fluorescent dye is embedded in a hydrophobic, oxygen-permeable matrix. The hydrophobic matrix serves the dual purpose of rendering the fluorophor more sensitive to oxygen quenching while excluding water. Aromatic hydrocarbons embedded in a silicone matrix have proven to be particularly well suited for oxygen sensing.[80-83] In other polymers, fluorophors tend to be less susceptible to quenching.[83] In general, sensitivity to oxygen is dependent on the microenvironment of the fluorophor.[79] With use, trace components of the sample may transfer to the fluorophor matrix, changing the microenvironment of the fluorophor and affecting response to oxygen even if the indicator itself remains stable.

A sensor based on perylene dibutyrate adsorbed on Amberlite® XAD4 has been characterized in some depth.[41] Perylene dibutyrate has the attractive feature that it is most efficiently excited at 468 nm, well out in the visible where plastic optical fiber can be used. The reagent phase is configured as shown in Figure 7d, with an oxygen-permeable porous polypropylene separating the immobilized indicator from the sample and separate fibers to conduct light to and from the indicator. The suitability of this sensor for in vivo oxygen measurements has been confirmed.

Because fluorescence quenching involves a decrease in emission intensity rather than a shift in spectral distribution, analytical response is not readily related to the ratio of intensities at two different wavelengths. Scattered excitation radiation can serve as a reference intensity.[41] Another approach has been to add a second oxygen-insensitive fluorophor to the reagent phase.[3] The ratio of the two fluorescence intensities serves as the measured parameter.

Efforts have been made to develop a single oxygen indicator that would show shifts in spectral distribution as a function of oxygen partial pressure. In principle, spectral shifts accompanying reversible oxygenation of immobilized metal complexes can be used for this purpose.[48] However, such complexes tend to be unstable because of slow, irreversible oxidation. Another approach has been to find an indicator that would have both oxygen-insensitive fluorescence and oxygen-sensitive phosphorescence bands. Bromonaphthalene derivatives on solid-phase cyclodextrins have the appropriate characteristics in dry environments, but moisture quenches the phosphorescence.[84] A related approach that would also avoid problems with intensity drift is to relate oxygen partial pressure to luminescence lifetime. As noted in Section III.C.4, however, this is expensive to implement unless a relatively long lifetime indicator is used.

Oxygen indicators are normally separated from samples by hydrophobic oxygen-permeable membranes made out of materials such as Teflon®. While this eliminates interferences from nonvolatile sample components, volatile quenchers can contribute to the signal. If optical oxygen sensors are to be used for critical care, quenching from volatile halogenated hydrocarbons used for anesthesia can be a problem. This problem can be dealt with using two fluorescent indicators which differ in their susceptibility to quenching by oxygen vs. halogenated hydrocarbons.[83] This represents a step in the direction of using arrays of sensors that differ in their selectivity to various analytes with appropriate mathematics to deconvolute the array of signals and yield individual concentrations. While such an approach is attractive in principle, it requires highly stable sensors with mathematically defined response characteristics.

An oxygen sensor based on chemiluminescence has also been developed.[51,52] While this

approach does not have the advantages of an equilibrium measurement, it is extremely sensitive and may be useful for trace oxygen measurements.

D. Alkali Metal Ions

If optical sensors for in vivo biomedical measurement of pH, pCO_2, and pO_2 prove to be practical successes, the development of electrolyte sensors is a logical next target. Ionophores that selectively bind various alkali metal ions are available and are used to impart selectivity to ion-selective electrodes.[85] Chromogenic ionophores that change optical properties upon binding alkali metal ions have been developed and can potentially serve as direct indicators in optical sensors.[86] However, these reagents are designed for use in mixed solvent systems. In water, the formation constants for alkali metal ion-ionophore complexes are much smaller because water is so effective at solvating the metal ion. For example, the log formation constants for 18-crown-6/sodium complexes decrease from 4.35 in pure methanol to 0.82 in water.[85] This is too small for detection of alkali metal ions in aqueous media at physiological levels.

An alternative approach based on ion pairing has been used to develop a reversible indicator system for sodium.[61] The components of the indicator include an ionophore immobilized on a solid substrate, an anionic fluorophor, and a cationic polyelectrolyte that quenches fluorescence from bound fluorophor. In the absence of alkali metal ion, a combination of electrostatic and hydrophobic interactions causes the anionic fluorophor to bind to the cationic polyelectrolyte. Added alkali metal ion complexes with ionophore forming a hydrophobic cation competitively ion pairs with the anionic fluorophor, drawing it away from the cationic polyelectrolyte and rendering it fluorescent. The free energy of ion pairing serves as a thermodynamic driving force facilitating the formation of the metal ion-ionophore complex.

