

Chapter 8

Chemical Sensing Using Indicator Dyes

Otto S. Wolfbeis

University of Regensburg, Germany

This chapter describes the design, fabrication, and properties of materials that respond to the presence of a chemical species by a change in their optical properties. Ideally, such effects are reversible. They may be detected by conventional methods of absorption, reflection, or luminescence spectrometry, and applied in various formats such as test strips and disposable tests, but preferably by making use of optical waveguides including optical fibers, integrated optics, capillary type devices, and the like. Specifically, we describe the design and use of appropriate indicator dyes, polymers, and additives, with a particular focus on materials for sensing pH, oxygen, carbon dioxide, ammonia, and certain ions. These materials (or their solutions in an appropriate solvent) may be deposited on various supports including simple plastic strips (e.g., by spin-coating), on fibers (e.g., by dip coating), inside porous materials (e.g., by soaking), on integrated waveguides or walls of disposable cuvettes (e.g., by spreading the solutions as thin films), inside capillaries (e.g., by passing the solution through the capillary), on the bottom of microtitre plates, or inside disposable vials.

8.1 INDICATORS

Indicators (*probes*) are synthetic dyes that undergo color changes on interaction with chemical species. The purpose of using a so-called *indicator chemistry* (i.e., a dye in or on a polymeric support) in optical sensing is to convert the concentration of a chemical analyte into a measurable optical signal. In other words, the indicator acts as a transducer for a chemical species that frequently cannot be determined directly

by optical means. This has an important implication in that it is the concentration of the *indicator* species that is measured rather than that of the *analyte* itself.

The chemistry of indicators is fairly established [1-4], but not optimized in many cases for sensing purposes. In fact, many indicators cannot be used in fiber-optic chemical sensors because of unfavorable analytical wavelengths, poor photostability, low molar absorbance, the need for additional reagents (such as strong acid or alkali, which are frequently used in conventional spectrometry in order to adjust for optimal conditions), or simply because they are not available in a purity required for sensing applications. The spectral range for the kind of dyes treated in this chapter extends from 350 to 900 nm. However, optical sensor systems are preferably operated between 450 and 800 nm. It is noted at this point that indicator dyes are available for numerous ions (including practically all metals ions and the proton), but not for most organic species of clinical or environmental significance.

On interaction with the target analyte, most indicators undergo a change in color or fluorescence (with one band appearing as the other disappears) rather than a change in intensity of one single band, which is only the case for certain (quenchable) fluorophores. Usually, both the complexed and uncomplexed indicator species have absorptions (but much less so emissions) of comparable intensity. Such indicators are referred to as two-wavelength indicators. They are advantageous over other indicators in that they lend themselves to two-wavelength internal referencing methods.

Fluorescent indicators, in contrast, are frequently of the yes/no type in that only one of the species (i.e., the complexed or the uncomplexed form) is fluorescent. In such cases, fluorescence intensity can be measured with no background resulting from the presence of a second species. Obviously, however, two-wavelength internal referencing is impossible. Measurements of decay time or polarization are then preferred over other internally referenced methods. Another disadvantage of fluorescent indicators results from the fact that they are prone to quenching by species other than the analyte. Finally, many fluorophores display low molar absorbance (when compared to color indicators), and are not excitable by green, yellow, or red LEDs, or by semiconductor lasers. On the other side, fluorescent indicators provide distinctly improved sensitivity (which is important in case of minute sensor size) because of the unsurpassed sensitivity of luminescence. Finally, luminescence offers a broad variety of techniques including measurement of intensity, lifetime, polarization, energy transfer, and combinations thereof, since processes occurring in both the ground and the excited state can be monitored.

There is a general trend visible now toward the use of longwave absorbing indicator dyes for the following reasons:

1. Shortwave emitting light sources are expensive and often require high power, while LEDs as well as diode lasers are inexpensive, easy to drive, and require low power.
2. Photodiodes are inexpensive photodetectors that—unlike PMTs—do not require

high voltage and display best sensitivity in the 600- to 900-nm range (with exceptions).

3. Many dyes suffer from photobleaching if exposed to blue or UV light.
4. Optical waveguides display measurable intrinsic absorption at below < 450 nm and this is particularly true for plastic waveguides: simultaneously, background luminescence increases.
5. Most biological matter has good permeability for light at > 600 nm and < 900 nm only, so this is the window at which *in vivo* sensors are preferably operated at.
6. Scattering of light usually decreases with λ^4 .

Consequently, present day fiber-optic chemical sensors preferably are based on LED light sources, photodiode detectors, glass waveguides, and indicator dyes absorbing in the 450- to 800-nm range.

8.1.1 pH Indicators

These are mostly weak acids (less often, weak bases) whose color or fluorescence is different in the dissociated and the associated (protonated) form, respectively [1.5]. Figure 8.1 shows the pH dependence of the excitation and emission spectra of the widely used fluorescent pH probe HPTS. It has two bands in the excitation spectrum, a fact that allows for two-wavelength excitation and hence internal referencing because the ratio of the fluorescence intensities obtained at an excitation of, for example, 405 and 460 nm, is independent of dye concentration, the intensity of the light source, and the sensitivity of the photodetector (unless they vary within the time required for making the two measurements).

An important parameter for characterization of a pH indicator is its pK_a value (i.e., the pH at which the dye is present in the undissociated and the dissociated form at 50% each). The pK_a is the negative log of the binding constant (which in turn is the inverse of the stability constant K_s):

$$pK_a = -\log \left(\frac{[\text{Ind}^-][\text{H}^+]}{[\text{H-Ind}]} \right) \quad (8.1)$$

where $[\text{H-Ind}]$ represents the concentration of the undissociated indicator molecule while $[\text{Ind}^-]$ denotes the concentration of the anion (the dissociated form which, in case of phenolic dyes, is more intensely colored), and $[\text{H}^+]$ is the concentration of protons (i.e., the negative antilog of the pH). At the transition point of the titration curve, $\text{pH} = pK_a$.

A typical titration plot as obtained from pH-dependent fluorescence emission spectra is shown in Figure 8.2, from which it is obvious that 1) pH indicators are most

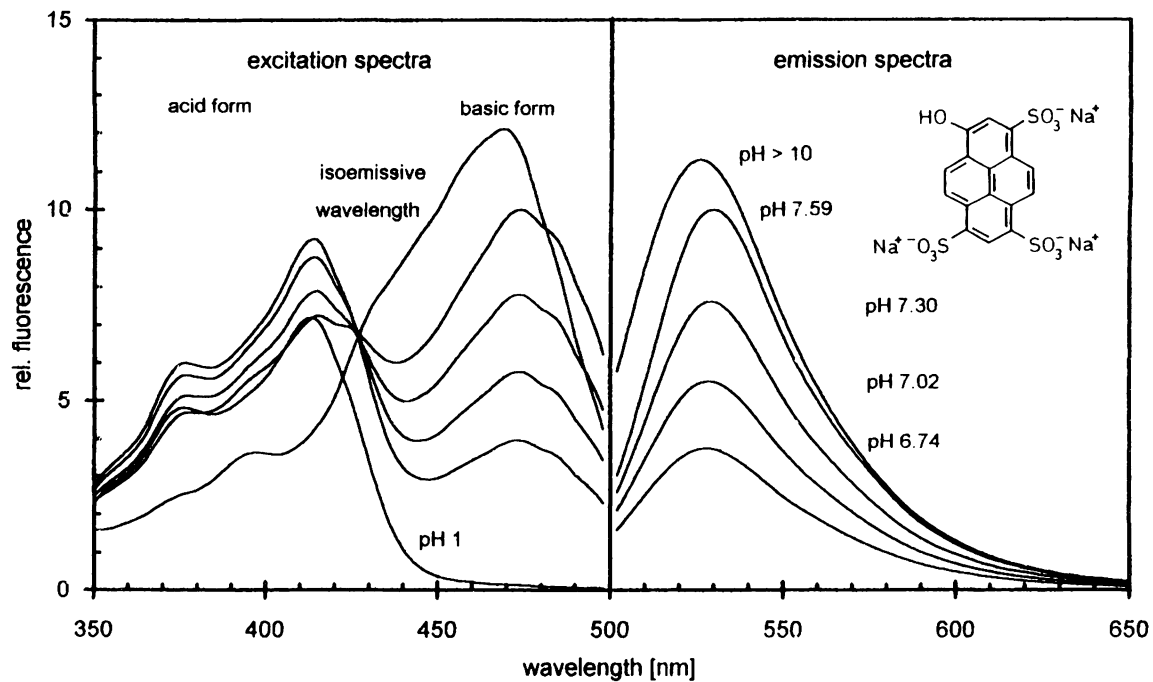


Figure 8.1 The pH-dependent fluorescence excitation and emission spectra of the pH indicator HPTS covalently immobilized on cellulose.

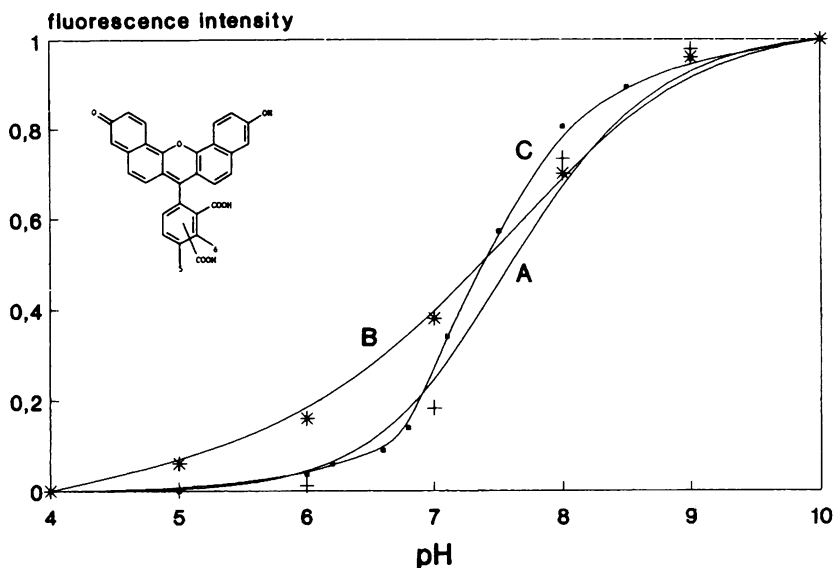


Figure 8.2 pH-Dependent fluorescence intensities ("titration plots") of the naphthofluorescein dye CNF (a) in dissolved form, (b) immobilized on cellulose, and (c) in a sol-gel.

sensitive at pHs near the pK_a , 2) their dynamic range covers a pH range of approximately $(pK_a \pm 1.5)$ units, and 3) the shape of the curve is different for the dissolved and immobilized forms of the dye.

The relation between pH, pK_a and absorbance (or fluorescence intensity) of the two species is given [5,6] by

$$pH = pK_a + \log \frac{[Ind^-]}{[H - Ind]} \quad (8.2)$$

pK_a 's may be determined by spectrophotometry or fluorimetry from a titration plot using (8.3):

$$pK_a = pH - \log \frac{(E_x - E_A)}{(E_B - E_x)} \quad (8.3)$$

where E_x is the absorbance (or luminescence intensity) at a given wavelength and a

certain pH, and E_A and E_B are the absorbances at this wavelength for the pure acid and base forms, respectively. The values E_A and E_B are obtained by acquiring the spectra of the indicator at pHs of $< (pK_a - 2)$ and $> (pK_a + 2)$, respectively.

The fact that optical pH sensors measure over a limited range of pH is disadvantageous but inevitable in view of the mass action law that governs response (see (8.2)). No single indicators are available that allow measurements to be performed over the pH 1 to 13 range (as can electrodes). Rather, different indicators have to be employed. The most important range is the one in the near neutral (physiological) pH range. However, few indicators only meet the requirements for use in pH sensors for physiological samples. Desirable properties include 1) an appropriate pK_a (7-8); 2) absorption/excitation maxima at or above 450 nm to allow the use of inexpensive waveguide optics and light sources; 3) high molar absorbance; 4) photostability and chemical stability; and 5), ease of immobilization. Tables 8.1 and 8.2 summarize some of the more common absorption indicators which, however, if immobilized on a solid support, may undergo significant shifts in both their pK_a values (and, hence, pH transition ranges) and less so their absorption maxima.

Fluorescent indicators have been applied more often than absorbance-based indicators in optical sensors ("optrodes"). The 7-Hydroxy-coumarins are pH indicators for cell studies, but have found little application in sensors because their spectral maxima are in the UV (or the blue) part of the spectrum. Fluoresceins, in contrast, form a widely used class of pH probes. Their popularity results from the close match

Table 8.1
Spectral Maxima (in nm) and pK_a Values of the Common Absorbance-Based Indicators for Physiological pHs

<i>Indicator</i>	<i>Amax of Acid/Base Form</i>	<i>pK_a</i>
Bromothymol blue	430/617	6.8
o-chlorophenol-indophenol	555/625	7.1
Chlorophenol red	460/530	6.3
Dibromo-xyleneol blue	ca. 420/614	7.6
a-naphthol-phthalein	428/661	6.7 and 7.9
Neutral red	527/453	7.4 and 5.9
Nitrazine yellow	460/590	6.5
Palatine chrome black	520/643	7.4
Phenol red	432/576	7.6
Phenoltetrachloro-sulfonaphthalein	435/575	7.0
Solochrome violet RS	515/562	4.35, 7.4, 9.35
Styryl acridine	455/684	7.50 *

* in plasticized pvc.

Table 8.2
Selected Longwave Absorbing pH Indicators

<i>Indicator</i>	<i>Color of Acid/Base Form</i>	<i>pK_a Value or pH Range</i>
Methyl violet	Yellow/blue	0.0–1.6
Malachite green	Yellow/blue-green	0.2–1.8
Cresol red	Red/yellow	1.0–2.0
m-cresol purple	Red/yellow	1.2–2.8
Bromophenol blue	Yellow/blue	2.8–4.8
Congo red	Blue/red	3.0–5.0
Bromocresol green	Yellow/blue	4.6
4-phenylazo-1-naphthylamine	Red/yellow	4.0–5.6
Bromocresol purple	Yellow/purple	6.3
Meta-cresol purple	Yellow/purple	7.4–9.0
4,4'-bis(4-amino-1-naphthylazo)- 2,2'-stilbenedisulfonate	Blue/red	8.0–9.0
Naphtholbenzein	Orange/blue	8.2–10.0
Ethyl bis(2,4-dinitrophenyl)-acetate	Blue/yellow	10.5
Alizarin yellow R	Yellow/red	10.0–12.0
Alizarin	Red/purple	11.0–12.4
Indigocarmine	Blue/yellow	11.4–13.0
Tetraethyl anilinesulphthalein	Blue/yellow	13.2

of their absorption with the emission of the blue LED and the 488-nm line of the argon laser and their availability in activated form (e.g., FITC), which facilitates covalent immobilization. Notwithstanding their popularity, many fluoresceins are poor pH probes in having small Stokes' shifts, overlapping pK_as, and limited photostability. The spectral properties of fluoresceins are similar to bilirubin and flavins, which therefore may interfere in blood and serum measurements. More longwave emitting fluoresceins therefore are preferred. Table 8.3 lists the most common fluoresceins along with their properties. The naphthofluoresceins and the SNARF and SNAFL dyes have dual emissions, which enables dual-wavelength measurements.

