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Solvatochromic indicators for fiber optic chemical sensors

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Solvatochromic indicators for fiber optic chemical sensors

Shakhsher, Ziad Munib, Ph.D.

University of New Hampshire, 1989

SOLVATOCHROMIC INDICATORS FOR
FIBER OPTIC CHEMICAL SENSORS

BY

Ziad M. Shakhsher
B.S., University of An-Najah, Nablus, 1982

DISSERTATION

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in Partial Fulfillment of
the Requirement for the Degree of

Doctor of Philosophy
in
Chemistry
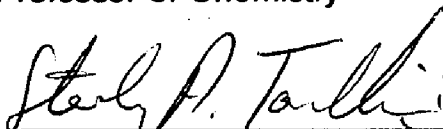
September, 1989

This dissertation has been examined and approved.
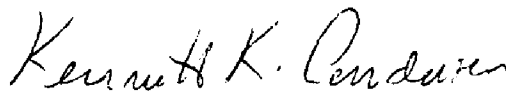
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Date

This Dissertation is Dedicated to my Parents

Munib A. and Hanan M. Shakhsher

Whose Love and Pray Made This All Possible

ACKNOWLEDGEMENTS

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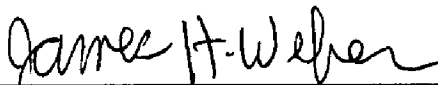
ABSTRACT

SOLVATOCHROMIC INDICATORS FOR
FIBER OPTIC CHEMICAL SENSORS

by

Ziad M. Shakhsher

University of New Hampshire, September, 1989

The goal of this thesis was to develop and evaluate solvatochromic indicators for fiber optic chemical sensors. Interaction with analyte modifies the polarity of the immobilized indicator environment leading to a shift in their fluorescence spectra. Three systems were studied.

In the first study a cationic fluorescent probe, 5-dimethyl-aminonaphthalene-1-sulfonamidoethyltrimethylammonium ion (DA^+), was equilibrated with unmodified- and hydrocarbon bonded-silicas. In solution and unmodified silica, maximum DA^+ fluorescence shifts to longer wavelengths with increasing percentages of methanol, tetrahydrofuran and acetonitrile in water. On hydrocarbon bonded silicas, however, DA^+ emission maxima in water occur at shorter wavelengths indicating a very nonpolar environment. This indicates that the organic fluorophor is excluded from the solvent and experiences primarily a surface environment. Added organic solvent competes with DA^+ for the hydrocarbon surface, causing it to interact more strongly with the solvent.

In the second study, 5-Dimethylaminonaphthalene-1-sulfon-

amidoethyltrimethylammonium ion (AEANS) immobilized on controlled pore glass, cellulose and poly(vinyl alcohol) (PVOH), was shown to responds to cationic surfactants including Dodecyl-, Tetradecyl- and Cetyl- trimethyl ammonium ions. The emission spectrum shifts to shorter wavelengths and increases in intensity with increasing surfactant concentration because surfactant forms an ion pair with AEANS causing its environment to be less polar. Both in solution and immobilized on solid substrates, AEANS responds more sensitively to cationic surfactant with longer hydrocarbon chain lengths. There is no significant response to anionic and nonionic surfactants. AEANS covalently bound to PVOH membranes was coupled to a fiber optic system for reversible *in-situ* determination of cationic surfactants.

The third system involved a betaine dye, 2,6-diphenyl-4-(3,4,6-triphenyl-N-pyridinio) phenolate (ET30), immobilized in a silicone rubber membrane. At low concentrations immobilized ET(30) fluoresces strongly. Fluorescence intensity decreases and spectra shift to longer wavelengths when the membrane is exposed to increasing percentages of methanol in water. Response is reversible with a response time of several minutes.

All systems studied undergo spectral shifts as a function of analyte concentration. This allows analyte to be related to an intensity ratio measurement at two wavelengths which compensates for changes in variables other than analyte concentration that affect the magnitude of the analytical intensity.

CHAPTER I

INTRODUCTION

A chemical sensor is a transducer that provides direct information about the chemical composition of its environment. It consists of a physical transducer coupled to a chemically selective layer. The fundamental transduction modes are classified as thermal, mass, electrochemical and optical (1). Chemical sensors should be easily applied to the sample, sensitive and highly selective for the analyte, respond reversibly to the analyte, be easily calibrated and not perturb the analyzed system.

