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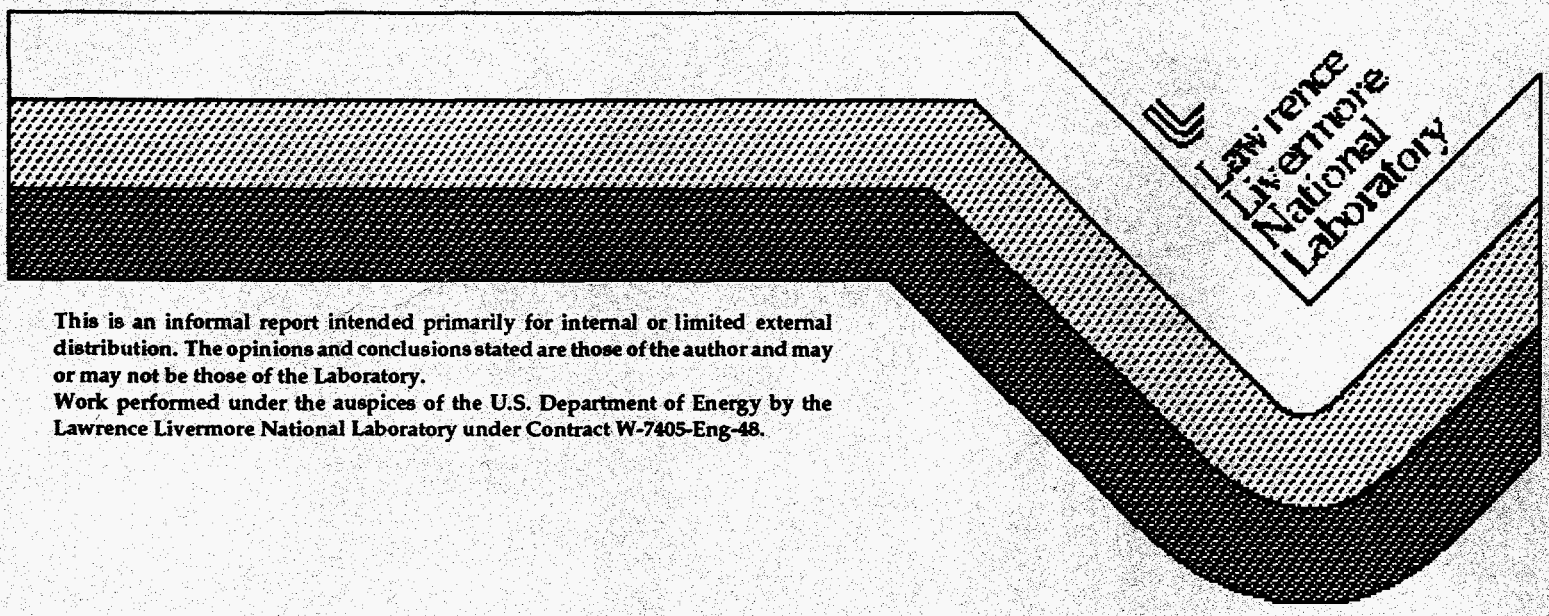

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Jesse L. Yow

DISCLAIMER

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Background

The initiative towards remediation of ground and waste water facilities requires, as the foremost step, identification and analysis of the numerous pollutant compounds present. In an effort to develop a field portable instrument for simultaneous monitoring of different contaminants, researchers at Tufts University have been subcontracted by the Department of Energy to design and fabricate a multianalyte fiber-optic chemical sensor. Fiber-optic chemical sensors, which allow remote detection of analytes have proven to be an excellent alternative to traditional methods of analysis. A number of optical sensors have been described based on different chemical transduction mechanisms and optical properties[1]. The small size of optical fibers, their insensitivity to electrical interference and the lack of the need for a reference sensor make these devices potentially suitable for remote applications[2-7]. Indicators that are sensitive to analyte concentration and exhibit changes in absorbance or luminescence form the basis of such sensors. Traditionally, most multianalyte sensors have been merely several individual sensors fabricated into a sensor bundle or array. This approach has limitations in the sense that the size of the array increases proportionally with each added individual sensing component. Researchers at Tufts University have demonstrated the capability of placing multiple indicator chemistries, which serve as discrete sensing sites, at the distal end of a single imaging fiber[8]. Typically an imaging fiber is 300-400 μm in diameter consisting of thousands of individual channels. The discrete sensing sites are the result of photopolymerizing different indicators in a polymer matrix on multiple regions of the fiber. By coupling the imaging fibers to a charge coupled device detector (CCD), one has the ability to spatially and spectrally discriminate the multiple sensing sites and hence monitor multiple analyte concentrations simultaneously. This report describes the development of the indicator chemistry and immobilization procedures developed for pH, Al^{3+} and hydrocarbons.