The ion-pairing approach was applied to sodium detection using the following reagents confined behind a dialysis membrane: (1) a sodium-selective ionophore immobilized by adsorption on silica, (2) the ammonium salt of 8-anilino-1-naphthalenesulfonic acid as the anionic fluorophor, and (3) the Cu(II) complex of poly(ethylenimine) as the cationic polyelectrolyte. Response to sodium ion was selective and reversible. Furthermore, the range of measurable sodium concentrations could be varied by appropriately choosing the concentrations of anionic fluorophor and cationic complex. In principle, this approach can be generalized to other ionophores and anionic fluorophors. However, in practice, it was found that some systems give an unacceptably slow response. Furthermore, the indicator system as reported is subject to slow deterioration due to leaching of the ionophore and fluorophor. Nevertheless, this represents a fresh and versatile approach to indicator development. A general discussion of the ion-pairing approach along with alternative implementation schemes is available.[87]

The possibility of using potential-sensitive fluorescent dyes in indicator systems for alkali metal ions has also been demonstrated.[6] These dyes are widely used to indicate potentials across cell membranes.[88] Selective response for a particular ion can be obtained by incorporating a neutral ionophore with the appropriate selectivity into the membrane. This is a general approach applicable with any neutral ionophore that can be incorporated into a cell membrane.

E. Glucose

There is also considerable biomedical interest in the development of a glucose sensor. The first competitive binding-based sensor developed by Schultz et al.[44] responded to glucose. A schematic of this sensor is shown in Figure 17. Detection is based on the displacement of dextran from concanavalin A by glucose. To get an optically measurable signal, dextran is labeled with fluorescein, and concanavalin A is immobilized on the interior of hollow fiber which fits over a single optical fiber. The immobilized concanavalin A is out of the zone

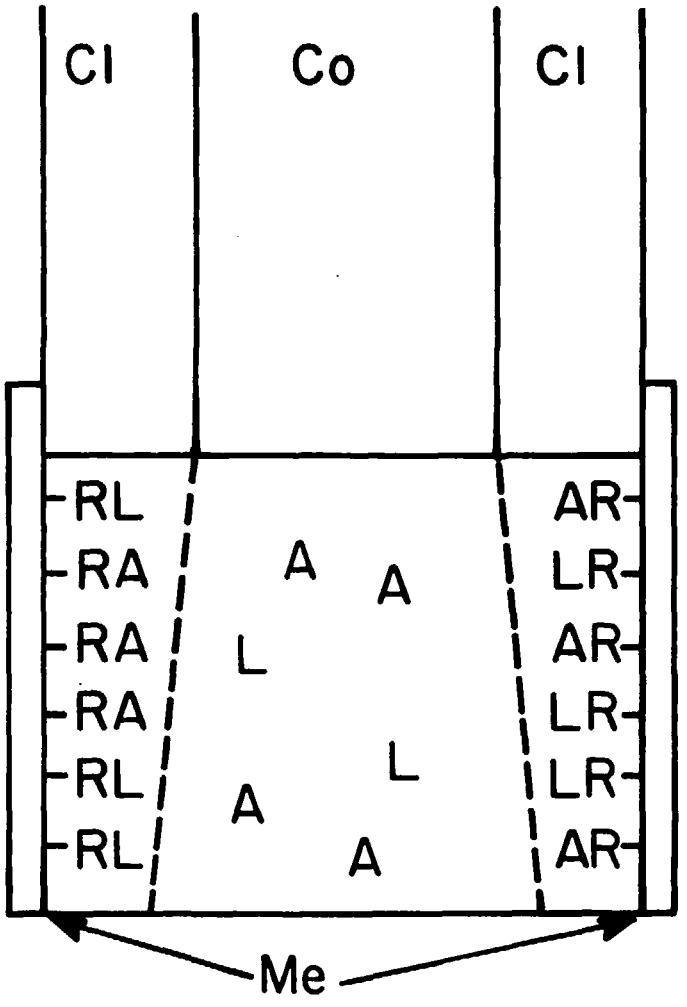


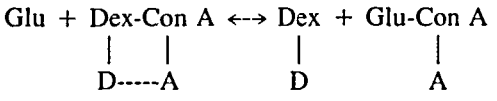
FIGURE 17. Schematic of competitive binding sensor for glucose. A = analyte (glucose), R = binding reagent (concanavalin A), and L = ligand (dextran). Me is a membrane permeable to analyte, but not to ligand. Co and Cl are the fiber core and cladding, respectively. The dotted line represents the area illuminated through the fiber.

illuminated through the optical fiber. Increasing glucose concentrations displace dextran from the concanavalin A, allowing the dextran to diffuse into the optical path where the fluorescein label is excited. Thus, increasing glucose levels are accompanied by increases in the intensity of fluorescein emission.