Electrochemical sensors such as ion-selective electrodes and ion-selective chemical field effect transistors are the largest group of chemical sensors(2-7). These kinds of sensors have some limitations, such as poor selectivity and susceptibility to electrical interference, and are not available for many chemical species.

Optical sensor development is of growing interest because optical sensors avoid some limitations associated with electrochemical sensors. Optical sensors are based on the change in the optical properties of a sensing element on the end of a fiber optic. These changes can involve absorbance, fluorescence, reflectance, chemiluminescence, or phosphorescence. Fluorescence is particularly well suited for measurements through fiber optics,

because it is sensitive and versatile and can be readily measured through a single optical fiber.

Optical sensors which have been developed include immuno-chemical sensors (8), ionic and pH sensors (9), gas sensors (10) and sensors for petrochemical applications (11).

Application areas include industrial process control, environment pollution monitoring, clinical chemistry, and various biomedical applications. When sensors are available , the sample can be analyzed *in situ* in real time, with minimum opportunity for contamination. Recently Seitz reviewed the principles of fiber optics and their applications in chemical sensing (12).

Fluorescent indicators can be classified into three different types based on their working principles. The first type is based on spectral changes of immobilized indicators as a result of ground-state reactions with the analyte, such as in acid-base equilibria. The second type is based on dynamic quenching. The third group is based on spectral changes of the indicator due to physical or chemical perturbations as a result of changes in temperature, pressure, viscosity or environment polarity. The last type is the subject of this thesis(13,14).

Fiber Optic Principles :

Optical fiber serves as a light or wave guide to transport light to and from the sensing element, an immobilized reagent phase which changes optical properties upon interaction with an analyte.

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Optical fibers are usually composed of two materials arranged coaxially as illustrated in figure 1.1. The inner part is a core of refractive index, n_1, surrounded by a layer of material of lower refractive index, n_2 called the cladding. A protective jacket of plastic or some other material is applied over the the cladding to increase the fiber's mechanical strength and to facilitate its handling.

Fiber optics are based on the phenomenon of total internal reflection. Light entering the fiber at an angle greater than the critical angle is transmitted through the fiber as a result of internal reflection at the core/cladding interface. The angle (a) of incident light which is accepted by the fiber transmitted, depends on the refractive indexes of the core and cladding and air (n_0).

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

Optical fibers consist of materials which are both flexible and transparent, such as glasses, plastics or silica. Fiber attenuation is expressed in terms of decibels (dB) per kilometer which is defined :

$$\text{Attenuation (dB)} = 10 \log P_1/P_2$$

Where;

P_2 : is the power entering the fiber.

P_1 : is the power leaving the fiber.

High fiber transparency in the visible region makes it possible

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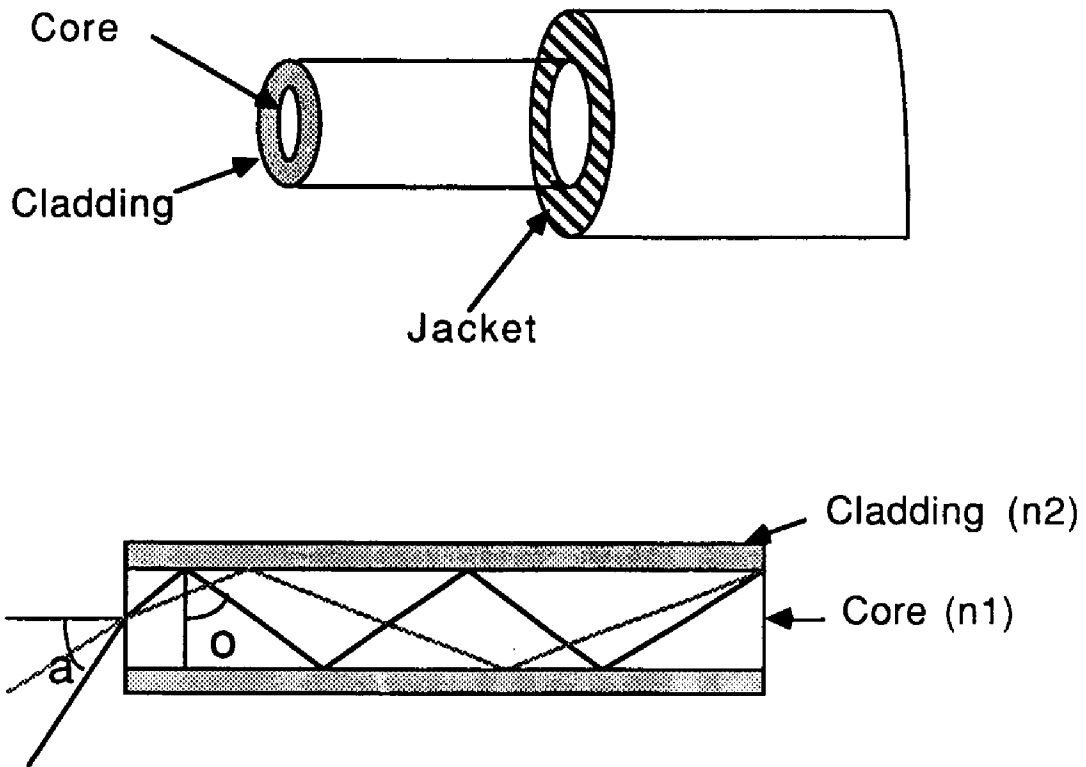
Figure 1.1 : Schematic of fiber optic :
 a: acceptance angle,
 n1 and n2 are the refractive indices for core
 and cladding, respectively,
 o: angle of incidence at core/cladding interface.