Introduction

There are several factors to be considered when immobilizing a ligand to serve as a sensor. While the fluorescence intensity is proportional to the total number of immobilized ligands, in practice, concentration quenching or inner filter effects may occur when ligands are spaced too closely together causing a reduction in intensity. Consequently, the amount of ligand immobilized should be the smallest possible amount that yields a sufficiently large fluorescence signal for the intended application. Immobilization introduces a new functional group on the ligand. This may affect the fluorogenic properties of the ligand to the extent that an otherwise non-fluorescent ligand may become fluorescent. This is especially observed for indicators which fluoresce upon binding to metal ions. In the present work, we have had success immobilizing ligands via photopolymerization along with the polymer at the activated distal end of the fiber. Fluorophores are functionalized to bind selectively at the sensing site. For instance, labile double bonds are introduced on the fluorophore by reacting the amine or acid chloride derivative of the indicator with acryloyl chloride.

The polymer matrix used is designed to facilitate mass transfer of the analyte to the immobilized indicator. Consequently, immobilizing the pH and Al^{3+} sensitive indicators requires the polymer to be hydrophilic so as to allow water and dissolved ions to easily penetrate into the matrix. Photopolymerizable hydrogels are most suitable for such applications. Polyacrylamide, poly-hydroxy ethyl methacrylate (HEMA) and poly vinyl pyrrolidone are some of the hydrogels that have been under consideration. HEMA has proved the most favorable because of its considerable porosity, high tensile strength, pH stability and the fact that it is easily photopolymerized at the tip of optical fibers. On the other hand, the different polymers used in case of the hydrocarbon sensor are primarily hydrophobic so as to allow only the volatile hydrocarbons to penetrate the matrix. Numerous siloxane based photopolymers have been identified for this application.

Indicator Chemistry

pH

Most pH indicators possess a limited dynamic range. As a result, multiple indicators must be employed to obtain sensors that cover a wider pH range[9-11]. Fluorescent pH indicators are typically weak acid dyes whose dissociated and undissociated forms have different absorption or fluorescent properties in the pH range of interest. In many cases, fluorescence occurs only from the excited state of the base form, e.g. fluorescein and certain coumarins[12-14]. Some indicators such as hydroxypyrene trisulfonic acid (HPTS) are fluorescent in both acid and

Parent Fluorophore	Typical pKa	Typical Measurement
Eosin	2.0-5.0	excitation ratio 450/520 nm
Rhodols (NERF)	4.5-6.0	excitation ratio 440/500 nm
Fluoresceins	5.0-7.0	excitation ratio 450/490 nm
8-Hydroxypyrene 1,3,6-trisulfonic acid (HPTS)	7.2-7.6	excitation ratio 450/400 nm
7-Hydroxy methylcoumarin	7.0-8.5	excitation ratio 390 nm
SNAFL®	7.0-7.8	excitation ratio 490/540 nm or emission ratio 450/500 nm
SNARF®	7.0-7.8	emission ratio 580/630 nm

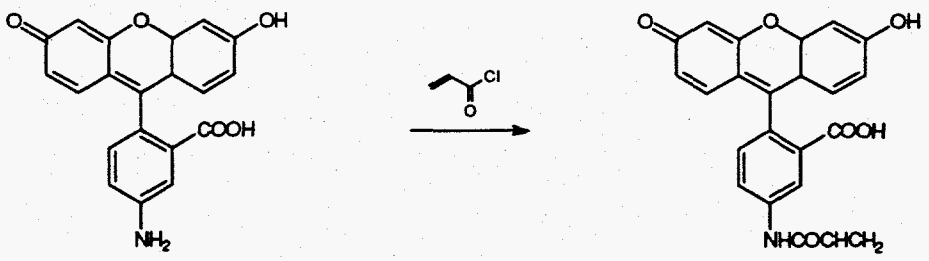
Table 1. Fluorescent pH indicators, classified by their parent fluorophores [16].