The competitive binding glucose sensor has several deficiencies. Because the competitive binding equilibrium requires an excess of dextran, there is significant background fluorescence. The total change in fluorescence with added glucose is a relatively small fraction of the total intensity. The response time is 10 min and the specificity for glucose is less than that of enzyme-based sensors.

It should be possible to improve the response characteristics of the competitive binding glucose by basing the optical detection on fluorescence energy transfer. This involves labeling dextran with a fluorescent donor and concanavalin A with an acceptor (or vice versa). When dextran is bound to concanavalin A, the distance between donor (D) and acceptor (A) should

be sufficiently short for significant energy transfer. Increasing glucose levels will displace the dextran, increasing the distance between donor and acceptor, thereby disrupting energy transfer. This is represented in the reaction below:



where the dotted line represents energy transfer. Increasing glucose levels will be accompanied by an increase in donor fluorescence and a decrease in acceptor fluorescence. This approach does not require diffusion from concanavalin A into the optical path and is amenable to intensity ratio measurement.

An alternative approach to optical glucose sensing is to use a reagent phase that includes glucose oxidase and a fluorophor sensitive to oxygen quenching.[76,79,89] Since the glucose oxidase-catalyzed oxidation of glucose consumes oxygen, high glucose levels reduce steady-state oxygen concentrations in the reagent phase, which leads to increases in observed fluorescence intensity. This approach is more selective than the concanavalin A-based sensor and can be generalized to determine other oxidase substrates. However, it requires a constant oxygen supply and involves a steady state rather than an equilibrium measurement.

F. Antigens

In principle, it is possible to design competitive binding sensors using immobilized antibodies as selective reagents and basing detection on displacement of a labeled antigen by the analyte. Because antibodies are available for a whole host of antigens, antibody-based sensors have been the subject of considerable interest.[90,91] The difficulty in designing such sensors is that the kinetics of antigen displacement are slow. One potential way to solve the response time problem is to use antibodies with relative weak affinities for the antigen of interest.[90] Since the rate of antigen-antibody association is more or less constant, a weaker binding affinity implies faster dissociation. However, this has yet to be demonstrated. Furthermore, weaker binding is likely to be accompanied by a loss in selectivity.

While practical reversible antibody-based sensors remain speculation only, rechargeable devices based on internal reflection have been demonstrated.[33] The antibody is immobilized on the surface of a fiber core. Fluorescence is excited by the evanescent wave of radiation propagating through the fiber. Because of the short penetration depth of the evanescent wave, fluorophor-labeled antigen is selectively excited when bound on the surface of the fiber core. Some of the resulting fluorescence propagates through the fiber which conducts it to a detector. After a measurement, the sensor is "recharged" by changing the pH to reduce the binding constant. Optical immunoassay by internal reflection techniques has been recently reviewed.[92]

G. Enzyme Activity Measurements

Enzyme activity can be measured through optical fiber using immobilized substrate as the reagent. The feasibility of this approach has been demonstrated for esterases using the trisodium salt of 8-acetyl-1,3,6-pyrenetrisulfonic acid immobilized on an ion exchange membrane as the reagent.[62] The enzyme hydrolyzes the nonfluorescent acetyl ester to the fluorescent phenol. The rate of increase in fluorescence serves as a measure of enzyme activity.

The reported reagent system for determining esterase activities has some attractive features. The fluorescent part of the substrate remains on the membrane in the field of view of the optical fiber, while acetate, the natural product of enzyme catalyzed hydrolysis, enters the sample. Also, the ionic substrate is mobile on the ion exchange membrane. Thus, after a measurement, new substrate migrates to the surface, effectively recharging the reagent as

long as substrate depletion and background fluorescence do not become excessive. This allows a single reagent to be used for several measurements, including a calibration.

H. Ammonia

Like CO_2, ammonia can be detected optically via its acid-base chemistry. The first report involved the detection of vapor-phase ammonia using a capillary tube as a waveguide with a thin layer of an oxazine dye as the reagent.[21] This system showed rapid reversible response, but was not characterized quantitatively.