to transmit light to and from remote locations over considerable distances without serious intensity losses.

Plastic clad silica, often referred to as PCS, is of greatest interest to analytical spectroscopists because it is transparent in the UV. Fibers typically have core diameters of 100 to 200 micrometers.

For chemical sensing applications, different types of fiber arrangements are used. A single fiber can be used as light transmitter and collector at the same time, as illustrated in figure 1.2. Fluorescence returning through the fiber is focused into a double monochromator which resolves fluorescence from scattered excitation radiation. A bifurcated arrangement is shown in figure 1.3. One arm transmits excitation light to the immobilized indicator phase, while the other arm transmits the light from the indicator phase to the detection system. Most bifurcated fiber optic sensors reported in the literature have used incandescent sources with filters for wavelength selection (12).

Advantages and Limitations of Fiber Optic Chemical Sensing :

Fiber optic chemical sensors have many potential applications in analysis as a result of the following advantages (15,16):
1) Unlike potentiometric electrodes, optical sensors do not require a reference signal.
2) Sensors can readily be miniaturized using single fibers. This allows them to be used with small samples as well as for in vivo

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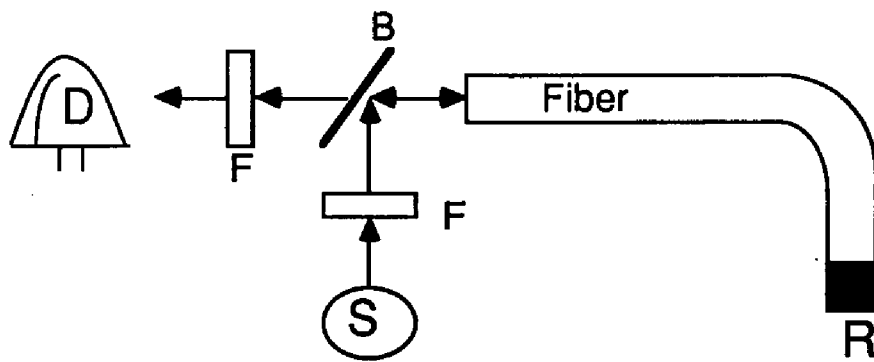
Figure 1.2 : Spectrometer for single-fiber measurments
using a beam splitter.
R : Indicator phase; S : Source; D : Detector;
F : Filter; B : Beamsplitter.
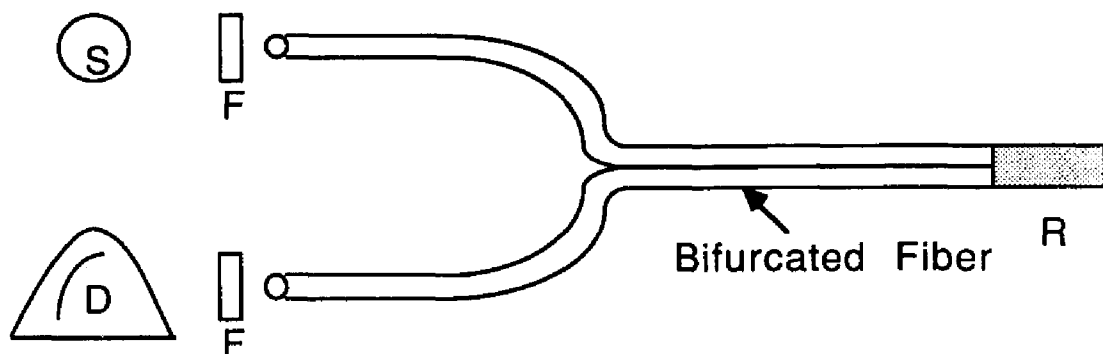
Figure 1.3 : Schematic of bifurcated fiber optic chemical
sensor. R : Indicator phase; S : Source;
D : Detector; F : Filter.