base forms[15], allowing pH changes to be followed by measuring the relative emission of both forms rather than only one. Most investigations of optical pH sensors have concentrated on developing sensors for biomedical applications because of the narrow pH range covered, with emphasis on achieving improved sensitivity and precision. As a result, fluorescent indicators developed outside this range are few. Table 1 lists some of the parent fluorophores used as fluorescent pH indicators.

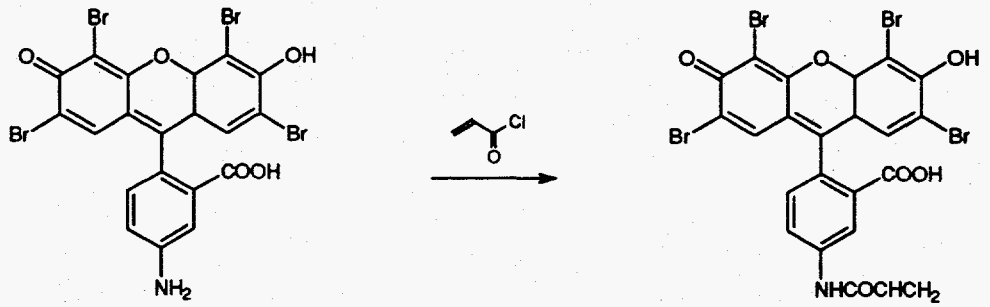
The multiple pH indicators used should have a strong absorption within the wavelength range 400-700 nm to allow the use of inexpensive optics. Furthermore, indicators should possess considerable photo- and chemical stability, lack of toxicity, a functional group capable of chemical immobilization. Certain coumarins have proven to be useful pH indicators. However the spectral properties are disadvantageous because of the low excitation wavelength. HPTS has many advantages including high fluorescence quantum yield, visible excitation and emission, large Stokes shift and ability for dual excitation for precise calibration. However, it is not suitable for the present application. This is due to the fact that it cannot be specifically functionalized for immobilization without sacrificing its fluorescent properties. Eosin and fluorescein have been identified as fluorescent indicators sensitive in the pH range 1-9 pH units and which may be easily functionalized to facilitate immobilization.

Both eosin and fluorescein are commercially available as the amine derivative. The amine is functionalized to a vinyl group via reaction with acryloyl chloride. The respective dyes, when photo-polymerized with HEMA at the distal end of the fiber, are immobilized in the polymer matrix. Prior to immobilization of the dye, the distal end of the fiber is functionalized to permit covalent bonding. The various steps developed, namely, functionalization of the dye, silanization of the optical fibers, and immobilization of the dye along with the polymer are shown below.

Functionalization of Indicators

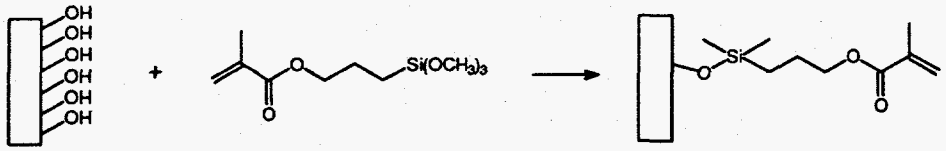


Fluoresceinamime



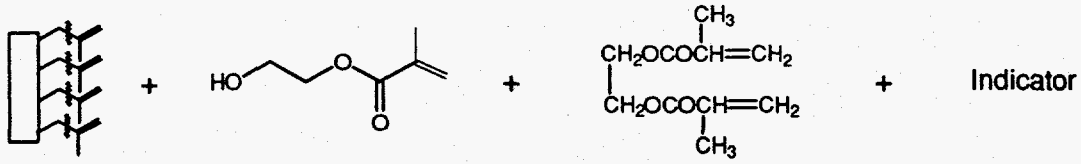
Aminoeosin

Silanization of Fibers



3-(trimethoxysilyl) propylmethacrylate

Immobilization



hydroxy ethyl methacrylate ethylenegylcol dimethyacrylate

Initiator, hv

Dye Immobilized on Fiber

Figure 1a shows the emission spectra obtained for eosin at different pH levels. The emission band at 580 nm shows a steady increase with increasing pH over the 2-8 pH range. As is evident, maximum sensitivity would be obtained with the emission being monitored at 545 nm. Similarly, in the emission spectra obtained for fluorescein (Figure 1b), the intensity of the 520 band increases with increasing pH. The basic forms of both eosin and fluorescein have higher quantum efficiencies leading to increased