Most pH sensors have been obtained by immobilization of pH indicators on hydrophilic supports such as cellulose, where shifts in pK_a due to immobilization remain small. More recently, pH sensors have been developed based on polymers like plasticized PVC or polyurethane. Classical indicators are insoluble in such polymers, but have good solubility in water. In order to make pH probes soluble in hydrophobic polymers, they have been made lipophilic by either eliminating charged functions such as sulfo groups or by introducing long alkyl side chains to render them more lipophilic. It is noted, however, that lipophilic pH indicators undergo massive shifts in their apparent pK_a when incorporated in lipophilic polymers, as shown in

Table 8.3
Absorption and Emission Maxima (in nm) as Well as pK_a Values
of Various Fluoresceins

<i>Indicator</i>	<i>pK_a(s)</i>	<i>Excitation/Emission Maxima</i>	
		<i>at pH 10</i>	<i>at pH 3</i>
Fluorescein	2.2, 4.4, 6.7	490/520	a)
Eosin	3.25, 3.80	518/550	+60/536
2',7'-dichlorofluorescein	0.5, 3.5, 5.0	502/526	+60/520
Dimethylrhodole	ca. 6.0	510/545	b)
5(6)-carboxy-fluorescein	ca. 6.4	505/530	a)
5(6)-carboxy-eosin	ca. 3.6	525/560	+65/540
Carboxy naphthofluorescein	ca. 7.0	590/665	510/565
SNARF ^(c)	ca. 7.6	560/625	530/575
SNAFI ^(c)	ca. 7.6, 7.3	550/620	515/540
Vita blue	ca. 7.5	610/665	524/570

a) No fluorescence.

b) A hybrid between fluorescein and rhodamine.

c) Registered trade name of Molecular Probes, Inc. (Eugene, OR).

Figure 8.3 for a pH-sensitive membrane made from plasticized polyurethane hydrogel. Such sensors are particularly easy to fabricate because they can be deposited as thin films from respective solutions ("cocktails") by conventional techniques.

8.1.2 Effects of Ionic Strength on pK_as

The ionic strength dependence of pH optrodes represents the major limitation for precise optical determination of pH [5–9]. The effect of ionic strength on a typical titration plot is given in Figure 8.4. The main sources of error are the effects of ionic strength and dissolved polyelectrolytes (viz. proteins), of added solvent, and of surface structural effects of optrodes. It has been concluded [7] that for thermodynamic reasons neither optical nor electrochemical sensors can measure pH precisely, but that, on grounds of error minimization in electrodes, the electrochemical measurements of ion activities are superior to the optical.

8.1.3 Metal Chelators

There are many types of dyes that form colored complexes (*chelates*) with metal ions [1–3] and therefore may be employed as indicators in optical sensors. However, the color reaction must be sufficiently selective and the value of the stability constant of the complex formed should be such as to make the reaction reversible in order to make the device a sensor rather than a single-shot probe. This appears to be a problem with most sensors for heavy metals [10].

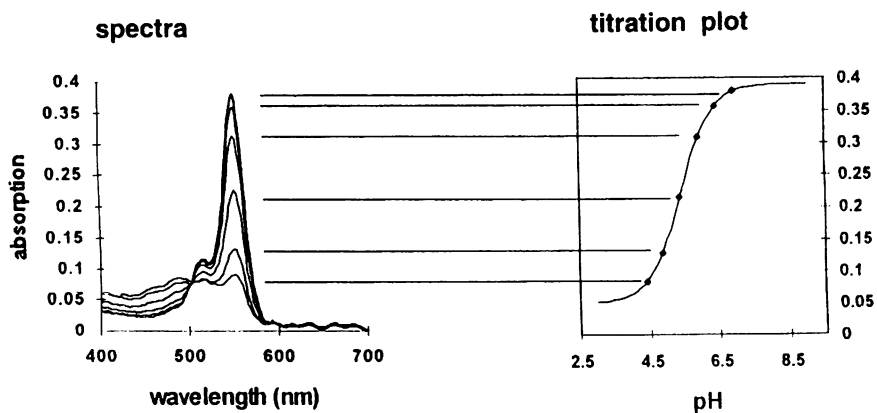


Figure 8.3 Titration plot di-iodofluorescein octadecylester incorporated into a membrane of polyurethane (plasticized with 66% NPOE). The apparent pK_o value is 7.3 (as opposed to ca. 4.2 in water).

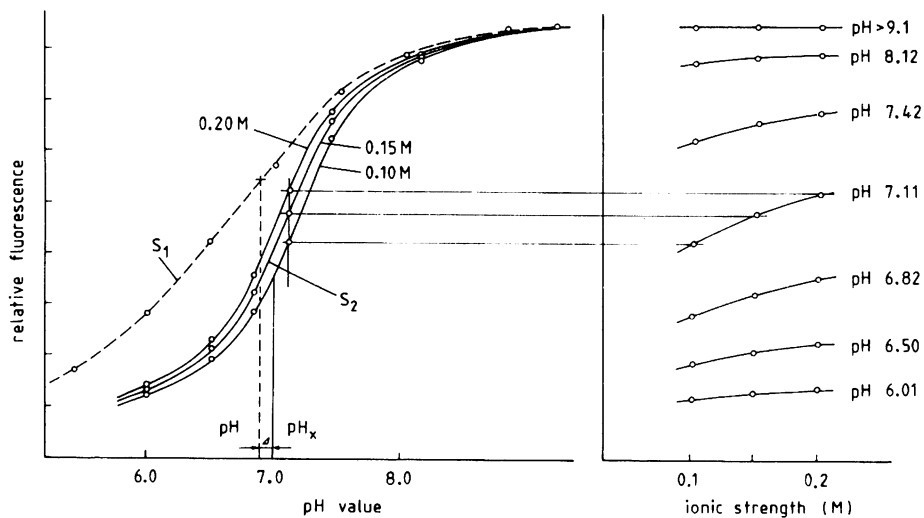


Figure 8.4 (a) Effect of ionic strength (NaCl in concentrations from 0.10 to 0.20 mM) on the work function of a pH sensor (S_2) and (b) effect on the accuracy of the measurement.

Metal indicators are usually salts of polybasic acids, which change color when the acidity of the solution is varied. It is therefore mandatory to buffer the pH of the sample solution when an indicator of this type is used. The theoretical basis of the use of metal indicators can be discussed in terms of the so-called conditional constant K'_s (8.4). When a metal ion M reacts with an indicator in a molar ratio of 1:1.

$$K'_s = [M - \text{Ind}]/([M][\text{Ind}']) \quad (8.4)$$

where $[\text{Ind}']$ denotes the concentration of the indicator, which is not bound in the complex M -Ind, and $[M]$ the concentration of the metal ion that is not bound to the indicator as $(M\text{-Ind})$. In view of (8.2), K'_s can be highly pH-dependent. A more extensive theoretical treatment can be found in [1].

Excellent textbooks and reviews on metal chelators are available [1–3], so there is no need to go into detail. The state of the art in optical probing of heavy metals has been reviewed [10]. Several manufacturers offer optical strip tests with reflectometric readout, but all act irreversibly. There is an obvious lack of optical indicators for alkali and earth alkali ions to work at near neutral pH. In view of the tremendous interest in sensing these species in clinical samples, alternative approaches have been made, which shall be discussed next.

8.1.4 Crown Ether Dyes (Chromoionophors)

This class of indicators dyes has attracted particular attention with respect to sensing alkali ions [11–15]. Chromoionophores incorporate two functions in one molecule, namely 1) that of a crown ether (or a more complex binding site) capable of binding alkali or alkaline earth ions (but also certain main group metal ions), and 2) that of a chromophore that is designed to bring about specific color changes. The chromophoric groups can bear one or more dissociable protons or can be nonionic (Figure 8.5). In the former, the ion exchange between the proton and appropriate metal cations causes the color to change, while in the latter the coordination of the metal ion to the chromophoric donor of the dye molecule induces a change of the charge transfer (CT) band of the dye. If complexation is associated with the release of a proton, the sensor obviously will have a pH-dependent response.

In the above CT-based systems, charges are shifted along the conjugated π -bonds of a chromophore or fluorophore. An alternative sensing scheme has been described that relies on an effect called photoinduced electron transfer (PET) [16–20]. In such systems, an electron is shifted from a tertiary nitrogen atom to a fluorophore through space (not along a chemical bond) as shown in Figure 8.6 (with an anthracene moiety acting as the fluorophore). This photoinduced process—which can only be observed in fluorescence—is suppressed if the free electrons of the nitrogen atom are blocked by binding to an ion such as potassium, or by a proton. This is a most promising and widely applicable sensing scheme.

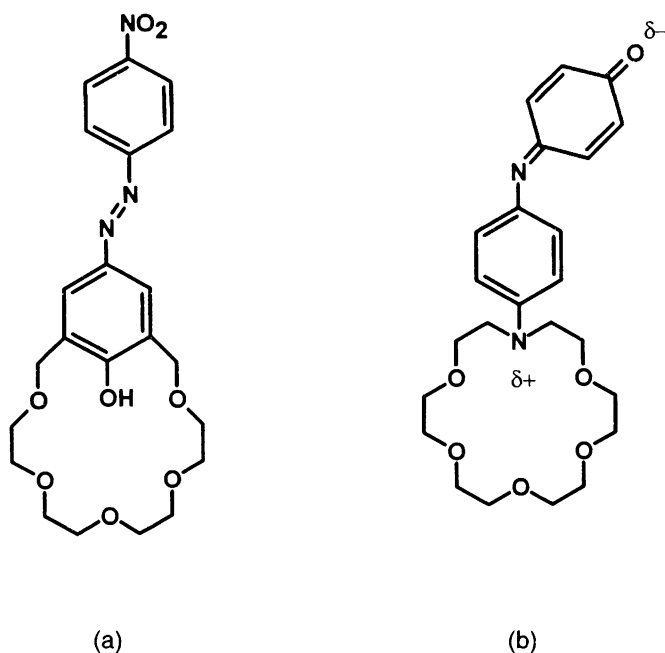


Figure 8.5 Chemical structures of (a) a typical dissociable chromoionophore, and (b) a nondissociable chromoionophore, both containing a cyclic polyether ring (the "crown") and a chromophore.

8.1.5 Chelators for Calcium and Magnesium

In recent years, indicator dyes have been developed for the clinically important bivalent cations calcium and magnesium [4,21], which are different from previous probes in that they chelate at physiological pH and in the concentration encountered in practice (see (8.4)). Hence, they meet the need for monitoring calcium or magnesium at physiological pH and over a wide range of concentrations.

8.1.6 Potential-Sensitive Dyes

These comprise a quite different class of dyes that respond to transport processes occurring at a sensor/sample interface [22]. Thus, they do not directly report the concentration or an activity of a chemical species. They provide an interesting alternative to sensors based on conventional chromogenic chelators. Potential-sensitive dyes (PSDs) (also referred to as polarity-sensitive dyes) are usually placed directly at the site where a "potential" is created by chemical means, usually via an ion carrier (such as valinomycin) incorporated into a lipid membrane.

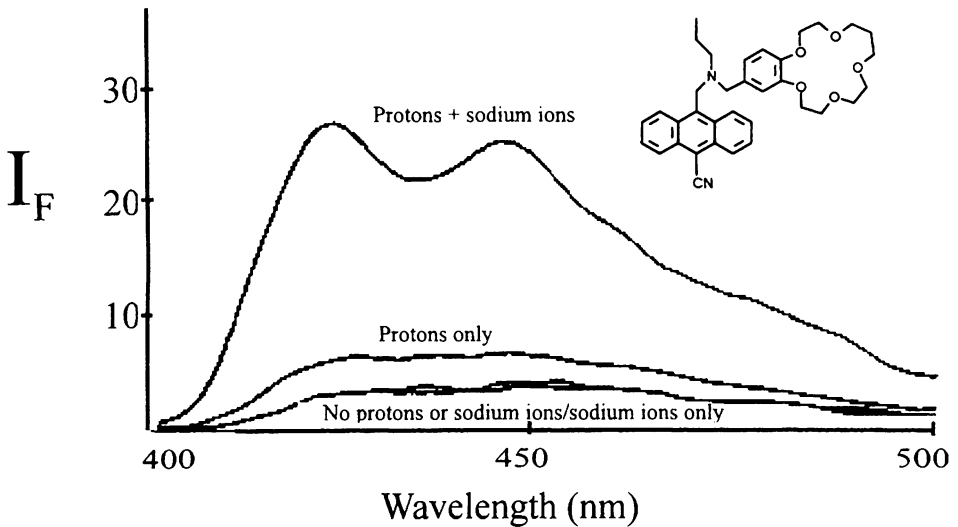


Figure 8.6 Chemical structure of a crowned fluorophore which, dissolved in methanol, on addition of 10 mM of sodium acetate gives a sixfold increase in fluorescence intensity. (Source: [20].)

The response of PSDs to electrolytes [22–26] is based on one or more of the following effects: 1) a field-dependent distribution of the dye between regions of different polarity within the lipid membrane, resulting in a solvatochromic effect; 2) changes in the otherwise homogeneous distribution of the fluorophore within the membrane when an electric field is created, leading, for example, to aggregation and self-quenching; 3) the *Stark* effect (i.e., a change in the absorption and emission spectrum of a fluorophore when an external field is applied); and 4) potential-induced changes in the solvation of the dye. The exact mechanisms of PSD-based sensors (and the relative contributions of the above effects) are not clear yet.

Figure 8.7 gives a schematic representation of the mechanisms occurring at the sensor/sample interface of a sensor membrane that fully reversibly responds to potassium ion. The dye is redistributed and undergoes a change in its microenvironment as a result of the transport of a cation into the membrane. Obviously, the kind of charge of the ion plays an important role in this process. It is assumed that the major effect results from the displacement of the PSD from an environment where it is strongly fluorescent to an environment where it is less fluorescent (or vice versa).

Another parameter to be considered is the hydrophilicity/lipophilicity balance (HLB) of the sensor membrane and the dye contained in it. If both the PSD and the polymer are highly lipophilic, the PSD will not be displaced into the direction of the aqueous sample and hence will not undergo a significant spectral change. The same is true if both are highly hydrophilic. It follows that the choice of the appropriate HLB of PSD and polymer dictates the relative signal change of such sensors.

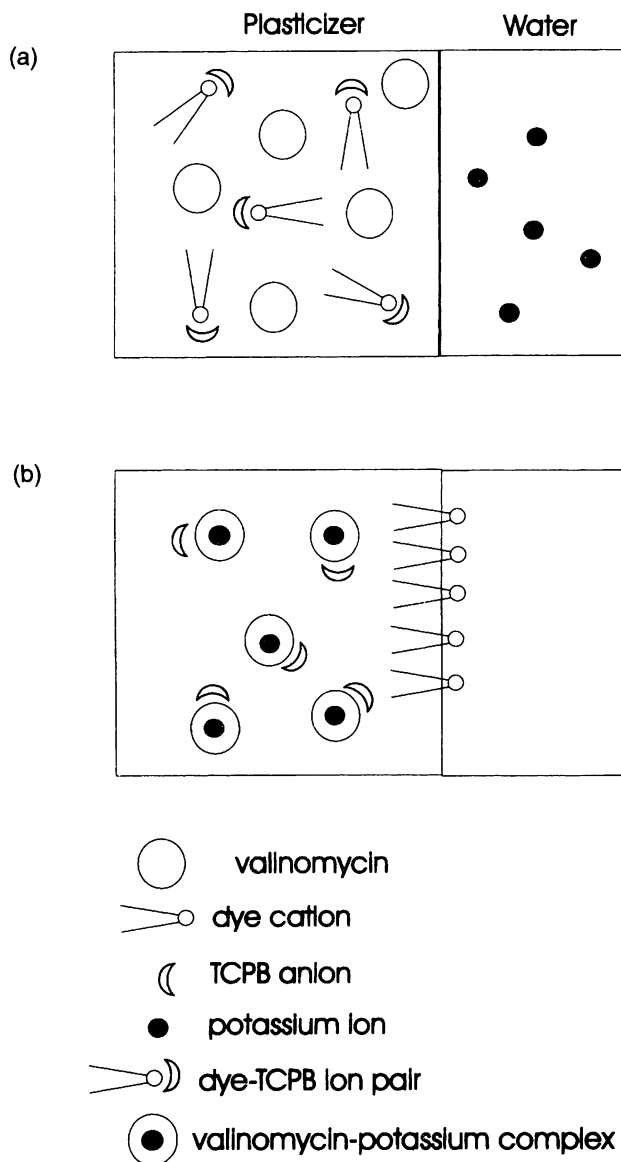


Figure 8.7 Schematic of the dye distribution in a sensor based on the use of a PSD (a) before and (b) after potassium ion has been carried from the water phase into the membrane phase.