measurements.

3) Because optical fibers have low loss, they transmit over a large distance. Currently, they are used for optical telecomunication. Also, they can be used for remote sensing to perform analysis of samples in locations that are hard to reach or hazardous.

4) Because the sensor signal is optical, it is not subject to electrical interferences.

5) Multiple analyses with a single central spectrometer can be performed using several fiber sensors placed in different locations coupled to the same instrument.

6) In most cases, optical sensors are nondestructive.

7) Multiwavelength information can be used to provide calibration stability or to allow simultaneous analysis of more than one analyte.

8) Optical sensors are simple in design and can be designed for easy replacement of indicators.

Fiber optic chemical sensors also have some limitations (15,16) :

1) They are subject to interference from ambient light. The interference can be reduced using dark surroundings or by modulating the optical signal, so that it can be resolved from ambient light.

2) Because the indicator and analyte are in different phases for indicator phase sensors, diffusion of analyte into the indicator phase limits the response time.

3) Highly selective indicators should be used, and the immobilization needs to be improved to get better selectivity and sensitivity. This

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is because most indicators suffer a reduction in sensitivity after immobilization or when dissolved in a polymer.

4) The indicator phase is subject to limited long-term stability because of photodecomposition. But this can be compensated for by relating the output signal to a reference intensity at another wavelength if there is a spectral shift as a function of analyte concentration.

Two Wavelengths Measurements :

The primary purpose of intensity ratio measurments at two wavelengths is to compensate for changes in any variables other than analyte concentration that affect the magnitude of the analytical intensity (16). These include fluctuations in source intensity, electronic drift in the detection system, background from the sample, changes in either the amount of indicator due to leaching or degradation of the optical properties of the indicator phase, temperature, quenching, ionic strength, and loss of intensity due to bends in the optical fiber which change the angle at which transmitted intensity strikes the core/cladding interface.

Analyses based on intensity ratio measurements at two wavelengths has been accomplished by different methods depending on the factor requiring compensation.

The first method is based on using a separate reference signal which compensates for instrumental fluctuation only, but does not compensate for indicator leaching or decomposition. This can be

done by selecting reference signals, such as :

1) Back-scattered excitation radiation.

2) Emission from a second fluorophor that is insensitive to the analyte.

3) Transmitted signal where no absorption occurs.

The second method is based on measuring the ratio of luminescence intensities at two different emission wavelengths. This compensates not only for instrumental fluctuations and variations in the optical properties of the indicator phase, but also for any loss of the indicator.

Indicator Immobilization Techniques :

Immobilization of indicators on solid supports attached to the fiber optic system is more practical than adding the indicator solution each time to a non-fluorescent analyte.

In order to produce a sensing layer with maximum sensitivity, the indicator, polymer support and immobilization chemistry should be chosen carefully (15). In fiber optical chemical sensors, the indicator is usually immobilized on a rigid, optically transparent polymeric support, which can be attached to the end of an optical fiber. The polymer should be permeable to analyte, and not be subject to physical changes under the operating conditions.

There are three important methods for incorporating the indicator in a solid phase. Reviews provide excellent coverage of all aspects of the chemistry and physics of immobilized reagents, enzymes and dyes (17-21).

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Mechanical (physical) immobilization involves inclusion of molecules in matrices that they can not leave. Chemical immobilization is performed by binding the indicator to a polymer. Indicators can be immobilized covalently or electrostatically.