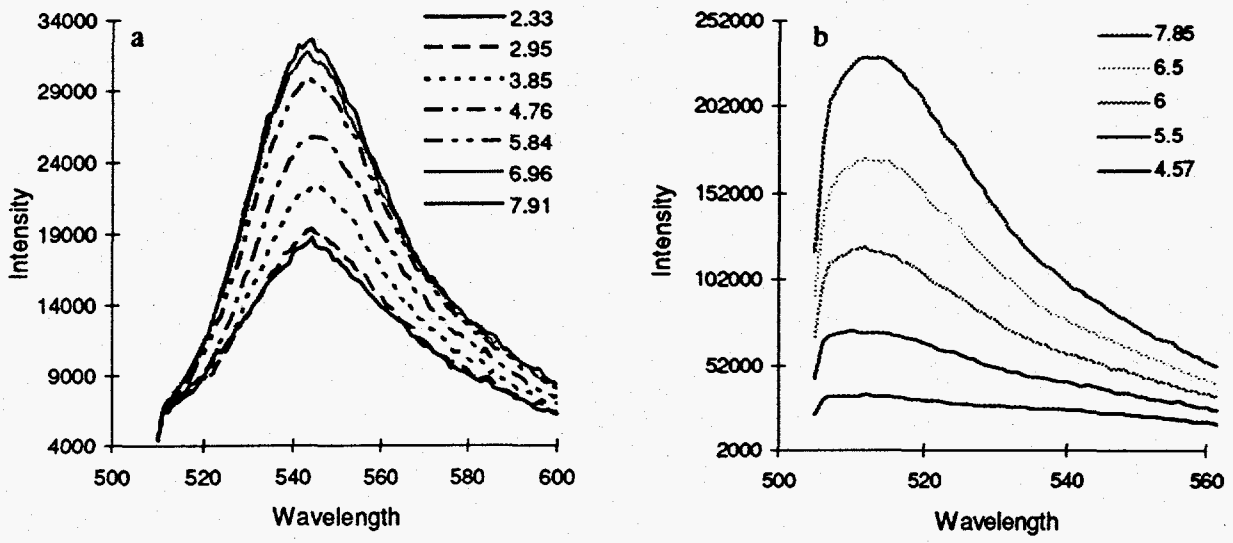


Figure 1. Emission Spectra of eosin (a) and fluorescein (b) at respective pH values

fluorescence at higher pH. In practice, to compensate for photobleaching, the fluorescence intensity is measured as a ratio for excitation at its excitation maxima vs excitation at a wavelength outside the excitation spectra. Shown in figure 2 are the typical intensity vs pH response for eosin and fluorescein immobilized on a fiber.