The rhodamine dyes comprise an important class of cationic PSDs and have been used in sensors for alkali ions [22,25]. They are nontoxic and highly fluorescent. Acridine Orange [23] and certain merocyanines [25,26] have been used as well. The preferred polymers are plasticized PVC, certain PVC-PVA-PVAc copolymers, and polyurethane hydrogels.

8.1.7 Quenchable Fluorophores

Both the fluorescence intensity and the decay time of certain fluorophores are reduced in the presence of so-called dynamic quenchers [27,28]. The process of dynamic quenching is fully reversible (i.e., the dye is not consumed in a chemical reaction). Hence, quenchable fluorophores comprise an important class of indicators for reversible sensing. In the case of dynamic quenching, the interaction between quencher (analyte) and fluorophore is in the excited state only. The relation between luminescence intensity (I) and decay time (τ) on one side, and analyte concentration on the other is described by the Stern-Volmer equation (8.5)

$$(I_0/I - 1) = (\tau_0/\tau - 1) = K_{SV}[Q] = K_q \cdot \tau_0 \cdot [Q] \quad (8.5)$$

where I_0 and I are the luminescence intensities in the absence and presence, respectively, of the quencher Q present in concentration $[Q]$, τ_0 and τ are the luminescence decay times in the absence and presence, respectively, of quencher Q , K_{SV} is the overall (Stern-Volmer) quenching constant, and K_q is the bimolecular quenching constant. At higher quencher concentrations, Stern-Volmer plots tend to deviate from linearity. Figure 8.8 gives typical Stern-Volmer profiles of the quenching of the luminescence of a dye by oxygen in various polymers.

Oxygen is known to be a notorious quencher of luminescence, and this is widely exploited for sensing purposes [28–35]. Interferences by ionic species can be eliminated by immobilizing the fluorophore in ion-impermeable materials such as silicone or polystyrene. This is discussed in more detail in the section on oxygen sensors. Other dynamic quenchers of luminescence include bromide and iodide [36], halothane (which quenches by virtue of the so-called heavy atom effect of bromine) [37], and the transition metals (which quench due to the presence of unpaired spins) [10,38].

8.2 POLYMERIC SUPPORTS AND COATINGS

Polymer chemistry forms an integral part of sensor technology since all “chemistries” rely on the use of one of the many polymers and related supports. In indicator-based sensing schemes, polymers are expected to be optically inert. Their function is that of 1) a solid support onto which indicator dyes are being immobilized and 2) a material possessing a certain permeation selectivity for the species of interest while rejecting others. The choice of polymer is mainly dictated by the above considerations, but also

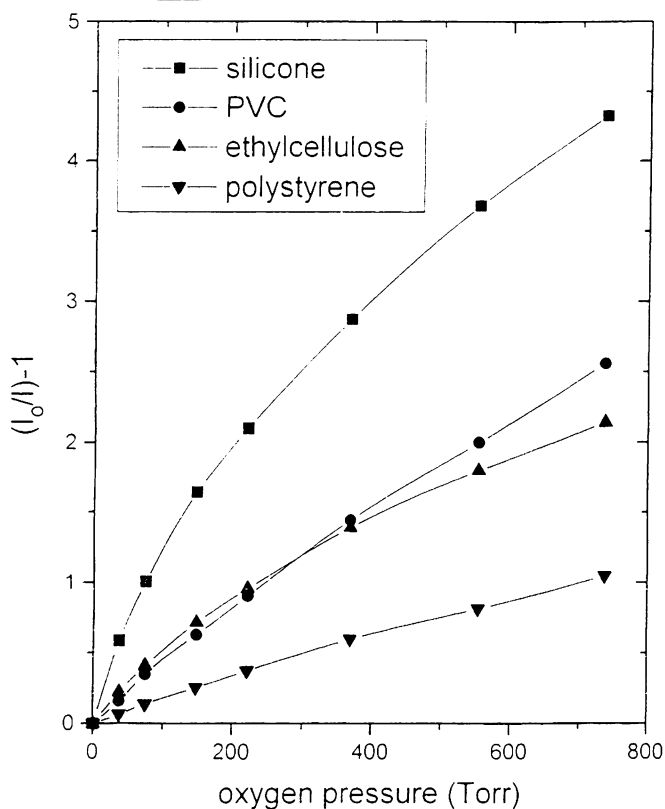


Figure 8.8 Stern-Volmer plots of the quenching by oxygen of the luminescence of ruthenium-tris(dpp) perchlorate dissolved in various polymers [29].

by the polymer's compatibility with the sample (e.g., blood). Polymer properties are compiled in various books and reviews [39–45] to which reference is made.

The choice of polymer material has a pronounced effect on the performance of the sensor. The response time, for example, will be governed by the diffusion coefficients of gases or ions, and the quenching efficiency by both the diffusion and solubility of the analyte in the polymer. Solubility and diffusion coefficients for various gas/polymer combinations have been compiled [46–48]. However, numerous new materials are available for which data are scarce. It is also known that copolymers and polymer mixtures do not necessarily display the properties that may be expected from averaging the data of the pure components.

Certain polymers such as polystyrenes and polyesters display intrinsic fluorescence under UV excitation, while poly(vinyl chlorides), poly(vinyl alcohols), and

polysiloxanes are fairly "clean." Most organic polymers have added plasticizers to make them softer and more permeable. Among these, esters of phthalic acid are fluorescent under UV excitation and can give rise to a considerable background signal. NPOE, in turn, is a plasticizer widely used in electrodes but acts as a dynamic quencher of the luminescence of many luminescent indicators.

8.2.1 Silicones

Silicones have unique properties [46,49-51] in possessing a higher permeability for most gases (including water vapor!) than any other polymer, but being impermeable to ions, including the proton. The selectivity of sensors for carbon dioxide, for example, results from the fact that interfering protons do not pass hydrophobic membranes and therefore cannot interact with a dissolved pH indicator.

Silicones also have excellent optical and mechanical properties, and unique gas solubility. In case of oxygen, it exceeds all other polymers. Numerous silicone prepolymers are commercially available and allow easy manufacturing of membranes, emulsions, suspensions, or other kinds of sensing chemistries. One can differentiate between one-component and two-component silicone prepolymers. The former cure in the presence of moisture (e.g., in air) by splitting off acetic acid, methanol, or amines (which are bases!). In two-component prepolymers, a catalyst is added to one component in order to cause an addition reaction of component A to component B to give a long chain polymer. The catalyst is usually contained in one of the prepolymers. Some catalysts have been found to act as quenchers of the fluorescences of charged indicators. Many silicones are of the room-temperature vulcanizing (RTV) type, and the respective prepolymers may be dissolved in aprotic solvents such as toluene or chloroform. This greatly facilitates handling.

Notwithstanding their advantages in terms of permeability and permselectivity, silicones, once formed, do not easily lend themselves to surface modification. Hence, covalent immobilization of indicators on cured silicone rubber is extremely difficult. Moreover, silicones have limited compatibility with other polymers and are difficult to glue onto many other materials (with the notable exception of glass, which has excellent adhesion to RTV silicones). As a matter of fact, certain sensor types described in the literature and based on silicone rubber materials in combination with other materials have extremely poor long-term stability because of material incompatibility, particularly if stored in buffer. Finally, silicones are very good solvents for most gases including oxygen. This may lead to a depletion of gas when the sample volume comes to lie below the hundredfold volume of the sensing layer.

The main application of silicone materials is in sensors for oxygen and other uncharged quenchers such as sulfur dioxide and chlorine, and as gas-permeable covers in sensors for carbon dioxide or ammonia. Silicones cannot be easily plasticized by conventional plasticizers, but form copolymers that may be used instead [52].

Blackened silicone is a most useful material for optically isolating gas sensors in order to make them insensitive to the optical properties of the sample [30].

8.2.2 Other Hydrophobic Polymers

Poly(vinyl chloride) (PVC), polyethylene, poly(tetrafluoroethylene) (PTFE), polystyrene, and ethylcellulose comprise another group of hydrophobic materials that efficiently reject ionic species. Except for polystyrene, they are difficult to chemically modify so that their function is confined to that of a "solvent" for indicators, or as a gas-permeable cover. However, the diffusion of analytes through, and the solubility of gases in such membranes is quite different from silicones and results in drastically limited quenching constants.

Plasticized PVC is the preferred matrix for ion sensors (including pH sensors) if provided with a carrier (such as a crown ether). Unless plasticized, PVC is not suitable for ion-sensing purposes. Useful plasticizers include DOS (dioctyl-sebacate), TOP (trioctyl-phosphate), DOP (dioctyl-phthalate), NPOE (nitro-phenyl-octyl-ether), and related long chain esters and ethers. Plasticizers are added in fractions up to 66% [22–25], and this can completely modify quenching constants and binding constants. Since NPOE is a notorious quencher of luminescence, trifluoromethyl-POE and cyano-POE (both of which do not quench due to the lack of nitro groups) have been suggested as alternatives [53], the former being commercially available. Water-equilibrated thin films of plasticized PVC, in fact, are not a homogeneous medium but rather may be imagined as a inhomogeneous system resembling a microemulsion as shown in Figure 8.9.

PVC is soluble in THF solvent only, and this represents a major disadvantage in view of the toxicity and flammability of THF. Modified PVC (PVC-CP, a copolymer of poly(vinyl chloride), poly(vinyl alcohol) and poly(vinyl acetate) is a useful alternative to PVC since it is soluble in the much less toxic solvent ethyl acetate, but otherwise displays very similar properties. Finally, carboxy-PVC (PVC-COOH) is a commercially available PVC copolymer containing free -COOH groups and has been used for immobilizing amines such as proteins [54].

Polystyrene (PS) has been used in sensors for oxygen [35] because the quenching constants are much smaller in PS than in silicone, which is advantageous in the case of luminescent probes with very long decay times (which makes them extremely sensitive to oxygen). PS is soluble in various organic solvents, including ethyl acetate and toluene. Polystyrene may be plasticized by the same materials as are PVC and PVC-CP.

8.2.3 Silica Materials

Glass is widely used for manufacturing optical fibers. It is unique in terms of mechanical stability, optical transparency, and complete impermeability to any analyte. Aside

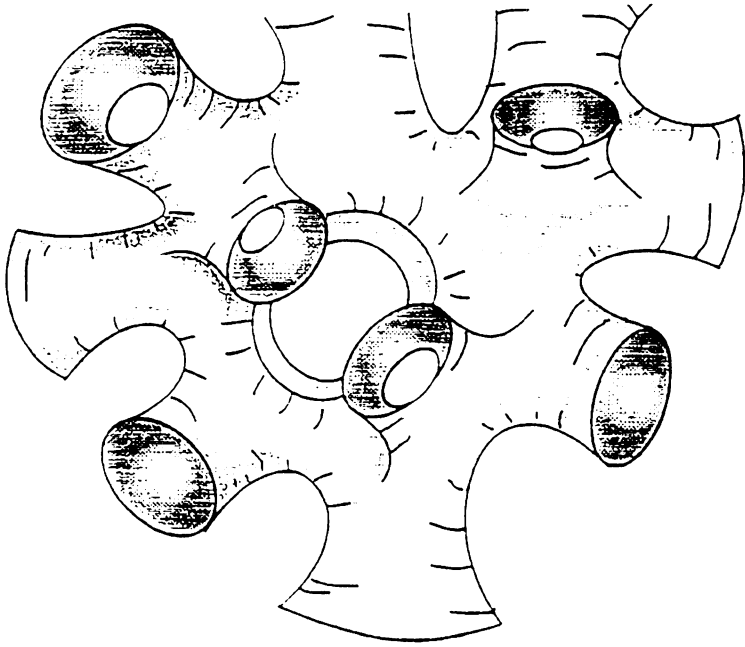


Figure 8.9 Schematic representation of the microinhomogeneity of thin films of water-saturated plasticized PVC. (Source: [22].)

from their function as a waveguide, glass fibers also have served as mechanical supports. Their surface may be made either hydrophilic or hydrophobic by treatment with a proper surface modification reagent [55,56]. Surface derivatization is usually performed with reagents such as amino-propyl-triethoxysilane, which introduces free amino groups onto the surface of glass to which dyes or proteins may be covalently attached. Glass does not measurably swell, but is difficult to handle in view of its brittleness. Many polymers have poor long-term adhesion to glass, which should be kept in mind when designing integrated optical chemical sensors.

Sol-gels form an attractive alternative to conventional glass [33,57]. They are obtained by hydrolytic polycondensation of tetraethoxysilane (TEOS) or related materials to give a fairly inert inorganic glassy matrix whose porosity and size of pore network can be varied to a wide degree by polymerization conditions, including time, pH, temperature, and silane:water ratios. Numerous organic dyes have been incorporated into sol-gel glasses at room-temperature conditions. Sol-gels support the transport of small molecules. Because they have no absorption in the near UV and visible,

sol-gels are well-suited for fabrication of dyed materials in the form of films, fibers, or monoliths.

8.2.4 Hydrophilic Supports

Hydrophilic supports are characterized by a large number of hydrogen-bridging functions such as hydroxy, amino, or carboxamide groups, or by anionic groups (mainly carboxy and sulfo) linked to the polymer backbone. Typical examples are the polysaccharides (celluloses), polyacrylates, polyacrylamides, polyimines, polyglycols, and the variety of so-called hydrogels. Depending on the degree of polymerization and crosslinking, they are water soluble or water insoluble. All swell in water. Throughout, they are easily penetrated by aqueous solutions and display poor compatibility with hydrophobic polymers such as silicone and polystyrene. Most hydrophilic polymer membranes are easily penetrated by both charged and uncharged low molecular weight analytes, but not by large proteins.

Cellulose in either the bead or membrane form has found widespread application as a support for indicators [4,39,58-64]. The ease of penetration by water results in short response times. Cellulose membranes as thin as 6 μm are commercially available, but require careful handling [62,63]. Beads are easier to handle, and after dyeing can be immobilized in a hydrogel matrix [64]. Aside from plain membranes, cellulose bound to polyester also is commercialized and has found application to pH sensing [62,63]. In addition to cellulose, other polysaccharides including dextrans and agarose have been used for dye immobilization to produce sensing chemistries for water-soluble analytes, but with no obvious advantages over cellulose. All saccharides are readily populated by bacteriae and algae.

Chemical modification of cellulose by introducing either hydrophilic or lipophilic groups results in entirely different but extremely useful materials. Celluloses also may be rendered with charged groups so to make them ion-exchangers. Such materials are offered by various manufacturers, albeit optimized for chromatography purposes. When cellulose membranes dry out, they become very brittle and are difficult to handle. Once dry, cellulose requires a considerable time to completely rehydrate and thereby undergoes considerable swelling, resulting in signal drift. Both the swelling rate and the hydration number are pH-dependent.