The indicator phase can be attached to the end of the optical fiber in a variety of ways as illustrated in figure 1.4;

a) The indicator phase is a membrane which is attached to the end of the optical fiber.

b) The indicator phase is a powder which adheres to a sticky membrane attached to the end of optical fiber.

c) The indicator phase is held in place by a membrane attached to the end of the optical fiber.

d) The indicator phase is held in a capillary tube which fits over the end of the optical fiber.

In order to avoid physical changes during operation, some indicator phases need to be formed under controlled conditions. For example, poly(vinyl alcohol) requires the appropriate amount of crosslinker to avoid contraction or expansion in contact with solvent (22). Other membrane materials, such as the silicone rubber, are less subject to physical changes.

It is important to recognize that immobilization has a significant effect on sensor chemistry. For example, immobilization can change indicator spectral properties. It is quite common to observe bathochromic shifts of a few nanometers upon immobilization as a result of both the immobilization chemistry and the nature of the substrate surface. In addition, there are some

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a) Fiber — Indicator/Membrane

b) Fiber — Indicator Phase / Membrane

c) Fiber — Seal / Indicator Phase / Membrane Tubing

d) Fiber — Capillary Tube / Indicator Phase
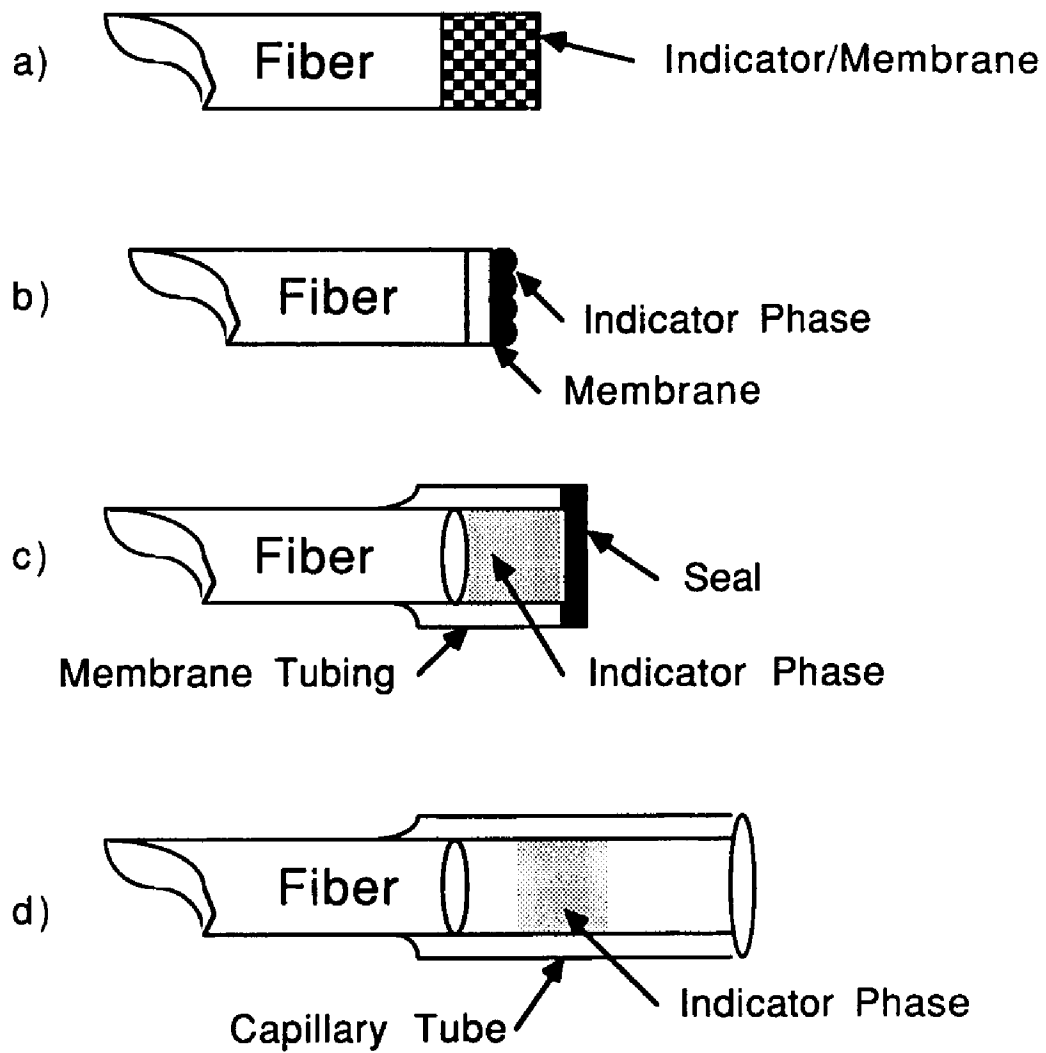
Figure 1.4 : Fiber optic chemical sensor configurations.