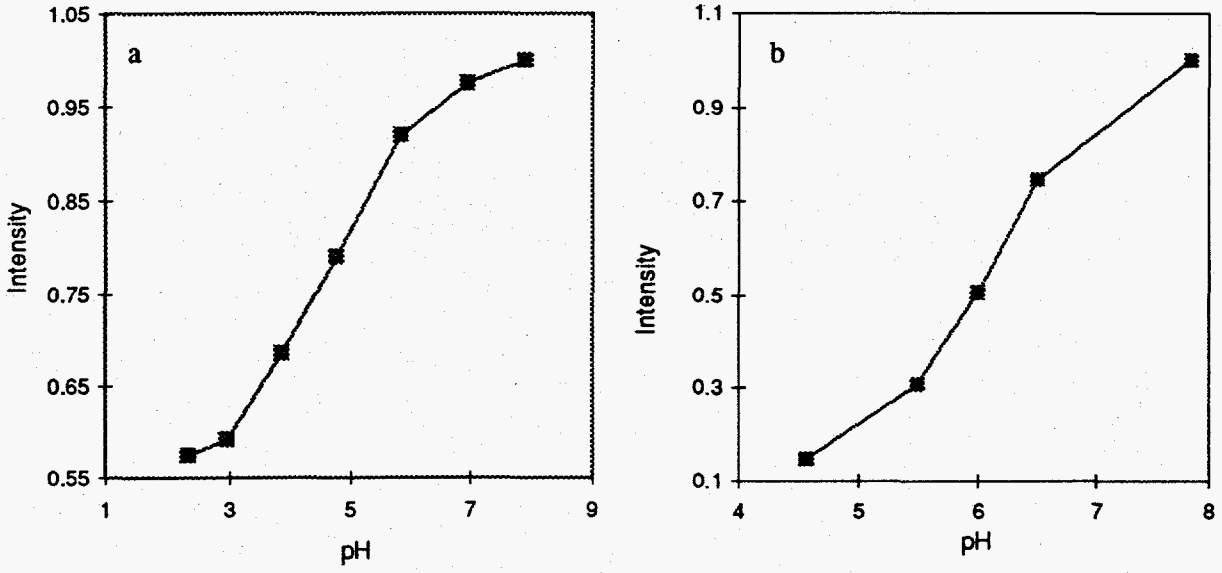


Figure 2. Normalized emission intensity vs pH plots for eosin (a) and fluorescein (b) immobilized on individual fibers with excitation at 490 nm.

Eosin shows maximum sensitivity in the 2-6 pH range, while fluorescein is most sensitive in the 5-8 pH range. Consequently, pH measurements over the range of 2-8 pH units can be achieved using immobilized eosin and fluorescein. Further research will now focus on developing fluorescent indicator chemistries to cover the higher end of the pH range (FY 1995 Milestone 4). In addition, poly-HEMA, the hydrogel currently being used to immobilize fluorescein and eosin, hydrolyses at pH > 9. As a result, a more rugged matrix also needs to be identified for such a application.

Al^{3+}

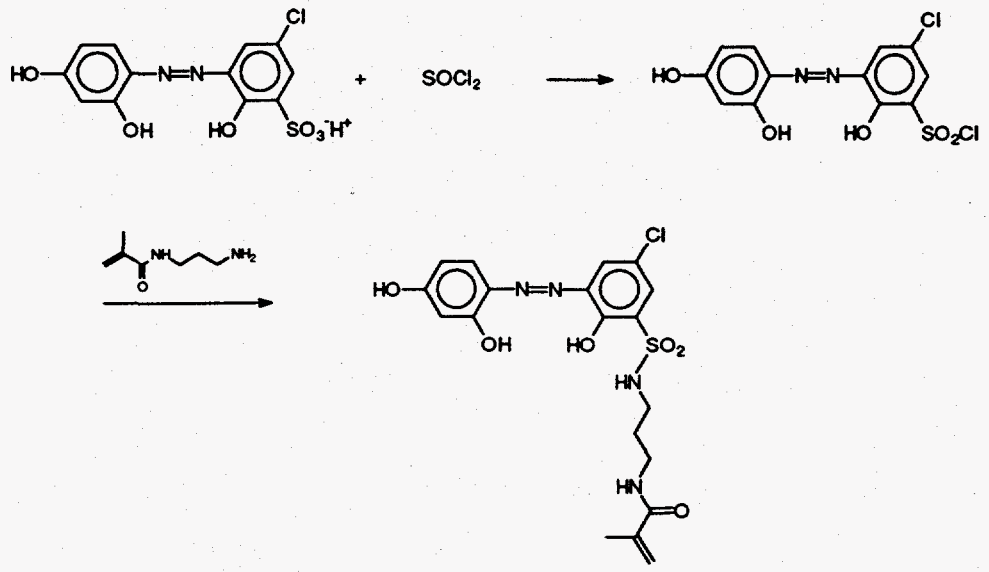
An aluminum ion sensor can be made by immobilizing a non-fluorescent ligand that forms a fluorescent complex in the presence of the metal ion. As mentioned earlier, concentration of the fluorophore and immobilization procedures play a crucial role in the fluorescence intensity of the indicator. Furthermore, complex formation for most ligands involves displacement of one or more protons. This means that the equilibrium for the indicator reaction is described by a pH-dependent conditional formation constant. The measurement, therefore, must be done at a constant pH or be corrected for variations in pH.