Polyacrylamides (PAAs), poly(hydroxyethyl acrylate) (poly-HEMA), poly(vinyl alcohols), poly(vinyl pyrrolidones), polyurethanes, and polyglycols [39,40,43,65] are good polymeric solvents for a number of indicators, but are water-soluble unless crosslinked. They can be retained on a support by cellulosic membranes, but dissolve quite an amount of water when in contact with aqueous samples. Crosslinked PAAs form mechanically stable and water-insoluble supports that are easily handled and chemically modified, but lack the good permeability of cellulose. PAAs are also available in bead form, and their surface can easily be modified by functional groups such as carboxy or primary amine. However, an excess of these functions may 1) introduce

a considerable buffer capacity, resulting in very long response time at the respective pH range and 2) establish an undesired Donnan potential.

Hydrogels are crosslinked macromolecular networks swollen in water or biological fluids and possess excellent biocompatibility, probably due to their high water content and special surface properties [65,66]. They are well-suited for pH and ion sensing, but covalent immobilization of indicator dyes is more tedious.

8.2.5 Diffusion and Permeation of Gases Through Polymers

The most important parameters for characterization of diffusion and permeation of gases through polymers are the diffusion coefficient D , the gas solubility S , and the permeation coefficient P . The permeation of small molecules through flawless and pinhole-free polymers occurs through consecutive steps of solution of a permeant in the polymer, and diffusion of the dissolved permeant through the inner free volume of the polymer, so that

$$P = D \cdot S \quad (8.6)$$

The temperature coefficients of P , D , and S can be represented in Arrhenius-type equations:

$$P = P_0 \cdot \exp(-E_p/RT) \quad (8.7)$$

$$D = D_0 \cdot \exp(-E_D/RT) \quad (8.8)$$

$$S = S_0 \cdot \exp(-\Delta H_S/RT) \quad (8.9)$$

where E_p and E_D are the respective activation energies, and ΔH_S is the solution enthalpy. Permeability P generally decreases with increasing density of the polymer, its crystallinity, and orientation. Crosslinking a polymer reduces P , as do added fillers (such as silicagel), while adding plasticizers can increase it. Humidity increases the P of some hydrophilic polymers. Permeation coefficients for numerous gases can be found in a useful compilation [46], and typical solubility data are compiled in Table 8.4.

It should be stated at this point that polymers are not ideal solvents, and that indicator dyes incorporated into polymers in almost any case have thermodynamic

Table 8.4
Solubility S (in $10^{-6} \text{ mol} \cdot \text{N}^{-1} \cdot \text{m}^{-1}$) of Simple Gases in Polymers at Room Temperature

<i>Polymer</i>	<i>Hydrogen</i>	<i>Methane</i>	<i>Nitrogen</i>	<i>Oxygen</i>	<i>CO₂</i>	<i>Water Vapor</i>
Poly(vinyl chloride)	12.0	76	11.0	13	214	39.200
Polycarbonate	0.3	—	—	2	58	758
Polystyrene	—	—	—	25	290	—
Poly(ethyl methacrylate)	—	—	34.0	38	500	110.000
Polyethylene (d 0.914)	—	49	10.0	21	116	—
Polyethylene (d 0.946)	—	23	5.4	8	9	—
PTFE*	—	—	0.7	1.3	5	—
Natural rubber	17.0	—	25.0	50	401	—
Silicone rubber**	33.0	—	89.0	138	310	High

* Teflon.

** Containing 10% (w/w) filler.

properties (such as pK_a values, lifetimes, or quenching constants) that are different from the respective data in solution. This represents a serious challenge in the design of materials for use in optical chemical sensors.

8.3 IMMOBILIZATION TECHNIQUES

Following the choice of indicator and polymeric support, the next step in sensor design involves immobilization of the dye in- or on- a support to result in the so-called sensing chemistry or working chemistry. Three methods are important for the preparation of sensing chemistries, viz., mechanical, electrostatic, and covalent immobilization. Several reviews cover all aspects of the chemistry and physics of immobilized reagents and dyes, proteins, and even whole cells [60.61.67.68]. Immobilization of dyes is not confined to reactions occurring in aqueous solutions, and may involve several steps. However, the immobilization chemistry should be kept as limited as possible, and procedures giving high yields at mild reaction conditions are highly preferred. Immobilization of most dyes results in a change of their spectral characteristics, pK_a values, binding constants, and-in particular-dynamic quenching constants. The changes reflect the various interactions that occur between neighboring dye molecules in, or on, the polymer; interactions between dyes and polymer, and electronic effects of covalent bonds on the chromophor.

8.3.1 Mechanical and Physical Immobilization

Methods for mechanical (physical) immobilization include 1) adsorption, 2) inclusion of dyes into spheres that they cannot leave (for example, into the void volume of polymers [69]) inside microspheres or the inner domains of sol-gels [33,57,70] or zeolites [71]; and 3) dissolution of indicators in a polymeric "solvent." Adsorption is the most simple technique, but of limited practicability. While many proteins, lipophilic dyes, plasticizers and detergents adsorb very well on moderately polar surfaces such as polystyrene, they also are slowly washed out into samples or buffers and tend to diffuse into other materials.

Mechanical immobilization is more attractive. A good example is provided by the incorporation of cationic oxygen probes into silicone rubber, where they do not dissolve because of their positive charge. To overcome this problem, they were first absorbed onto silica gel particles, which then were dispersed into silicone prepolymer, which in turn was cured in air [72]. Alternatively, they may be deposited on fillers contained in silicones [73]. In another example, it has been shown [69] that copolymerization of acrylamide with methylene-bis(acrylamide) in the presence of phenol red leads to microspheres with the dye firmly bound to the polymer. Such nondiffusible forms of pH indicating dyes are obtained by emulsion copolymerization of phenol red with aqueous acrylamide in the presence of emulsifier and toluene under nitrogen to give microspheres that are useful for optical pH sensing.

Another method of immobilization involves the use of indicators that are highly lipophilic and hence dissolve in lipophilic polymers from which they are not readily washed out because of their much better solubility in the lipophilic phase. Typical examples include oxygen-sensitive polymers incorporating lipophilic nonionic dyes [34,35], lipophilic pH indicators dissolved in plasticized PVC [74-76] and lipophilic ion pairs (i.e., a pair of positively and a negatively charged organic species, one of which is an indicator) [30,77,78]. Such coatings are particularly easy to make and highly reproducible because fabrication only involves dissolution of the dye in a polymer solution, and casting this "cocktail" onto the surface of a waveguide. However, dyes may slowly diffuse into other materials wherein their solubility is better.

8.3.2 Electrostatic Immobilization

If a surface of a rigid support contains charged functions (such as sulfo groups or quaternized ammonium groups), it is capable of binding ions of opposite charge. Sulfonated polystyrene, for instance, binds cations with varying affinity. This effect is widely used for separation of anions or cations from a solution, and for enrichment of traces of ions. Cations subsequently may be displaced from the solid phase by strong acid, and anions by strong base. Many indicators are either cations or anions, and consequently may be immobilized this way.

Ion exchangers are commercially available and may be classified into "strong" and "weak" forms. This refers to the affinity of the material for the respective cations

or anions. Both membrane- and bead-type ion exchangers are available. In order to firmly bind organic ions, the use of strong ion exchangers is preferred in order to prevent washout over time. Typical examples of indicators immobilized this way include bromothymol blue (on anionic polystyrene) [79,80] and hydroxypyrene-trisulfonate on cationic styrene [81].

The major advantages of electrostatic immobilization are the ease of the procedure and its reproducibility. Dye loading can be easily governed by the time of immobilization. The fabrication is very simple in that the charged polymer is immersed, for a defined period of time, into a solution of the dye. Because the indicator molecules are situated at sites on the surface of the polymer that are easily accessible to protons, but often not easily accessible to proteins, the corresponding pH sensors are said to display no protein error [81]. Many ion exchange materials show pH-dependent swelling and this may cause slow drifts in intensity and, even worse, the work function.

8.3.3 Chemical (Covalent) Immobilization

Covalent immobilization is the preferred method because it results in dyes that are firmly bound, via a covalent bond, to the polymer backbone and hence cannot be washed out by a sample. On the other side, the methods are more tedious than previous ones in that they require the presence of reactive groups on both the dye and the polymer, and at least one must be activated to freely undergo a chemical reaction with the partner. Numerous methods of surface modification (and activation) of polymers exist and can yield materials capable of covalently binding indicators via their reactive groups. With respect to reproducibility, it is preferred, though, to make use of pre-activated commercial materials. Excellent reviews have been given on the immobilization of metal chelators on cellulose [60] and of pH indicators on various materials including celluloses for use in optical-fiber sensors [61]. Among those, we find the Remazol procedure (which is the preferred method for making commercial pH paper strips) to give best results in case of celluloses [62] and related hydroxy polymers. The respective chemical bonds are shown in Figure 8.10.

Covalent surface modification of quartz, sol-gel, silica gel, conventional glass, and even metals such as iron and platinum, and elemental carbon, is almost exclusively performed with reagents of the type $(RO)_3Si-R'$, with R being ethyl or methyl, and R' being 3-aminopropyl, 3-chloropropyl, 3-glycidyloxy, vinyl, or a long chain amine [55,56,82]. An alternative reaction sequence that introduces amino groups involves the use of epichlorohydrin (which reacts with hydroxy groups) and then ammonia. The resulting materials then are easily reacted with the indicator or peptide to be immobilized. Porous glass with various types of organofunctional extension arms is commercially available and has been widely used for the design of waveguide biosensors.

From our experience, the recommended procedure for immobilizing dyes possessing $-COOH$ groups onto amino-modified surfaces is via the N-hydroxy-succinimidoyl (NHS) esters of carboxylic acids, which is highly reproducible and

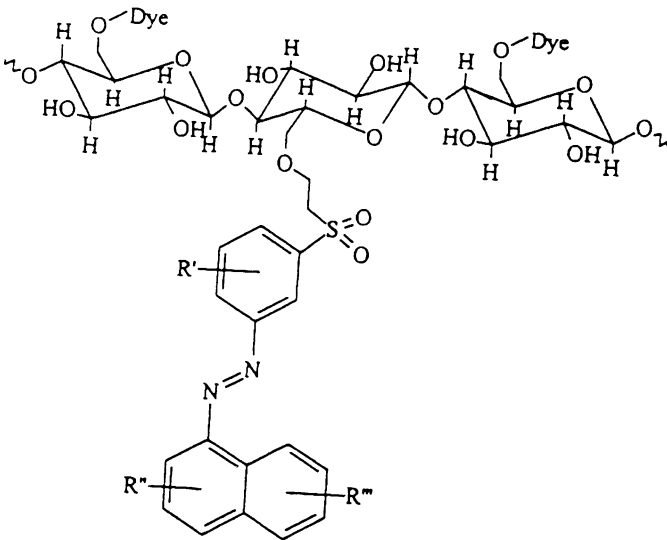


Figure 8.10 Schematic of a covalent link (the $-SO_2-CH_2-CH_2-O-$ group) between a dye and a cellulose backbone. Substituents R' , R'' and R''' can be varied to govern color and pK_o of the indicator dye.

proceeds under controlled and moderate conditions at room temperature. It is also recommended to use spacer groups (of a typical length of 6 carbon atoms) when immobilizing dyes or proteins so as to minimize undesired interactions between dye and support. A final method of immobilization of dyes is based on photopolymerization of dye-doped monomers, or by copolymerizing dyes possessing polymerizable groups with a monomer, typically acrylamide [69,83,84].

8.4 pH SENSORS

The kind of pH optrodes covered in this section is based on pH-dependent changes of the optical properties of an indicator-dyed layer attached to the tip or surface of an optical lightguide through which these changes are detected. The dye reversibly interacts with the protons of the sample to result in a pH-dependent absorption, reflection, or fluorescence. A selection of suitable dyes is given in Section 8.1.1., while suitable polymers are discussed in Section 8.2. Because the indicator dye and the sample are in different phases, there is necessarily a mass transfer step required before a constant signal is obtained. This leads to relatively long response times. Photobleaching and leaching, interferences by ambient light, nonideal optoelectronic equipment, the lack

of violet LEDs, and inexpensive blue lasers are further problems encountered in development of fiber-optic pH sensors.

Numerous optical sensors for pH have been reported [5.64]. They differ mainly in the kind of chemical transducer and the optical sensing scheme employed. Most work so far was on sensors for physiological pHs (i.e., from 5 to 8). In the past years, however, the working range has been extended to other pHs as needed in certain industrial applications because it has been recognized that pH optrodes have the potential of becoming useful in special fields of application where potentiometric methods fail or because they can offer considerable economic and sampling advantages.

One of the limitations of optical sensors is their sensitivity to changes in ionic strength (IS: a parameter for total ion concentration) at constant pH (see Section 8.1.2). The error in pH measurement caused by the IS of a sample also depends on the charge of the dye and is largest if the IS of the calibrant is highly different from that of the sample. The theory of the IS dependence of optical pH sensors has been described [5-9.64] and has resulted in a sensor for measurement of IS [9].

The preferred polymers for use in optical sensors are cellulose and related hydrophilic supports (see Section 8.2.4 and below). More recently, alternative solid supports for indicator dyes have been found. Sol-gels, for example, have excellent compatibility with glass fibers and may be deposited on both the distal end of a fiber, or may even replace the cladding of a waveguide [57.85-87]. Other materials that have been used more recently include rather hydrophobic ones such as plasticized PVC into which a fully lipophilic dyes were incorporated to give sensors with pK_a around 7.5 [54.88]. However, such sensors have pK_a values that strongly depend on the charge and quantity of additives, and on the fraction of plasticizer added [89]. Lipophilic pH probes (such as certain eosins) may be incorporated into Langmuir-Blodgett films to give pH-sensitive lipid bilayer membranes with pK_a 's quite different from the respective data in aqueous solution [90]. Recently, it was discovered that films of polypyrrol display pH-dependent absorptions between 600 and 1.000 nm [91]. Finally, it was shown that certain (nonsilicious) optical pH sensor materials are much more resistant to pH than are glass electrodes [63].

8.4.1 Absorbance and Reflectivity-Based Sensors

In the case of absorbance-based pH sensors, the Lambert-Beer law can be applied that relates absorption with the concentration ($[D]$) of the dye species

$$A = \log(I_0/I) = \epsilon \cdot [D] \cdot l \quad (8.10)$$

where I_0 and I , respectively, denote the intensity of transmitted light in the absence and presence of the dye at the analytical wavelength, l is the effective path length, and ϵ the molar absorption coefficient ($\text{cm}^{-1} \cdot \text{mol}^{-1}$) at the given wavelength.

In case of reflectance-based pH sensors and only absorption from the alkaline

(longwave absorbing) species occurring at the analytical wavelength, the absorbance can be described by

$$A = \log(k \cdot I_o^{\text{ref}}/I) = (A_{\text{max}}/10(-\Delta + 1)) \quad (8.11)$$

where $k = I_o/I_o^{\text{ref}}$, $\Delta = \text{pH} - \text{pK}$, and $A_{\text{max}} = T \cdot \epsilon \cdot l$. A is the absorbance at a given pH, and A_{max} is the absorbance of the completely dissociated dye. I is the transmitted light intensity at the analytical wavelength, and I_o^{ref} is the transmitted reference light intensity. I_o^{ref} can be measured at any wavelength where the intensity of multiple reflected light is independent of pH. Typically, it is measured at the isobestic point or at a wavelength at which neither form absorbs. The reference measurement is frequently needed to compensate for optical and instrument variations. A_{max} , k , and pK are intrinsic constants of the sensor. Other theories for reflectometric sensors do exist as well [92].