indicators that do not fluoresce in solution but become fluorescent when immobilized on a solid substrate (23).

The response time of these sensors depends on both the mass transport of the analyte into the indicator phase and the kinetics of the interaction between analyte and the indicator. The dominant factor determining the response time is usually diffusion of analyte in the indicator phase. For continuous sensing, the interaction of indicator with the analyte must be reversible.

Luminescent Solvatochromic Indicators :

The optical properties of luminescent solvatochromic indicators depend on environment polarity as schematically illustrated in figure 1.5. The ground and the excited states are both stabilized by interactions with a polar solvent. The relative stability of ground and excited states depends on the molecule polarity. For the dansyl molecule, the magnitude of this effect is much greater for the excited state than for the ground state. In the excited state, there is a transfer of charge from one end of the molecule to the other, resulting in a highly polar excited state which interacts strongly with a polar media. The strong interaction between the excited state and a polar medium also promotes radiationless return to the ground state. This is why intensities are lower in a polar medium. This behavior is well known (24).

Fluorescent derivatives of 5-dimethylamino-1-naphthalene-sulfonic acid collectivly known as dansyl compounds, are well known
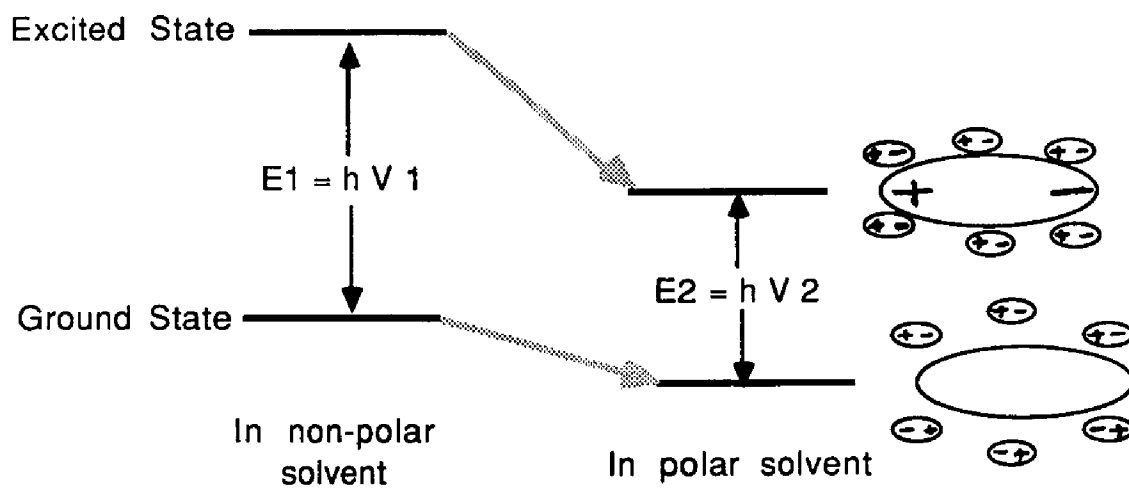
Figure 1.5 : Schematic diagram of solvatochromic effect.
Large ellipse : dye molecule,
Small ellipse : solvent molecule.

solvatochromic fluorophors that undergo large wavelength shifts. Betaine dyes are another important class dyes which exhibit solvatochromism. The absorbance of these dyes shifts with polarity. Betaine dyes have been used to characterize the environment polarity in micelles, microemulsions and phospholipid bilayers (25-28). They have been applied to measure the polarity of binary acetonitrile/water and methanol/water mobile phases used in reversed-phase liquid chromatography (29-35).