Sensors for aqueous ammonia have been developed using internal solutions containing ammonium ion in the presence of an optical pH indicator. Devices have been developed using both colorimetric and fluorimetric indicators.[56,93]

I. Halide Ions

Two approaches to halide sensing have been tried. The first uses silver fluoresceinate as the reagent.[26] The reagent itself is nonfluorescent because of the quenching effect of the silver ion. Halide ions combine with the silver ion freeing the fluorescein anion so it can fluoresce. Sensitivity depends on the solubility product for formation of the silver halide salt and follows the order I > Br > Cl. This approach involves irreversible reagent consumption and will be sensitive to variations in the rate of mass transfer of fluorescein away from the surface.

The other approach is based on halide quenching of fluorescence from an immobilized cationic fluorophor.[59] Response is reversible but not very selective. Sensitivity of this approach is greatest for the highest atomic number atom, following the order I > Br > Cl.

J. Nonalkali Metal Ions

Nonalkali metal ions complex more strongly than alkali metal ions. They can be sensed optically using immobilized ligands that change color and/or fluoresce upon complex formation. One approach is to use a nonfluorescent ligand that forms a fluorescent complex. Al(III) and Be(II) can be sensed based on reversible formation of fluorescent complexes using morin covalently immobilized to cellulose.[57,94] Another reagent that has been evaluated is the sodium salt of 8-hydroxyquinoline-5-sulfonic acid (8HQS) immobilized electrostatically on an ion exchange resin.[95] This ligand forms fluorescent complexes with Mg(II), Zn(II), Al(III), and Cd(II). It was demonstrated that the observed response was consistent with literature values for 1:1 complexes between metal ions and 8HQS. Experiments involving other ligands are described in a general discussion of optical metal ion sensing.[65]

Metal ion sensing based on fluorigenic ligands is subject to several limitations. Selectivity is limited, depending on the relative affinity of the ligand for various metal ions. The approach is restricted to metal ions that form fluorescent complexes, i.e., diamagnetic metal ions with low atomic numbers. However, other metal ions can potentially be determined by their quenching effect on fluorescent ligands.[96] Complex formation for most ligands involves displacement of one or more protons. This means that the equilibrium for the indicator reaction is described by a pH-dependent conditional formation constant. The measurement must either be done at constant pH or corrected for variations in pH.

In spite of the limitations, there are many potential applications for metal ion sensing in contexts where interferences are not a problem. Although examples have yet to be reported in the literature, it should be possible to devise sensors which are stable with respect to calibration based on spectral shifts accompanying complexation and intensity ratio measurements.

K. Ionic Strength

Two systems have been reported for optical measurement of ionic strength. One is based on the use of two pH indicators whose pKas differ in their ionic strength dependence.[64,69]

The difference in pH values measured with the two indicators is the parameter related to ionic strength. This approach is most likely to be useful in the context of an ionic strength correction to measured pH values. It is effectively a two-sensor array used to measure two parameters.

The other approach is based on the interaction of two dissolved polymers, poly(ethylenimine) and dextran, confined behind a dialysis membrane.[97] At low ionic strengths, the two polymers bind to each other. Increases in ionic strength cause them to dissociate. The most interesting feature of this system is the use of fluorescence energy transfer to get an optically detectable signal. The dextran is labeled with fluorescein and the poly(ethylenimine) is labeled with sulforhodamine 101. When the two polymers associate, the average distance between fluorescein and sulforhodamine 101 is short enough so that energy is transferred from fluorescein to sulforhodamine 101. Upon dissociation, the average distance increases and the degree of energy transfer decreases. The measured parameter is the ratio of fluorescein to sulforhodamine fluorescence intensities when fluorescein is excited. Fluorescein emission increases and sulforhodamine emission decreases as the degree of energy transfer decreases. While this is not a practical system for ionic strength measurement, the energy transfer approach to labeling can be used with competitive binding-based sensors, offering the attractive feature that the measured parameter is an intensity ratio.

For direct optical measurement of ionic strength, the most practical approach is likely to be based on changes in the optical properties of a charged porous solid, e.g., an ion exchange resin, which will swell with increasing ionic strength.

L. Potential

In principle, potential can be sensed using immobilized redox indicators and measuring optically the ratio of reduced-to-oxidized indicator. Such a measurement could provide highly stable potential measurements. However, the dynamic range would be limited to about 100 mV or less centered around the standard potential of the indicator. To date, this application has not been developed. However, immobilized redox polymers with viologen as the indicating group have been used to detect oxidizing gases on a rechargeable basis.[98] An attractive feature of this application is the extremely high stability of the redox polymer which was used.[99] The instrumentation involves an LED source and a coated capillary tube as described elsewhere.[21]

M. Adsorption Sensors

An optical sensor that responds to polyaromatic hydrocarbons (PAHs) in water has been prepared by removing the cladding of a fiber and derivatizing the core so that it is coated with octadecyl groups. Adsorption of PAH onto the coating modifies the refractive index at the surface of the core. This is detected as a change in the intensity of helium-neon laser light transmitted through the fiber.[37]

A similar approach has been used to detect organic vapors adsorbed onto a polymer film-coated capillary tube.[35]

N. Titrations

Colorimetric or fluorimetric endpoints of titrations may be detected through fiber optics.[100-102] This may be useful in automating titrations. Of particular interest is the use of a blue-LED as the light source in an acid-base titrator.[102] LEDs with emission maxima in the visible at wavelengths as short as 480 nm have recently become available, offering the spectroscopist an extremely inexpensive source with adequate intensity for many applications.