One of the first absorption-based pH-sensitive "chemistries" that had been developed made use of phenol red, which was incorporated into polyacrylamide beads [69,93] to give a sensor material with a pK_a of 7.92 ± 0.02 at zero ionic strength, and a pK_a of 7.78 ± 0.02 at 0.25M ionic strength. The temperature coefficient of the system, expressed as the change in pH indication per °C was 0.017 between 20 and 40°C, and a change of 0.01 pH units was observed per 11% change in ionic strength over the range of 0.05 to 0.3M. The response time for the signal to drop to 63% of its initial value is 0.7 min [93]. A schematic of the sensor is shown in Figure 8.11.

Fiber-optic pH sensors for sea water monitoring were obtained [94] by immobilizing phenol red on XAD-type ion exchangers. The dyes were adsorbed onto the polymer by placing the dry beads in a 0.1% indicator solution in methanol for four hours. While easy to fabricate, this material tends to undergo a pH-dependent swelling, which causes long-term drifts and to release the dye at high ionic strength.

Sensors for process control and physiological studies are based on thymol blue and bromophenol blue [95], cresol red [96], bromocresolgreen, and bromothymol blue adsorbed on cellulose strips [97], or on related absorber dyes [79-82,98-102]. The temperature coefficients of the XAD-immobilized bromothymol blue and thymolphthaleine, respectively, between 25 and 45°C are 0.013 ± 0.003 and 0.015 ± 0.003 per °C. The response time for 63% of the total signal change to occur is 1 min.

The preferred method for making pH-sensitive optical materials is clearly via covalent immobilization. Mohr & Wolfbeis [24] have designed a general logic for making pH sensors for various pH ranges, starting from a single precursor that was reacted with various components to give azo dyes with widely varying pK_a 's. These were covalently immobilized on cellulose-coated polyester films to result in sensors with pK_a 's ranging from 0.5 to 11.3. Because of the stability of the dyes and of the covalent bond, the sensors are stable over years, have long operational lifetimes, and achieve response times in the order of 30 to 60 sec because the active layer (which is

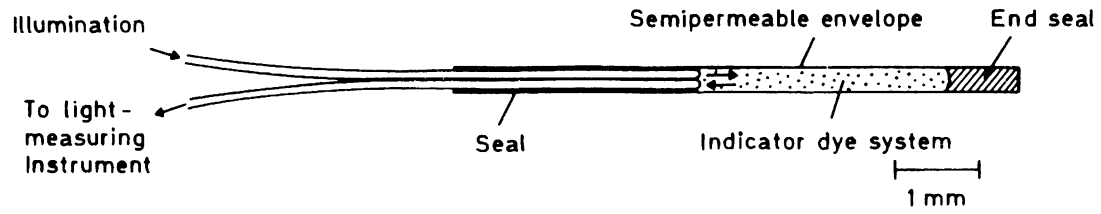


Figure 8.11 Fiber-optic pH sensor with reflective pH indicator chemistry contained in a semipermeable envelope at the tip.

a film of cellulose acetate on polyester, [103] is ca. 1 μm thick only. Table 8.5 summarizes the various types and $\text{p}K_a$'s of the such membranes.

Polyphthalate esters (like Mylar) are the preferred materials for depositing sensor chemistries to obtain planar sensors or sensor spots. Such sensors are now being made in large quantities for use in blood gas analysis, both continuously and single shot. Sensors are made by coating the polyester films with the respective materials by methods such as spin coating, or spreading (frequently using dissolved materials) as they are known in the film industry (see Figure 8.12), and sensor spots are then punched out to be used as either planar sensors (e.g., in disposable cassettes) or at the tip of an optical fiber.

8.4.2 Fluorescence-Based Sensors

Fluorescence is particularly well-suited for optical sensing owing to its sensitivity. For weakly absorbing species (i.e., when $A < 0.05$), the intensity I_f of fluorescent light returning from the sensor tip is proportional to the intensity of the exciting radiation, I_0 , and the concentration ($[D]$) of the fluorescent dye in the sensor:

$$I_f = k' \cdot I_0 \cdot \phi \cdot \epsilon \cdot l \cdot [D] \quad (8.12)$$

where l is the length of the light path in the sensing layer, ϵ is the molar absorptivity, ϕ the quantum efficiency of fluorescence, and k' the fraction of total emission being measured. At constant I_0 (8.12) can be simplified to give

Table 8.5
Absorption Maxima of Acid and Conjugate Base Forms and
 $\text{p}K_a$ Values of Dyed Cellulose Sensor Membranes (at 21°C)
for Various pH Ranges

Membrane	λ_{max} (nm) (Base Form)	λ_{max} (nm) (Acid Form)	$\text{p}K_a$
M-1	553	460	9.37
M-2	535	501	9.26
M-3	541	473	7.55
M-4	517	491	7.83
M-5	518	455	7.34
M-6	476	487	11.28
M-7	474	479	10.68
M-8	481	488	10.64
M-9	507	509	3.68
M-10	492	518	-0.5
M-11	486	503	2.24

Source: [24].

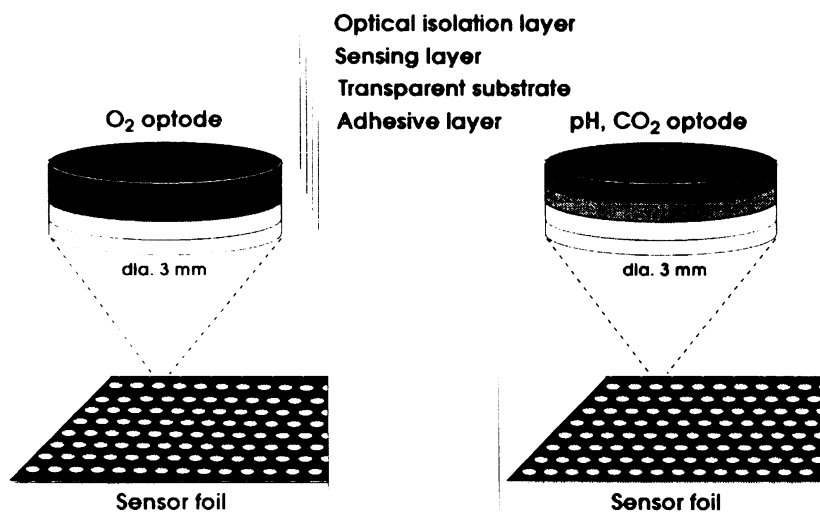


Figure 8.12 Cross-sections through sensor foils for mass fabrication of optodes for oxygen and pH/CO₂.

$$I_f = k \cdot [D] \quad (8.13)$$

where $k = k' \cdot I_0 \cdot \phi \cdot \epsilon \cdot l$. Seitz [61], as well as Leiner & Hartmann [64], derived equations to relate fluorescence intensity with actual pH, and the former also has addressed calibration issues.

A variety of fluorescent indicators is known [85], but only a few meet the requirements of an excitation maximum beyond 450 nm, to allow the use of inexpensive and flexible plastic fiber optics as light guides, and of light-emitting diodes as excitation sources (see Table 8.3). Large Stokes' shifts are also desirable in order to conveniently separate scattered excitation light from fluorescence using inexpensive optical filters. Further desirable properties include photostability, the presence of functional chemical groups suitable for covalent immobilization, and the lack of toxicity.

One of the first pH fluorosensors was obtained [81] by electrostatic immobilization of hydroxypyrene trisulfonate (HPTS) on an anion-exchange membrane. It allowed for measurement of pH in the range 6 to 8 by relating the ratio of fluorescence intensities emitted at 510 nm and excited at 405 nm (specific for the acid form) and 470 nm (specific for base form). The ratio was not affected by source fluctuations and slow loss of reagent, all of which can affect a single intensity measurement. The sensor showed an approximately 10% loss in intensity after four hours of continuous illumination. An important observation is the effect of indicator loading on the response curve of a sensor. With increasing indicator amounts being immobilized, the relative signal change becomes smaller and the pK_a is shifted to lower values [61].

Two related optical sensing materials for measurement of near-neutral pH values were also described in some detail [8.9]. HPTS and the pH probe 7-hydroxycoumarin-3-carboxylic acid (HCC), respectively, were covalently immobilized on surface-modified controlled porous glass (CPG). Analytical excitation and emission wavelengths were, respectively, 410 and 455 nm for the HCC-based sensor, and 465 and 520 nm for the HPTS based sensor. On CPG, the pK_a values were distinctly lower than those determined in solution, and the HPTS-based sensor was more sensitive to ionic strength than the sensor with the coumarin dye.

In an alternative approach [64,104], HPTS was covalently bound to a hydrophilic cellulose matrix, which then was deposited at the distal end of a single optical fiber. The cellulose matrix was further covered with an opaque cellulose overcoat, which provides both mechanical integrity and optical isolation from environmental optical interferences. Fluorescence intensity again was measured at 520 nm under 460-nm and 410-nm excitation (see Figure 8.1). The ratio of the two signals can be related to pH, and is relatively insensitive to optical throughput.

Other fluorescence-based pH sensors use immobilized fluoresceins [59,105,106] and naphthofluoresceins [85] on cellulose films or in sol-gels. Rather than attaching preformed sensing materials onto single fibers, the sensor chemistry has been deposited directly on the end face of a fiber via thermal or photopolymerization [84,107]. In a typical experiment, a fluoresceinamine was copolymerized with acrylamide and N,N'-methylenebis(acrylamide) onto the distal end of surface-modified single glass/glass optical fibers of 100/140- μm diameter. The polymer-modified single fiber sensors had a response time of < 10 sec. One disadvantage is the poor sensor reproducibility due to the difficulty in controlling the polymerization process, the need for an argon ion laser, and a rather sophisticated detection system.

One of the trends in the design of optical-fiber probes is miniaturization. A near-field fluorescence optical technique was applied to design submicron-sized pH sensors [108]. Multimode fibers were drawn into submicron fiber tips and coated with aluminum to form minute optical-fiber light sources with a distal-end pH chemistry. Such sensors have excellent spatial resolution and very short response times. Long-wave absorbing fluorophors (such as the SNARF dyes) immobilized on dextrans were employed for measurement of physiological pH. Similarly, oxygen-sensitive coatings have been deposited at the tip of fiber only 15- to 40- μm thick, and the resulting sensors were used to measure oxygen profiles in marine sediments. Figure 8.13 shows the tip of such a fiber with and without the black optical isolation.

8.4.3 Energy Transfer-Based Sensors

Jordan et al. [109] developed a single fiber-optic pH sensor based on energy transfer from a pH-insensitive fluorophore, eosin (the donor), to a pH-sensitive absorber, phenol red (the acceptor). The dyes were co-immobilized with acrylamide on the distal end of a surface-silanized single optical fiber. The pH-sensitive layer had a thickness of approximately 10 μm . The emission spectrum of eosin overlaps with the absorption

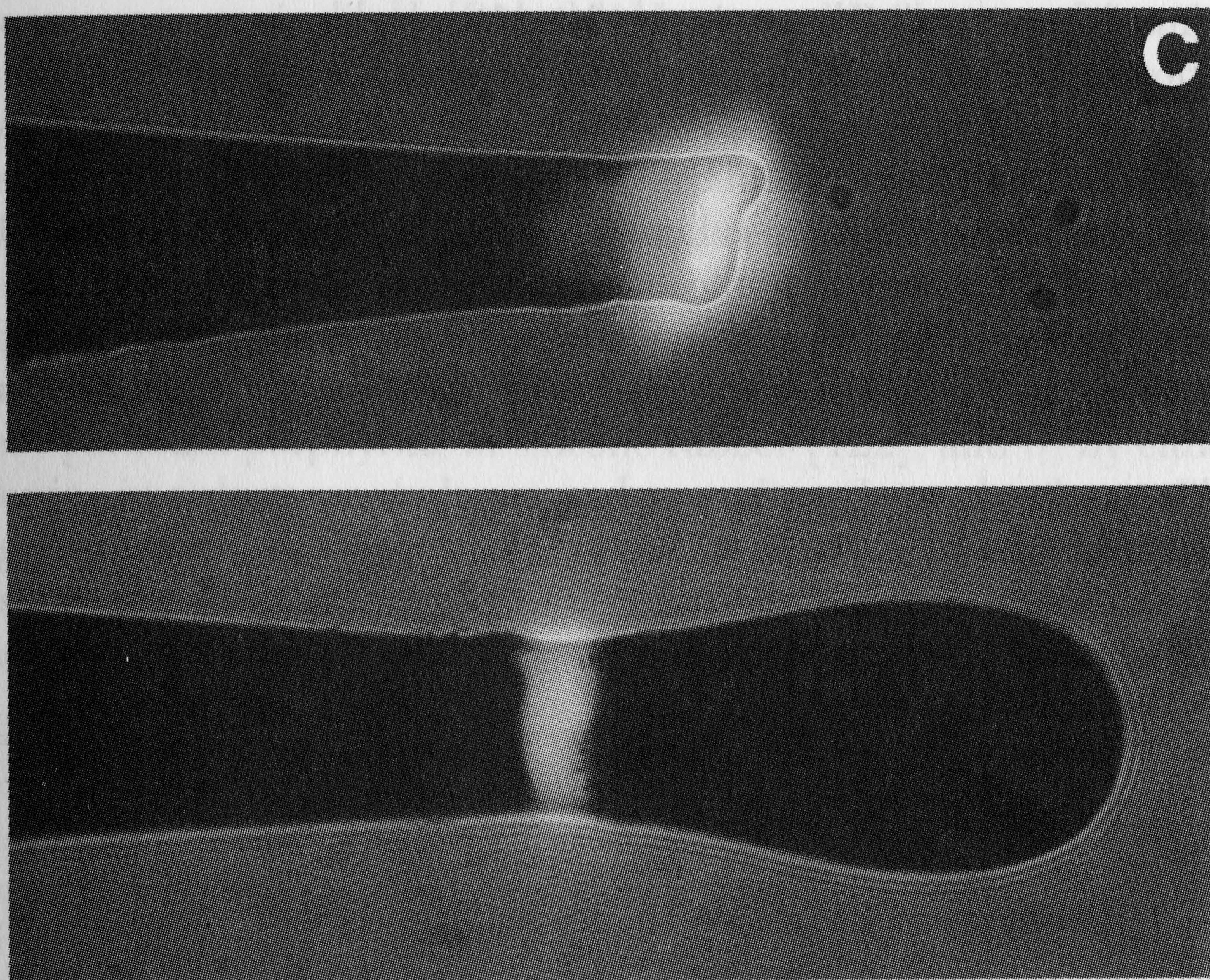


Figure 8.13 Oxygen micro-optrode (tip diameter ca. 15 μm) showing the red luminescence of the oxygen-sensitive chemistry without optical isolation (top) and with an optical isolation (a black silicone; bottom). (From: I. Klimant, V. Meyer and M Kühle, *Limnol. Oceanogr.* 40 (1995) 1159.)

spectrum of the basic form of phenol red. As the pH increases, the concentration of the base form of phenol red increases, resulting in an increased energy transfer from eosin to phenol red and in a diminished fluorescence intensity of eosin. Thus, changes in the absorption of phenol red, as a function of pH, are detected as changes in the fluorescence signal of eosin. The intensity of the fluorescence signal was observed with a photon-counting detector at the emission maximum of eosin (546 nm), after passing a dichroic mirror, a longpass filter, and a monochromator. The precision was ± 0.008 pH units, when measured with standard buffer solutions. The time required for 63% response is 4 to 5 sec for a pH change from 7.1 to 6.5. Some photobleaching of the base form of phenol red has been observed when continuously exciting the sensor over a 10-min period. Because the efficiency of energy transfer depends on the sixth power of the average distance of the two dyes, such sensors are highly sensitive to leaching and bleaching, particularly if this occurs at different rates.