Solvatochromic indicators have been used to study the physical properties of organic solvents (36-39). They have been used to determine water content in organic solvents (40-43), and organic impurities in organic solvents (44). Chemical species that cause a significant change in polarity can be detected with solvatochromic indicators.

Solvatochromic indicators are widely used to probe polarity of various environments. Fluoroscence techniques have become widely popular for studying the surface microenvironment of reversed phase chromatographic materials. Stahlberg and Almgren investigated the change in the polarity of chemically modified silica surfaces as a function of solvent composition (45). The same approach has previously been used to study perfluorinated cationic exchange membranes (46). Covalently immobilized dansyl derivatives have been used to study the interaction of various solvents with hydrocarbon bonded silica surfaces (47-49). Dowling and Seitz used an anionic organic probe to study ion pair interactions with hydrocarbon bonded silica (50).

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Carr and Harris used fluorescence techniques to study the interaction of organic modifiers with stationary reversed phases used in reversed phase liquid chromatography (51). This interaction affects the hydrocarbon layer arrangement on the surface of hydrocarbon bonded silicas. The polarity of the immobilized indicator environment depends on the extent of hydrocarbon layer rearrangement which is highly sensitive to solvent composition.

Surfactants :

Surfactants are chemical species with hydrophobic tails and hydrophilic heads. Surfactants are widely used in both aqueous and non-aqueous systems as textile softeners, dispersants, emulsifiers, wetting agents, sanitizers, dye fixing agents, foam stabilizers and corrosion inhibitors. Furthermore, surfactants are used in research to improve analytical methods. For example, cationic surfactants have been used in analytical chemistry to control the linear response range in spectrophotometric methods for the determination of metal ions and to reduce pH effects and improve sensitivity (52-55).

Cationic surfactants are amphipathic species in which the hydrophilic head groups carry positive charges. Cationic surfactants have been analyzed by a variety of methods including spectroscopy (56-59), potentiometry (60,61) and chromatography (62-66). Spectroscopic methods are based on the change in optical properties of an indicator as a result of surfactant and indicator interactions. Surfactants cause the intensities from fluorescent indicators to

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increase and their maximum emissions to shift to shorter wavelengths. Anilinonaphthalenesulfonate compounds (ANS) were used to study the behavior of cationic surfactants in water (67). Martin found that fluorescence shifted to shorter wavelengths and increased in intensity when an ionic polyelectrolyte was added to a cationic dansyl fluorophore solution (68).

Thesis Goals :

The goal of this thesis was to develop indicators for fiber optic chemical sensors based on solvatochromism. The first project involved a cationic dansyl derivative immobilized on bare silica and various hydrocarbon-bonded silicas. The primary objective was to develop an indicator to sense changes in solvent composition. It was found that the wavelength shifts accompanying changes in solvent polarity depend on the nature of the surface and how it interacts with the solvent.

The second project was to develop an indicator for cationic surfactants using an immobilized anionic dansyl derivative. Ion pairing between the surfactant and the indicator enhances fluorescence intensity and causes it to shift to shorter wavelength. Response depends on the structure of the surfactant and the extent to which the ion pair interacts with the indicator substrate. This indicator was used in a fiber optical sensor for surfactants.

The third project was to characterize the response of an immobilized betaine dye to changes in environment polarity.

CHAPTER II

EXPERIMENTAL

INSTRUMENTATION :

SLM 8000 Spectrofluorometer :

The instrument used for measuring fluorescence spectra was an SLM 8000/8000s photon counting spectrofluorometer (SLM-AMINCO Instruments, Inc. Urbana, II.). The major components of the SLM spectrofluorometer are illustrated in figure 2.1. The source is a 450 watt xenon lamp (LH-450). The excitation wavelength is selected by a double monochromator with two concave holographic gratings (MC 460). Excitation radiation is focussed onto the sample by lenses.

The sample compartment for the cell contains a cuvet holder which can easily be moved in all directions. Emission spectra are resolved by a single monochromator with a concave holographic grating (MC 320) placed on the channel A side. The emission wavelength for channel B is selected by placing a 50*50 mm filter in the filter holder. The detectors are photomultiplier tubes with independent variable voltage supplies for channels A and B.