Aluminum determination using fluorescence measurements has been widely studied and numerous methods have been proposed[17]. Aluminum forms fluorescent complexes with morin, salicylidene-2-thiophenol, and a variety of hydrazones and Schiff's bases. To date, all sensors designed for Al^{3+} use the parent fluorogenic compound in solution or adsorbed on a solid support like cellulose or an ion exchange resin[18]. Immobilization of the indicator in such a non-specific manner often leads to a deactivation of the Al^{3+} binding sites and an increase in background fluorescence. Furthermore, as the complex formation is not reversible, the initial increase in fluorescence intensity observed in the presence of Al^{3+}, rapidly plateaus once the binding sites get saturated.

We have designed a sensor that not only has the indicator chemically immobilized on the distal end of the optical fiber but also exploits the diffusion of the Al^{3+} ions through the polymer matrix to extend the

life of the sensor. Lumogallion, a non-fluorescent azo compound, forms stable fluorescent complexes with Al^{3+} in slightly acidic media showing an emission maxima at 580 nm for excitation at 490 nm[19].

Functionalization of Lumogallion



Lumogallion is functionalized as outlined above to provide labile double bonds which are co-polymerized photochemically along with the polymer matrix. Al^{3+} binds irreversibly to lumogallion. Consequently, the concentration of Al^{3+} is measured as a function of the rate of increase in fluorescence. The rate is not only dependent on the formation constant of the complex but also on the rate of diffusion of the Al^{3+} ions through the polymer matrix.

The response curve obtained for different concentrations of Al^{3+}, using lumogallion immobilized in HEMA is shown in Figure 3a. This figure plots the fluorescence emission intensity of lumogallion as the optical sensor is immersed in solutions of increasing Al^{3+} concentration. It was expected that the rate of increase in fluorescence intensity would be higher at higher Al^{3+} concentrations as the rate of diffusion is directly proportional to concentration. On the contrary, as is evident from Figure 3b, the rate change of fluorescence intensity decreases with increasing concentration. It is postulated that at higher concentrations, Al^{3+} forms a different solvated species which leads to slower diffusion to the indicator sites and hence a