8.4.4 pH Sensors Based on Measurement of Decay Time

In intensity-based pH sensors, it is usually the ratio of two signals (obtained at two wavelengths) that is related to pH. An alternative self-referenced method is based on

measurement of fluorescence decay time [110–112]. Unlike in oxygen sensors (where the population of the excited state of a single indicator is reduced due to collisional quenching by oxygen), decay-time pH sensors are based on the measurement of the relative contributions of the acid and base form of an indicator to the total decay time. Such sensors are likely to have excellent operational lifetime, but like all 2-wavelength methods cannot compensate for the most serious problem of indicator-based sensing (i.e., the temporal drift of the work functions). Like in all optical sensors, long-lived probes are desired for reasons of instrument simplicity and resolution.

8.5 OXYGEN SENSORS

8.5.1 Indicators and Polymeric Supports

Oxygen has almost exclusively been sensed via quenching of luminescence [28], while photometry plays a minor role. Chemiluminescent methods are irreversible and require addition of a reagent. The variety of indicators known for oxygen include polycyclic aromatic hydrocarbons such as decacyclene and perylene dibutyrate, and longwave absorbing metalorganic complexes of ruthenium, osmium, palladium, and platinum, which will be discussed below. Some have long-lived excited states (up to 1 ms), which makes them useful for lifetime-based oxygen sensors. Certain dyes also undergo quenching of their *phosphorescence* when absorbed on solid supports. These include tryptophan, benzoflavine, chlorophyll, and hematoporphyrin [113–115].

In order to obtain an oxygen-sensitive material for use in optical sensing, it is necessary to immobilize the oxygen-sensitive dye in a polymer subsequent to being deposited on a waveguide. However, most indicators do not have appropriate functional groups suitable for covalent immobilization, so that physical immobilization or immobilization on ion-binding membranes usually is preferred. In addition, it has been found that the Stern-Volmer quenching constants (K_{SV} in (8.5)) are reduced by 30 to 50% on covalent binding of a dye onto a rigid surface.

A simple way for immobilizing lipophilic oxygen indicators is to dissolve them in hydrophobic polymers such as poly(vinyl chloride) (PVC) or silicone. Silicone has excellent oxygen permeability and solubility, but is a poor solvent for most dyes. PVC, on the other hand, is a good solvent for most polycyclic aromatic hydrocarbons (PAHs), but has slow oxygen diffusion. In order to make PAHs more lipid-soluble and less water-soluble, they may be fitted with tertiary butyl groups, which results in a five- to twentyfold improved solubility in silicones and other materials [37]. Cross-linked poly(hydroxyethyl methacrylate) is another solvent that retains polycyclic aromatics such as diphenylanthracene [116] or ruthenium-tris(dipyridyl) [117].

The widely used oxygen indicator ruthenium-tris(bipyridyl) may be immobilized electrostatically on cation exchange membranes or physically adsorbed on particles entrapped in silicone [72]. The incorporation of decacyclene into a Langmuir-Blodgett quadruple layer has been reported as well [90], and an excellent sensitivity

and fast response to oxygen was observed. However, LB films are not stable on contact with many samples encountered in practice.

Oxygen-sensitive luminescent coatings also were obtained by 1) dissolving pyrene in dimethylformamide solvent [118], polyethylene [119] or poly(dimethylsiloxane) copolymers [52]; 2) by incorporating osmium-organic complexes on silica in silicone rubber [32]; 3) by embedding phosphorescent metal complexes of ferrone in silicone rubber films or binding them to anion exchanger beads [120]; 4) by dissolving camphor-quinone in PMMA, PVC, or polystyrene [121]; 5) by incorporating luminescent platinum porphyrins in silicone rubber [122]; and 6) by either dissolving porphyrins in polystyrene/toluene and spreading the cocktail as a film [35] or depositing porphyrins on various solid supports [123]. Certain (histidinato)cobalt complexes undergo reversible changes in absorption on exposure to oxygen, but are nonluminescent [124].

The quenching by oxygen of the luminescence of a ruthenium complex dissolved in various types of silicones was investigated by various groups. Since the work function was found to vary over time for quite a while, it was concluded that such sensor materials need to be recalibrated after prolonged storage. The type of matrix has a pronounced effect on the overall performance of such sensor materials, and decay kinetics are more complex in being multi-exponential, the various species being differently susceptible to quenching by oxygen [29,122,125,126]. Figure 8.14 shows the effect of oxygen on each of the three lifetimes of an oxygen sensor membrane composed of the dye Ru(dpp) in plasticized PVC. Physical models have been established to interpret the observed quenching processes [29,122,125-127].

8.5.2 Oxygen Sensors Based on Measurement of Luminescence Intensity

The first oxygen fluorosensors were based on the use of PAHs [119,128-134]. Fluoranthene, pyrene, benzoperylene, and decacycene are typical indicators, but all suffer from interferences by halothane, sulfur dioxide, chlorine, and nitrous/nitric oxide. Typical polymeric solvents are silicone rubber, plasticized PVC, polystyrene, poly(hydroxyethyl methacrylate) (p-HEMA), or porous glass. Detection limits are in the order of 1 torr (as with most types of fluorescence-based oxygen optrodes). Not unexpectedly, the diffusion of oxygen through poly(dimethylsiloxane) (PDMS) and p-HEMA is quite different, the diffusion constants being $3.56 \times 10^{-25} \text{ cm}^2/\text{sec}^{21}$ in PDMS (at 25°C), but 1.2×10^{-26} only in PHEMA (at 20°C).

Peterson and others [133] found perylene dibutyrate adsorbed on polystyrene beads to be a viable sensing material. It has excitation and emission maxima of, respectively, 468 and 514 nm. It is stable, and is efficiently quenched by oxygen, thereby allowing a resolution of ± 1 torr up to 150 torr. The sensor measures the ratio of scattered blue light and green fluorescence. Others have used decacycene dissolved in PDMS [37,129]. Interferences by the inhalation narcotic halothane were eliminated by covering the sensor with an 8- μm layer of teflon, which also acts as an

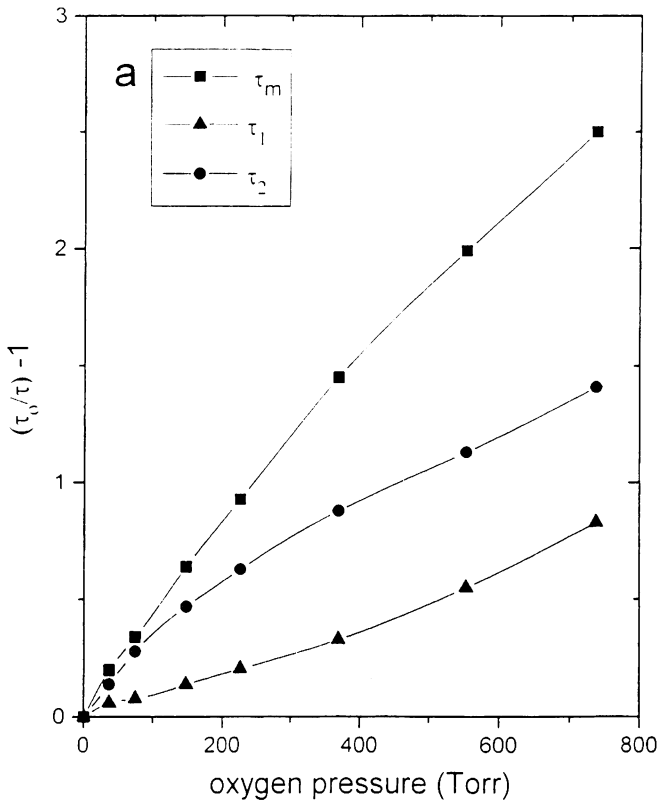


Figure 8.14 Effect of oxygen partial pressure on each of the three components of the decay of the luminescence of an oxygen sensor. (Source: [29].)

optical isolation [30,37]. An interesting application of this sensor is for simultaneous determination of oxygen and an interfering quencher such as halothane [37].

Semiconductor lasers have unique advantages over other types of conventional and laser light sources: they have a fairly large output (sufficient for most fiber applications), a narrow bandwidth, and a low price. Unfortunately, their wavelengths do not match the absorption spectra of PAHs. Okazaki and others [134] therefore have frequency-doubled the 780-nm emission of a semiconductor laser to obtain a 390-nm line with 50-pW intensity, which is suitable to excite benzo(g,h,i)perylene. The beam was launched into a fiber that guides light to the sensing material (the indicator dissolved in silicone grease) at its end. A second fiber was used to collect fluorescence at 430 nm. Oxygen was determined in the 0 to 30% range at atmospheric pressure.

In recent years, metalorganic complexes have become the preferred type of probes for oxygen. They have long lifetimes (0.2-1.000 μ s) and therefore are efficiently quenched by oxygen. Their excitation wavelengths range from 450 to 600 nm.

which makes them excitable by LEDs, and some display extraordinarily large Stokes' shifts. For example, ruthenium-tris(bipyridyl) [31,72] and the respective tris(phenanthroline) complex [135] were adsorbed onto silicagel particles and then entrapped in silicone polymer. The fluorescence of the resulting material is strongly quenched by oxygen, but a Stern-Volmer plot is nonlinear. Bacon & Demas [136] found ruthenium(II)tris(4,7-diphenyl-1,10-phenanthroline) (Ru(dpp)) to be a most viable oxygen probe that can be incorporated into a silica-filled silicone polymer. The resulting membranes, ca. 100 μm in diameter, were found to be useful for optical oxygen sensing and were investigated with respect to the effect of oxygen on fluorescence intensity and lifetime. The respective Stern-Volmer plots are practically superimposable, but not linear. Ru(dpp) is one of the most widely used oxygen probes at present.

Table 8.6 summarizes figures of merit for longwave oxygen probes. Among those, the metallated ketoporphyrins [35] look to be almost ideal indicators by virtue of their longwave absorptions and emissions, a 150 to 200 nm Stokes' shift, full compatibility with diode light sources, excellent photostability that by far exceeds all other probes of Table 8.6, solubility in various polymers, long decay time, compatibility with LEDs and diode lasers, 15% quantum yield, and the fact that they are uncharged. A generalized chemical structure, with Me representing the metal ion, is given in Figure 8.15.

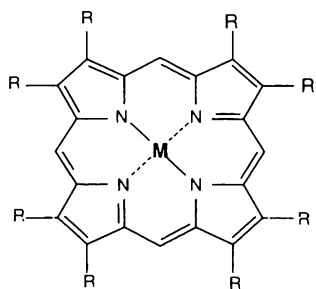
Most of these indicators are highly specific if applied as a solution in a lipophilic organic polymer. No, or only small, interferences are found for water vapor, nitrogen, noble gases, carbon monoxide, carbon dioxide, methane, and higher alkanes at realistic pressures, although these usually can pass the polymer membranes. Potential interferents are sulfur dioxide (a notorious quencher), halothane (an inhalation narcotic), chlorine, and nitrogen oxides (except N_2O). Transition metal ions and heavy atoms also quench, but usually cannot enter the polymer membrane and therefore remain inert.

Table 8.6
Figures of Merit for Longwave Luminescent Oxygen Probes

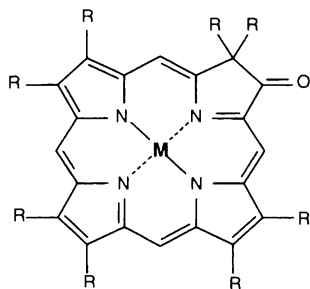
<i>Probe</i>	<i>Excitation/Emission Maxima*</i>	<i>Decay Time**</i>	<i>Ref.</i>
Ru(dipy)	460/605	ca. 0.8 μs	[31]
Ru(dpp)	460/610	ca. 4 μs	[136]
Os-bis(2,2',2''-terpy)	650/710	270 ns	[32]
Pd-octaethylporphyrin	545/670	990 μs	[137]
Pd-keto-porphyrin	600/780	480 μs	[35]
Pt-keto-porphyrin	590/760	64 μs	[35]
Al-Ferrone	390/600	unknown	[120]
Camphorquinone	470/560	ca. 1 ms	[121]

* In nanometers

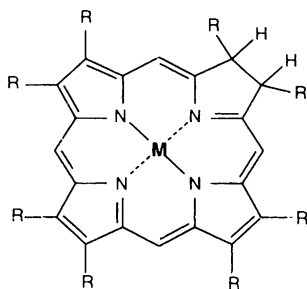
** Under nitrogen.



(a)



(b)



(c)

Figure 8.15 Chemical structures of luminescent oxygen probes: (a) porphyrins; (b) ketoporphyrins; and (c) chlorins. The central metal atom preferably is Pt or Pd.

8.5.3 Oxygen Sensors Based on Measurement of Decay Time

Equation (8.5) predicts that oxygen not only affects the intensity of the luminescence of a luminophore, but also its decay time τ (see Figure 8.14). The long decay of metalorganic oxygen indicators renders them particularly suitable for sensors based on measurement of decay time. In the first sensor of that kind [31], phase fluorimetry was applied to measure the oxygen-dependent phase shifts of a sinusoidally excited luminophore and a frequency-modulated blue LED served as a light source. Compared to former sensor types, decay time-based sensors display decisive advantages: They have negligible signal drift arising from leaching and bleaching because the decay time is independent of fluorophore concentration (in a first approximation). Secondly, they display excellent long-term stability because the system is internally referenced. Finally, no drift arising from light source intensity and photodetector sensitivity fluctuations is to be expected because it is not the absolute intensity that is measured, but rather the phase shift between excitation and fluorescence.

Numerous other reports on oxygen sensors based on measurement of decay time exist [31–35, 138–140]. However, the aim of this chapter is on materials rather than methods and hence a discussion of decay time-based sensors is beyond its scope. An interesting application of decay time-based sensors is in transcutaneous oxygen sensing [140] where intensity-based systems are clearly inferior due to the strongly varying fluorescence background and light permeability of skin.

8.5.4 Oxygen Sensors Based on Measurement of Energy Transfer

An interesting type of oxygen sensor has been described [132] that is based on electronic energy transfer from a donor (whose fluorescence is quenched by molecular oxygen) to an acceptor (whose fluorescence is less affected by oxygen). Pyrene was employed as the donor, and perylene as the acceptor. The fluorescence emission band of the donor shows good overlap with the absorption band of the acceptor. When excited at 320 nm, the two-fluorophore system showed strong fluorescence at 476 nm, where pyrene itself is nonfluorescent. Although perylene is not efficiently quenched by oxygen, the system strongly responds to oxygen because fluorescence is quenched with an efficiency that by far exceeds the quenching efficiency for pyrene or perylene alone. There is an almost fourfold increase in the quenching constants of the energy transfer system (as compared to the conventional system). A fiber-optic oxygen sensor was developed by incorporating the two dyes into a silicone polymer matrix that had been attached to the end of an optical fiber. Oxygen was detected in the 0 to 150-torr range with a 0.5 to 3-torr resolution.

8.6 SENSORS FOR CARBON DIOXIDE

Traditionally, *gaseous* carbon dioxide (CD) has been assayed via infrared absorptiometry. The preferred method for measuring *dissolved* CD is via electrochemistry by

measuring changes in the pH of a buffer solution retained in front of a pH electrode by a CD-permeable but proton-impermeable polymer. Two important indicator-based methods exist. The first (named the *Severinghaus* method) works by analogy to the electrochemical approach (i.e., via a change of pH of an immobilized buffer). The other (so-called plastic type) sensors are based on a hydrated pH indicator anion entrapped (without any buffer!) in a proton-impermeable polymer.