O. Chlorinated Hydrocarbons

An integrating indicator for volatile chlorinated hydrocarbons has been developed.[103] A strongly basic pyridine reagent is separated from the sample by a membrane that is permeable

to volatile chlorinated hydrocarbons, but impervious to water. The chlorinated organics react with the indicator reagent to form a fluorophor. The rate at which fluorescence increases is measured through optical fiber. Response has been demonstrated in a chloroform-contaminated well.

P. Humidity

Humidity has been sensed optically based on the change in color from blue to pink accompanying hydration of cobalt chloride.[31,58] Evanescent wave excitation was used in both studies. The instrumental arrangement in Figure 13 has been successfully used to monitor humidity.

Q. Albumin

Immobilized bromocresol green has been used as a rechargeable indicator for albumin.[50] Albumin binds the base form of the indicator, effectively decreasing its pKa. Thus, the presence of albumin can be detected as an increase in the absorption of the base form of the dye, provided the actual pH is held constant at an appropriate value (3.8 for serum albumin measurements). An important aspect of this application is the use of an LED as a light source with a semiconductor detector, allowing for very low cost detection in a flowing system. The concept has been extended to the analysis of enzyme substrates, including penicillin G, urea, and D-glucose, by coimmobilizing the acid-base indicator and the appropriate enzyme.[49]

R. Substrate Analysis

Immobilized enzymes can be used as reagents for the determination of substrates that are converted into optically detectable products. The concept has been demonstrated for the determination of *p*-nitrophenyl phosphate using immobilized alkaline phosphatase to catalyze hydrolysis of the substrate to the colored *p*-nitrophenoxide product.[38] The measured signal involves a steady state where the rate of product formation is balanced by the rate at which product diffuses away for the fiber optic surface. In principle, this concept is widely applicable. For example, it can be used for enzymes that catalyze the reduction of NAD to the fluorescent product NADH. However, it requires controlled initial conditions and thus is not likely to be applicable to *in situ* measurements.

S. Remote Measurements without an Indicator

Optical fiber is routinely used to couple light into locations that are not easily accessible or are otherwise hostile or inconvenient for optical measurements. Examples include measurements in living cells, in samples exposed to strong magnitude fields, and in flames and plasmas. Fibers are also useful in coupling optical energy into small volume cells such as detectors used for high performance liquid chromatography. Some applications are cited in a review on optical waveguide techniques.[104] Fiber optics has also been used in various ways for Raman spectrometry.[105]

While fiber optics has been used for spectroscopic measurement for many years, interest in using fibers to transmit light over long distances has developed relatively recently as fibers with excellent transmission characteristics have become available. Remote measurements over distances as great as 25 m have been demonstrated in the UV using a pulsed UV laser and plastic-clad silica fiber.[106] In the visible, where fiber transmittance is much higher, fluorescence has been observed over several hundred meters using an argon ion laser as the excitation source.[26] Remote measurements have been successfully used for remote on-line measurements of uranium concentrations.[107] An interesting feature of this application is the use of lifetime measurements to compensate for different degrees of fluorescence quenching in the sample matrix. A commercial spectrometer designed specifically for measurements through fiber optics has been described along with remote sensing applications.[108,109]

VI. A LOOK TO THE FUTURE

There is increasing interest in a variety of approaches to optical sensing. The common theme of this research is the desire to do spectroscopic analysis directly in the sample on a continuous basis rather than bringing samples back to the laboratory for one-shot measurements. *In situ* continuous measurements offer major advantages. They provide more information which is available in real time. Furthermore, sample integrity is maintained. Because of these advantages, the surge of interest in optical sensing will continue for many years to come.

The development of optical-sensing systems requires expertise in several areas including indicator synthesis, polymer chemistry for preparing indicator substrates, analytical spectroscopy, and optical engineering. The interaction of experts in these different areas will be required to enhance the rate of sensor development. The first major commercial application will almost certainly be in vivo continuous measurement of oxygen, CO_2, and pH. However, other applications are likely to follow.

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