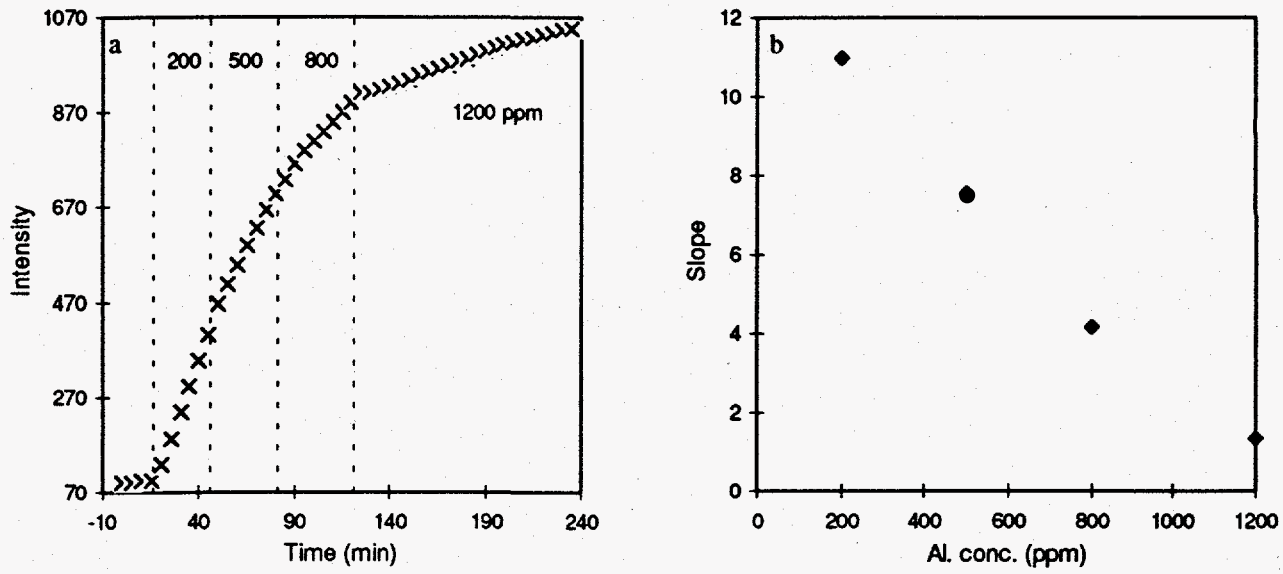


Figure 3. a) Fluorescence emission intensity vs time plot for Lumogallion immobilized on an optical fiber at varying Al^{3+} concentrations. b) Plot of the slope (rate of increase in intensity with time) vs Al^{3+} concentration.

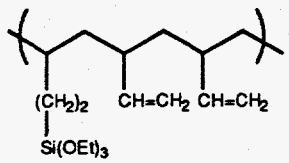
lower increase in fluorescence intensity. Alternatively, the hydroxyl groups on the poly-HEMA matrix could bind to the Al^{3+} and hence compete with the complex formation. Either of these mechanisms works to our advantage as they prolong the life of the sensor and makes it more sensitive at lower Al^{3+} concentrations.

Hydrocarbons

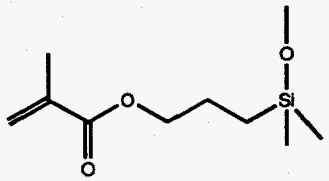
Organic vapors are partitioned to varying extents into a polymer membrane depending upon the polarity of the polymer matrix. Neutral membrane probes have spectroscopic properties that are sensitive to changes in the microenvironment, thus providing a transduction mechanism for monitoring the adsorbtion of organic vapors in the polymer membrane. Such a probe immobilized in a membrane exhibiting solvachromic behavior in their fluorescence spectra forms the basis of the hydrocarbon sensor. Construction of such a sensor typically involves deposition of a suitable polymer containing a membrane

probe at the distal end of the fiber via photopolymerization or dip coating. Different dye polymer combinations are being identified for maximum spectral variations when exposed to organic vapors.

Siloxanes and certain modified polybutadienes are known to form gas permeable membranes which absorb low levels of organic vapors reversibly. Numerous siloxane based polymers with varying porosity and polarity are being tested. PS078.5 and PS851 are two such representative polymers obtained from Polysciences, Inc. The chemical structures of these polymers are shown below.

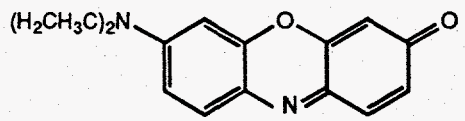


PS078.5 triethoxysilyl modified, polybutadiene

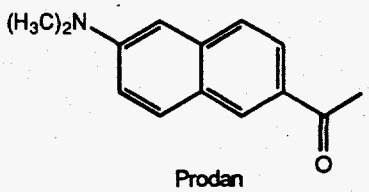


PS851 (97-98%)dimethyl-(2-3%)methacryloxypropyl) methyl siloxane coplymer

Nile Red and Prodan are two of the fluorescent dyes under review. Nile Red is a phenoxazine micro-environment sensitive dye which is almost non-fluorescent in water and polar solvents but shows a large blue shifted fluorescence depending upon the polarity of the medium. This dye is incorporated in photopolymerizable siloxanes and immobilized on optical fibers.



Nile Red



Prodan

Figure 4a shows the emission spectra of Nile Red immobilized in PS078.5 on an optical fiber in the presence and absence of acetone vapor. As can be seen, the emission maxima is red shifted in the presence of acetone. The emission spectra of Nile Red is also sensitive to the nature of the organic vapor. Shown in Figure 4b are the emission spectra of Nile Red immobilized in PS851 on an optical fiber in response to different organic vapors.