8.6.1 Severinghaus-Type Sensors

Such sensors are obtained by entrapping a buffer (such as a 10-mM hydrogencarbonate solution) along with a dissolved indicator (such as bromophenol blue or HPTS) in a gas-permeable polymer (such as silicone). The response of this sensor to CD occurs as a direct result of the proton concentration in the buffer in the sensitive material, which is related to the concentration of CD through the following series of chemical equilibria:

1. $\text{CO}_2(\text{aq}) + \text{H}_2\text{O} \rightleftharpoons \text{H}_2\text{CO}_3$ (hydration)
2. $\text{H}_2\text{CO}_3 \rightleftharpoons \text{H}^+ + \text{HCO}_3^-$ (dissociation, step 1)
3. $\text{HCO}_3^- \rightleftharpoons \text{H}^+ + \text{CO}_3^{2-}$ (dissociation, step 2)

These are governed by the following equilibrium constants:

$$K_h = [\text{H}_2\text{CO}_3]/[\text{CO}_2](\text{aq}) = 0.0026 \quad (8.14)$$

$$K_1 = [\text{H}^+][\text{HCO}_3^-]/[\text{H}_2\text{CO}_3] = 1.72 \cdot 10^{-4} \quad (8.15)$$

$$K_2 = [\text{H}^+][\text{CO}_3^{2-}]/[\text{HCO}_3^-] = 5.59 \cdot 10^{-11} \quad (8.16)$$

In most studies on CD sensors, the total analytical concentration of carbon dioxide (i.e., $([\text{CO}_2]_{\text{aq}} + [\text{H}_2\text{CO}_3])$), has been related to the response.

In the first optrode for CD, Lübbers and Opitz [118] followed the changes in fluorescence intensity of a membrane-covered solution of 4-methyl-umbelliferone in a carbonate internal buffer as a function of the partial pressure of CD. The internal buffer (also containing the indicator) was covered with a 6- μm PTFE membrane, which is permeable to CD but impermeable to protons and other ionic species. The ratio of fluorescence intensity at 445 nm measured under excitation at 318 nm and 357 nm was related to pressure. The same scheme was applied to construct a compact instrument (5 by 6 by 14 cm in size) for CD. It consisted of a blue LED as a light source, the longwave-absorbing indicator hydroxypyrene-trisulfonate (HPTS)

dissolved in bicarbonate and placed behind a PTFE layer, two optical filters, and two photodiodes for detection of light.

Zhujun and Seitz [141] used HPTS in bicarbonate, covered with a silicone membrane, to sense CD. Fluorescence was measured with a bifurcated fiber system. Complete response occurs within a few minutes. Both sulfite and sulfide were found to interfere (probably as their membrane-diffusible forms SO_2 and H_2S). The equation used to relate the CD partial pressure to hydrogen ion concentration is

$$[\text{H}^+]^3 + N[\text{H}^+]^2 - (K_1 \cdot C + K_w)[\text{H}^+] = K_1 \cdot K_2 \cdot C \quad (8.17)$$

where N is the internal bicarbonate concentration. K_1 , K_2 , and K_w are the dissociation constants of carbonic acid ((8.15) and (8.16)) and water, respectively. The value C is the analytical concentration of CD (in both the hydrated and unhydrated form). It was shown that, within a limited range, there is linearity between CD pressure and $[\text{H}^+]$ according to

$$(\text{H}^+) = K_1/C \cdot N \quad (8.18)$$

In practice, the internal HCO_3^- concentration should be such that the CD concentrations of interest yield pH changes in the range of the pK_a of the indicator. In the case of the widely used HPTS with its pK_a of 7.3, the external pCO_2 should adjust a pH between 6.5 and 8.0 in the internal buffer.

Heitzmann & Kroneis [142] prepared CD-sensitive fluorescent membranes by soaking cross-linked polyacrylamide beads with a solution of HPTS in bicarbonate, and embedding them in silicone rubber matrix deposited at the tip of a fiber (Figure 8.16). The response to CD was varied by adding different quantities of bicarbonate, carbonate, and HPTS, all of which act as buffers. The poly(acrylamide) beads may be omitted so that an emulsion of the HPTS/carbonate solution in silicone rubber is obtained. These sensors have excellent long-term performance, but when stored in media of low pCD they tend to become destabilized. It takes several hours to obtain a stable baseline again after having been exposed to higher pCD levels. This is probably due to some dehydration and also a shortage of water molecules available for reaction (1) which, in turn, results in some contraction and expansion of the droplets and beads because of osmolarity effects.

Typically, a 20-mM bicarbonate solution would act as an internal buffer. With a given indicator, it is the choice of buffer that primarily determines the slope of the response curve. The slopes of the response curves also are governed by the pK_a of the indicator and the total ionic strength of the internal buffer. Unfortunately, high buffer concentrations result in very long response times. These are further prolonged with increasing thickness of the sensor coating, additional covers, slow kinetics of the hydration, and-in particular-slow dehydration of CD. For the system described above, the response for a change from 0 to 5% CD was 15 sec for 90% of the final

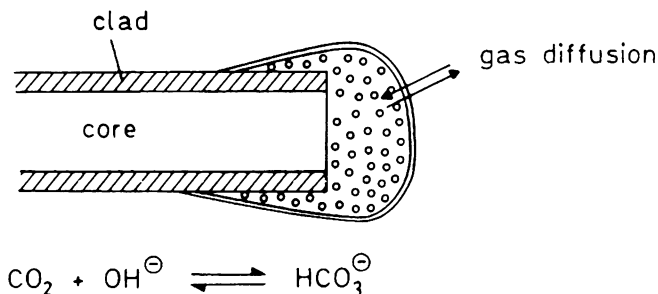


Figure 8.16 Optical sensor for carbon dioxide with a buffer solution emulsified into a drop of silicone placed at the tip of a fiber and covered with a gas-permeable black overcoat.

value. The reverse change required approximately 30 sec. It should be noted that most other optical CD sensors described so far have a much slower response. The addition of carbonic anhydrase can accelerate response time [143].

A CD sensor with an indicator directly immobilized on the fiber has also been described [144]. A fluorescent dye was covalently bonded onto the glass fiber tip, so the miniature size of the sensor was preserved. The fiber was then coated with a silicone-carbonate copolymer that rejects protons, whereas CD can pass. Direct casting of the polymer onto the sensor chemistry imposed major problems, and it was decided to use a preformed cover membrane. When two sensing layers with different spectral properties, and being selective for oxygen and CD, respectively, are attached to the end of a fiber, a single fluorosensor for both species can be obtained [145]. Blue excitation results in two fluorescences: The green fluorescence reports CD, while the red fluorescence reports oxygen.

Vurek and others [146] devised an absorbance-based CD sensor that relies on the principle of a previously designed pH sensor. An isotonic solution of salt, bicarbonate, and phenol red was covered with a CD-permeable silicone rubber membrane. The device uses two fibers: one carrying the input light, the other reflected light. The sensor responds over the physiological range and its performance was demonstrated *in vivo*. Similarly, a fluorescein-based CD-sensitive system was reported by Hirschfeld and others [147].

Leiner and others [148] have developed planar CD-sensitive chemistries for mass fabrication (Figure 8.12). Polyester membranes served as planar supports onto which a pH-sensitive material was fixed using a proton-permeable glue. After soaking with a bicarbonate buffer, the cellulosic material was covered with hydrogel and, finally, with a CD-permeable silicone-polycarbonate copolymer. CD, permeating into the internal buffer, changes the internal pH and hence causes fluorescence intensity to change. The sensing membranes can be prepared in large sheets that later can be punched into small (i.e., 1-5 mm) spots and placed at the distal end of a fiber. They

also can serve as sensor sheets in measurement of surface pCD of the skin. Given the low costs for the fabrication of such sensor spots, another important application is in disposable kits for determination of CD along with oxygen and pH in blood. Figure 8.17 shows the design of such a kit.

A mechanically highly resistant and sterilizable sensor for CD was obtained by covalently immobilizing a commercial pH probe on a thin cellulose film, soaking it with buffer and covering it with a proton-impermeable film of silicone rubber [149]. The top layer also contained a highly reflective material that acts as an optical isolation. CD was measured over the 0 to 760-torr range, and effects of buffer capacity and buffer pH studied in detail. Response times for dissolved CD are in the order of several minutes, and no cross-sensitivity to pH was observed at all. Sensor sterilization with hydrogen peroxide did not affect the calibration graph. An interesting sensing scheme for CD that also is applicable to decay time-based sensing makes use of tris(pyrazinyl)thiazole complexes of ruthenium(II) whose luminescence is quenched by the protons formed by reaction of CD with water [143,150]. The probe was immobilized onto anionic dextrane gel soaked with phosphate buffer (8.5), and the resulting fiber sensor measured CD between 0 and 760 torr.

8.6.2 Plastic-Type Sensors

This type of sensor for carbon dioxide is being constructed without using an internal aqueous buffer solution. Rather, the pH-sensitive dye is placed directly in the organic polymer in the form of its anion, the cation usually being a quaternary ammonium ion. Mills and coworkers have described various types of plastic-type film sensors for CD. They are made by casting a cocktail composed of a pH indicator anion (D^-), an organic quaternary cation (C^+), and a polymer such as ethyl cellulose, all dissolved in

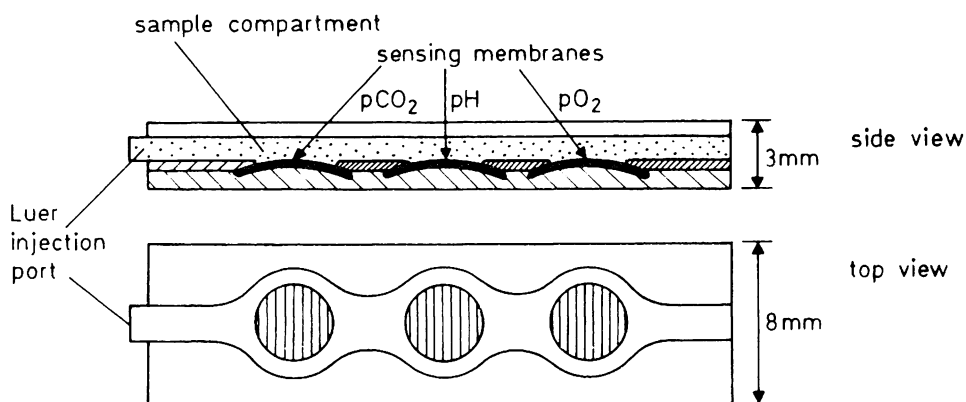


Figure 8.17 Top and side view of a disposable kit with three sensor spots (pH, oxygen, carbon dioxide) integrated into a compartment accommodating a calibrant or a blood sample.

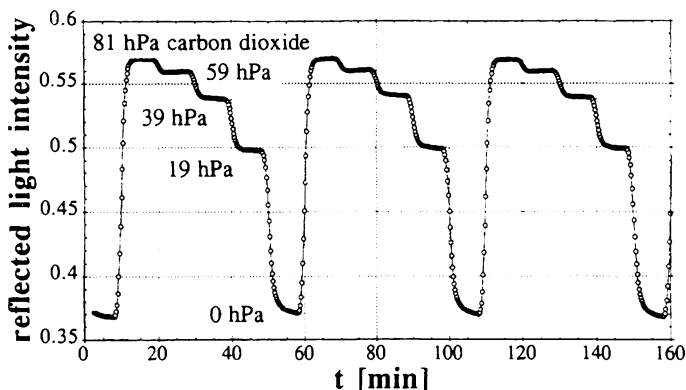


Figure 8.18 Changes in the reflectivity of a sensor for carbon dioxide with the partial pressure of carbon dioxide in water solution. (Source: [156].)

an organic solvent such as toluene onto a solid support and evaporating the solvent. Such films undergo very fast color changes on exposure to even small concentrations of CD [151–153], as can be seen from Figure 8.18. The response can be fine-tuned by variation of the components, in particular the quaternary ammonium base [154,155], and silicones have been suggested as an alternative to other polymers when measuring dissolved CD [156].

The scheme was extended to an energy-transfer fluorosensor composed of an absorber dye (*m*-cresol purple) and an inert fluorophore (sulforhodamine 101) entrapped in an ethyl cellulose film. CD modulates the decay time of the fluorescence of the rhodamine dye, and this serves as the analytical information. The sensor has an excellent long-term stability, is compatible with the 635-nm laser diode, and has response times in the order of seconds. A major problem may arise, though, if the two dyes leach/bleach at different rates in view of the extremely strong distance-dependence of the energy transfer process. In addition, all plastic-type sensors require storage in the complete absence of even traces of acidic gases, which tend to irreversibly deactivate plastic film sensors with their inherently low buffer capacity.

Another bufferless CD sensor material was reported that was prepared by dispersing fluorescein in poly(ethylene glycol) and then depositing it at the distal end of an optical fiber [157]. Evaporation of the solvent is reported to be negligible. The dynamic range is from 0 to 28% (v/v) for CD, with a detection limit of 0.1%. Full response is achieved within 10 to 20 sec. The outer membrane, ca. 10- μm thick, is composed of poly(ethylene glycol)s with molecular weights of 200 and 1540 Dalton, respectively, in a 20:80 (w/w) ratio.

8.7 AMMONIA SENSORS

Three major optical sensing schemes are known for ammonia. In the first, the absorption of light in the NIR by ammonia is exploited in plain fiber sensing. This approach is not very sensitive and response depends on humidity, but sensors are simple in design and display good stability. Absorption can be measured in both the transmission mode (in a gas cell) or by the evanescent wave technique. The latter, however, even more strongly depends on the relative humidity of the gaseous sample due to adsorption of water on the sensor/sample interface. The method cannot be applied to aqueous samples. In the second approach, ammonia is reacted with a dye such as ninhydrin to yield a purple coloration. This is an irreversible reaction, so that the "sensor" actually is a single-shot probe. In the third approach, the basic properties of ammonia are exploited: It is capable of changing the color of pH indicators immobilized on a waveguide. Only the third sensing scheme is both indicator-based and reversible and will be discussed here.

A reversible optical waveguide sensor for ammonia vapors was reported [158] that consists of a small capillary glass tube fitted with an LED and a phototransistor detector to form a multiple reflecting optical device. When the capillary was coated with a thin solid film composed of a pH-sensitive oxazine dye, a color change occurred on contact with ammonia. The instrument was capable of reversibly sensing ammonia and other amines. Vapor concentration from 100 to below 60 ppm ammonia were easily and reproducibly detected. A preliminary qualitative kinetic model was proposed to describe the vapor-film interactions. The method was applied to design a distributed sensor for ammonia [159].

Ammonia sensors based on the same principle as electrochemical ammonia sensors (viz., the change in the pH of an alkaline buffer solution) have been reported by various groups: Arnold and Ostler [160] followed the changes in the absorption of an internal buffer solution to which p-nitrophenol was added. Ammonia passes by and gives rise to an increase in pH, which causes a color change of the indicator to occur. Wolfbeis and Posch [161] entrapped a fine emulsion of an aqueous solution of a fluorescent pH indicator, which simultaneously may act as a buffer, in silicone rubber. Alternatively, 0.001M aqueous ammonium chloride may be used as internal buffer. The buffer strength strongly determines both response time and slope of the response curve. Detection limits are in the order of 5 to 20 μM , and equilibration is very slow, particularly in the back direction and with aqueous sample solutions. Another type of fluorescent sensor for ammonia was obtained by entrapping a 50- μM solution of a carboxyfluorescein in an ammonium chloride buffer in front of a fiber optic. The device was extremely sensitive and used for measurement of extracellular ammonia [162].

Hydrophilic ammonia sensor films were obtained by immobilizing bromothymol blue in a hydrophilic polymer and measuring the changes in reflectance

induced by ammonia in the gas phase [163]. The working range was from 1.5 to 30 mM, and possible interferents were investigated. Similar films have been used in a portable photometric ammonia gas analyzer [164].

Shahriari and others [165] developed a new porous glass for ammonia detection whose structure imparts a high surface area to the fiber core. Ammonia vapors penetrating into the porous zone pretreated with a reversible pH indicator produce a spectral change in transmission. The resultant pH change is measured by in-line optical absorbance and is said to be more sensitive than sensors based on evanescent wave coupling into a surrounding medium. The signal can be related to the ambient ammonia concentration down to levels of 0.7 ppm. In order to speed up response time, a porous plastic material, exhibiting very high gas permeability and liquid impermeability, was used in another type of ammonia sensor [166]. The porous plastic fibers were prepared by copolymerization of a mixture of monomers (methyl methacrylate and triethylene glycol dimethyl acrylate), which can be cross-linked in the presence of an inert solvent (such as octane) in a glass capillary. After thermal polymerization, the plastic fibers were pulled out of the capillaries and used in the sensor.

Ammonia, being a basic gas, causes the color of appropriate pH indicators to change. This is exploited in a plastic-type ammonia sensor that works by analogy to the respective sensors for carbon dioxide, except that pH-changes go in the other direction. Again, silicone is the preferred material, but usual dyes are insoluble therein. As a result, they have to be chemically modified, for example, by making a lipophilic (silicone-soluble) ion pair composed of the dye (usually on anion) and an organic cation (such as cetyl-trimethyl-ammonium ion). The silicone matrix acts as a perfect barrier for hydrogen ions ("pH"), which would interfere, and the resulting sensors display very low limits of detection (LODs) that range from 20 to 100 ppb [167]. Figure 8.19 shows a typical response. The method has been extended to fluorescence where LODs are even lower [168], and the coating was applied in an integrated optical disposable [169].

A fluorescent type of sensor for monitoring ammonia in air was obtained by impregnating porous cellulose tape with a solution of cosine bluish, p-toluenesulfonic acid, and glycerol [170]. On exposure to ammonia, the fluorescence of the dye at 550 nm increases and is proportional to the concentration of ammonia gas at constant sampling time and flow rate. One hundred ppb of ammonia were detectable and interference studies revealed a remarkable selectivity, although acidic gases are likely to reverse the response of ammonia. Obviously, the sensor is inadequate for detection of ammonia in water.

Generally, all types of ammonia sensors based on pH effects also respond to other uncharged amines such as methylamine, pyridine, or hydrazine because they are strong bases, too, and can pass almost all polymers used in ammonia-sensitive materials. Secondly, all acidic gases including CO_2 , SO_2 , and HCl, but also vapors of organic acids such as acetic acid, will interfere once the sensor is loaded with ammonia. Hence, the specificity of such sensors is limited. One way to overcome

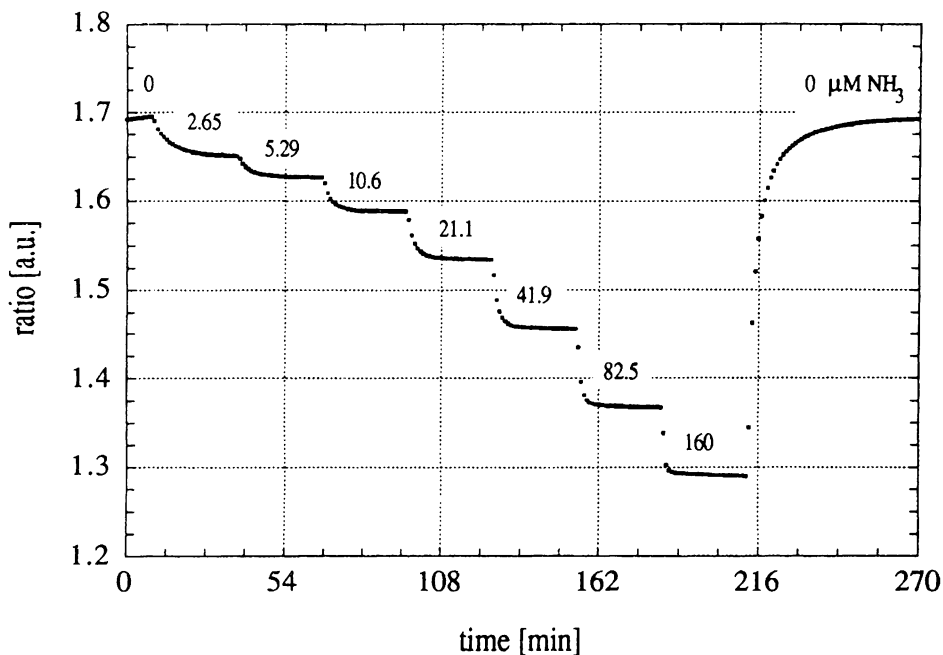


Figure 8.19 Response of an optical sensor to micromolar levels of dissolved ammonia. (Source: [167].)

interferences by acidic species is to make the sample strongly alkaline (if possible), which converts the acids into their nondiffusible salt forms.

8.8 ION SENSORS

Several schemes exist for sensing ions. They are based on either the use of 1) so-called chelators (i.e., dyes that bind a metal ion and thereby undergo a change in color: 2) ions carriers (i.e., uncolored, frequently cyclic ethers or esters that are capable of specifically binding (alkali) ions and to transport them into lipid sensor films): 3) chromoionophores (which, in essence, are a combination of a dye with an ion carrier, both contained in the same molecule): or 4) enzymes that undergo metal-induced change in their optical properties or activity.

8.8.1 Chelator-Based Ion Sensors

In this scheme, ions are determined, making use of so-called indicator dyes that undergo a binding reaction with ions, preferably of multiple charge. This reaction is

accompanied by a change in the absorption or fluorescence of such "chelators." Numerous chelators exist [1-4,10], but most bind irreversibly or with a high or low pH so that they cannot be used for continuous sensing at near-neutral pH or at pHs, which are strongly different from the sample to be monitored, but rather act as single-shot probes. The respective sensor materials are obtained by immobilizing an indicator dye in an ion-permeable matrix such as cellulose or a hydrogel. A major disadvantage is based on the fact that for practically each ion, a different dye, and hence a different analytical wavelength, has to be applied.

Oehme & Wolfbeis have reviewed the state of the art in optical probing (as opposed to continuous sensing) of heavy metals (HMs) [10]. Aside from reporting on existing probes for the main group HMs (mainly copper, zinc, cadmium, mercury, silver) and for the transition HMs (Fe, Cr, Mn, Co, Ni), they also discuss unspecific probes (i.e., sensors for *total* HMs). Unfortunately, practically all existing sensors for HMs are different in terms of dye (i.e., analytical wavelengths), method of immobilization, and polymeric support, a fact that is highly disadvantageous and does not allow simple optoelectronic sensor systems to be designed that can be applied to all sensor chemistries. A uniform protocol would be highly desirable, but is unlikely to exist. A detailed discussion of all these sensor materials-which have found their most widespread application in the form of tests strips-is, however, beyond the scope of this chapter.

8.8.2 Sensors Based on the Use of Ion Carriers and Chromoionophores

No chelators are available for the clinically important alkali ions including potassium, sodium, and lithium, to work at pH 5-8 and to cover the clinical ranges, which are 110-180 mM for sodium, 1-10 mM for potassium, 0.4 to 2 mM for calcium, and 50 to 170 mM for chloride. While certain probes have become available in recent years from commercial sources, these are designed for cytological studies where much lower ion concentrations are encountered. Hence, these probes cannot be used to measure ions in extrastitial fluids. Hence, other sensing schemes have to be applied. The most general approaches make use of organic "hosts," capable of binding an ion (the "guest") inside its cavity or cyclic structure. If incorporated into a polymeric matrix (such as plasticized pvc), the host may even extract the guest from an aqueous sample phase. Typical hosts for use in ion sensing include the natural antibiotic valinomycin (which binds potassium ion) and numerous synthetic carriers (such as crown ethers, podands, and coronands) that organic chemists have synthesized and are known to bind alkali and earth alkali ions [16,171,172].

8.8.2.1 Sensors Based on Ion Exchange and on Coextraction

While a cation can be extracted from an aqueous into a lipid phase by a guest carrier, the counterion (the anion) usually cannot, and the process therefore would come to a quick end for reasons of electroneutrality. If, however, at the same time a proton can be released from the membrane (in exchange for the cation), then a complete ion

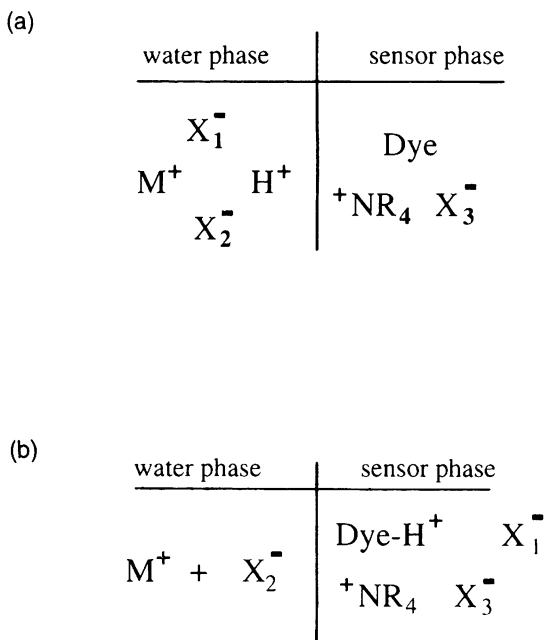


Figure 8.21 Distribution of charges in the water phase and the sensor phase (a) before and (b) after a coextraction process has occurred. The value X_1^- (unlike X_2^-) is highly lipophilic and, hence, extractable. For reasons of electroneutrality, a proton is coextracted, which protonates the indicator dye, which in turn results in a change in the optical properties.

modifications of the reflectometric methods for potassium using valinomycin as the carrier and based on either fluorescence energy transfer [178] or the inner filter effect using fluorescent beads [179] have been described.

The scope of both sensing schemes (i.e., ion exchange and coextraction) have been widely enlarged by the work of Simon and coworkers who discovered the beneficial effect of added lipophilic anions (such as the tetraphenylborates), which improve selectivity and response time [74,180–184], but also affect the work function. Numerous ions, gases, and neutral organic analytes can be analyzed now if appropriate carriers or receptors are found [185,180]. In fact, the sensing scheme is limited only by the selectivity and affinity of the molecular receptor.

More recently, synthetic crown ethers were found to represent an attractive alternative to natural ion carriers such as valinomycin and nonactin [183], and disposable ion-sensing probes for determination of blood electrolytes were obtained by incorporating respective ionophores (“carriers”) along with a blue anionic dye into

thin films of plasticized PVC. Again, the principle is based on ion exchange (in the case of cations) and coextraction (in the case of chloride), respectively. At constant pH, the color given by the sensor films can be related to the concentration of ions by equations derived from the Lambert-Beer and mass action laws [184].

By combining a dye moiety with an ionophor, so-called chromoionophors (or fluoroionophors) can be obtained. Both chromophores [11,186] and fluorophores [13,187] have been covalently linked to crown ethers, such that an ion-binding oxygen atom or nitrogen atom is part of the π -electron system of the dye. (In this context, the term crown ether refers to podands, coronands, and cryptands.) The event of binding the ion brings about a change in color, and more strongly in fluorescence, because the free electrons of the crown are now occupied by the alkali ion and cannot fully participate in the π -electron system of the chromophore or fluorophore. In most crown ethers, the binding constant (i.e., the turning point of the titration curve) strongly depends on the fraction of water in the solvent, because water acts as a competitive solvating agent for the crown.

The coextraction and ion-exchange sensing schemes are most promising because the selectivity to certain ions is exclusively governed by the carrier and the additives, while the dye (and hence the optical system) can be the same throughout. Their major disadvantage is the strong pH-dependence, which makes them not applicable to samples of unknown pH.

8.8.2.2 Sensors for Anions

Anions are more difficult to detect with high specificity than are cation because of the lack of adequate optical probes and the lack of selective carriers for anions. Usual carriers display a selectivity that follows the so-called Hofmeister pattern according to which lipophilic anions like perchlorate, salicylate, and iodide are more readily extracted than "hard" anions like fluoride and bicarbonate. Frequently used anion carriers include tetraalkylammonium salts, tri-alkyltin chloride, and certain porphyrins. They have been used both in coextraction-based sensors and in PSD-based sensors. Halides are known to quench the fluorescence of certain heterocyclic fluorophors and this has resulted in the design of respective sensors that cover the 1 to 100-mM concentration range (Figure 8.22) and hence are not suitable for trace analysis.

8.8.2.3 Sensors Based on Potential-Sensitive Dyes

An entirely different approach was introduced by Wolfbeis & Schaffar [188,189] in that the binding of ions, and their transport into a membrane, which results in the formation of an interface potential, was monitored by optical means using a potential-sensitive dye (PSD). The scheme has been extended [190]. Initially, Langmuir-Blodgett (LB) films were used as supports, because the potential (V/cm-1) is very high only if sensing films are very thin, typically a few nanometers in the case of LB films. Because of the poor stability of LB films, plasticized PVC was used in more

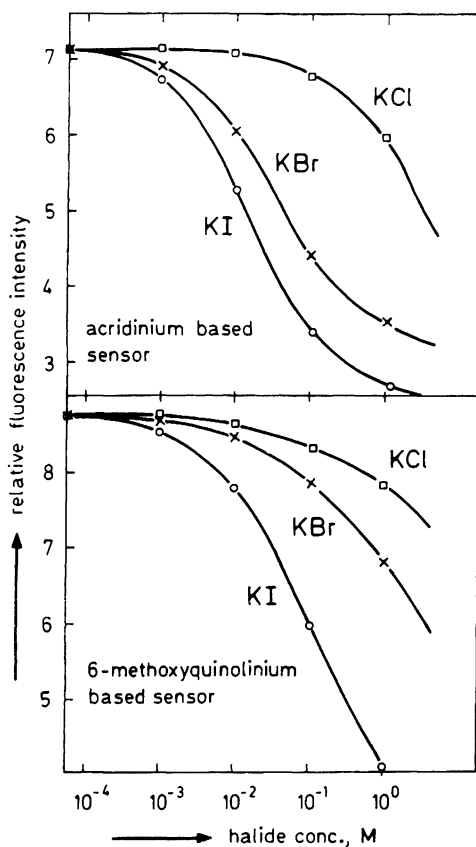


Figure 8.22 Dynamic quenching of the fluorescence of immobilized acridinium ion (top) and quinolinium ion (bottom) by chloride, bromide, and iodide, respectively.

recent work [22]. However, total signal changes do not exceed 50% in the best case, and a moderate pH-dependence still exists. The PSD-based scheme works best for such anions as nitrate [24], where the hydrophilicity-lipophilicity balance (HLB) seems to be better established (see Section 8.1.6). A schematic of the processes occurring at the sensor/sample interface is shown in Figure 8.7. A fairly specific carrier is known for nitrite anion and a sensor has been designed based on its use [191].

An apparently alternative sensing scheme for potassium was introduced by Kawabata and others [192,193] by making use of the same components except for another dye. It can be assumed, however, that the alkylacridinium dye used in this work in fact acts as a PSD and undergoes a voltage-induced partitioning. This is supported by the fact that all PSDs are both electrochromic and solvatochromic.

Sensors based on PSDs can be used to monitor, in a continuous and virtually pH-independent fashion, ions for which respective carriers are known. Both the plasticizer and the concentration of the borate counterion have a distinct effect on the work function [193]. The details of mechanisms that lead to a response of a PSD are still unknown and probably result from more than one single effect. Based on several findings, including turbidity effects, fast response, and relatively large signal changes, it was concluded that membranes have a microstructure that cannot be described by a model of a single homogeneous layer, but rather a complex microemulsion.

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