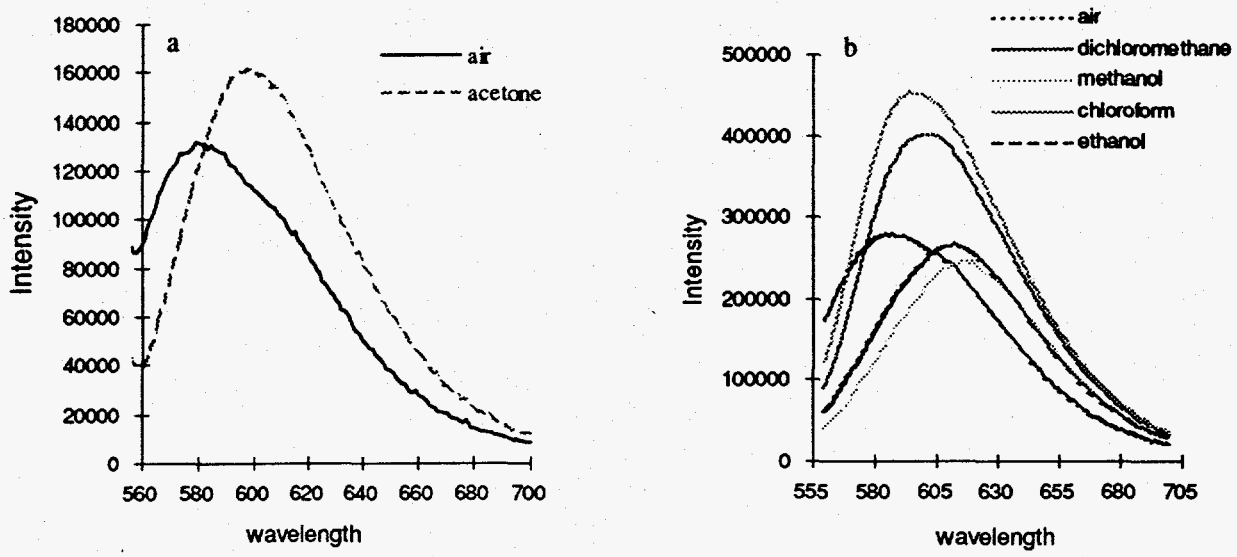


Figure 4. Emission spectra of Nile Red immobilized in PS078.5 (a) exposed to air and acetone and immobilized in PS851 (b) when exposed to different vapors.

The varying polarity of different hydrocarbons is responsible for the solvachromic shifts in the Nile Red fluorescence. Prodan has both an electron donor and an acceptor substituent, resulting in a large excited state dipole moment and extensive solvent polarity-dependent fluorescence shifts. Figure 5 shows the spectral and intensity variations in the emission spectra of Prodan in different solvents. As indicated

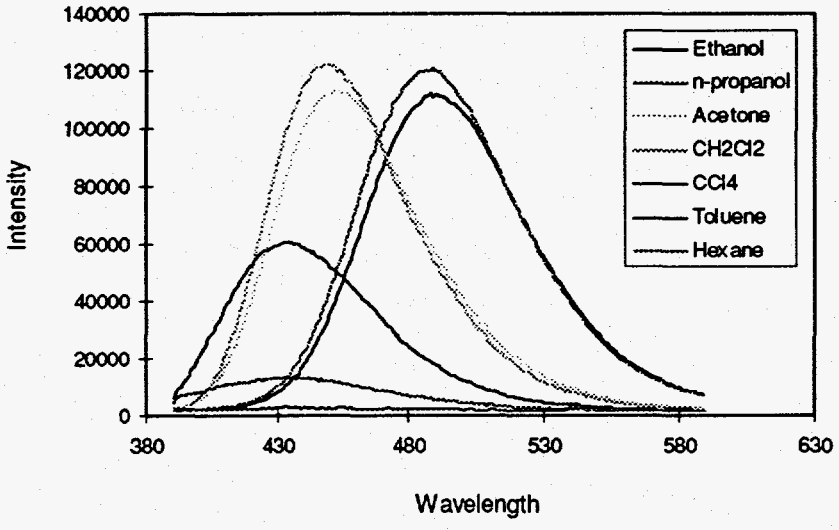


Figure 5. Emission spectra of prodan excited at 350 nm in various solvents

earlier, the varying polarity of the hydrocarbons is responsible for the spectral shifts.

Multianalyte sensors containing an array of different dye-polymer combinations could ultimately be used to identify unknown organic vapors. By developing a recognition pattern based on a neural network, the array, taken as a whole, could provide enough information to identify an unknown hydrocarbon.

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