Data acquisition is controlled electronically by an SPC 822/823 electronic unit. Excitation and emission wavelengths are
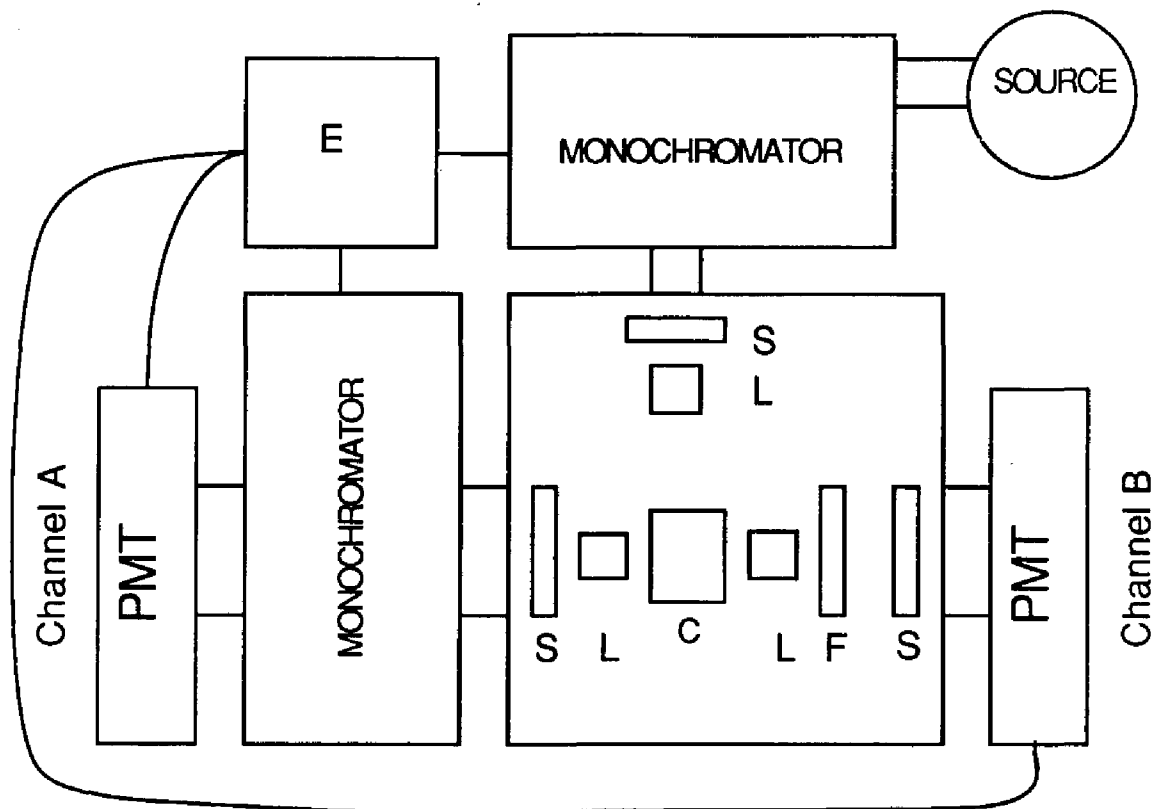
Figure 2.1 : Schematic of the SLM 8000/8000S Spectrofluorometer.
C : cell holder; F : filter holder; S : shutters; L : focusing
lenses; E : Power supplies for photomultipliers (PMTS),
monochromator controller, data acquisition electronics.

selected by an SMC-220 monochromator controller. The output signal of the photomultiplier tubes is controlled by SLM electronics. Hard copies of the spectra are obtained on a Hewlett-Packard plotter (HP7470) interfaced to the SLM 8000.

The instrument for measuring pH was an Orion Digital ion analyzer/501 with a glass combination electrode.

Absorption spectra were measured with a Bausch and Lomb 200 UV spectrometer.

Fiber Optic/Fluorometer System :

Fiber optic chemical sensors were prepared using a bifurcated fiber optic bundle to transport light to and from the immobilized indicator phase. In order to selectively excite the indicator and to resolve fluorescence from scattered excitation radiation, the fiber optic bundle was attached to the SLM 8000 spectrofluorometer as shown in figure 2.2. All parts in the sample compartment were removed. The directions of the optical lenses were reversed for both excitation and emission channels. The lens housings were attached to the arms of the bifurcated fiber optic by means of light-tight aluminum fittings and O rings.
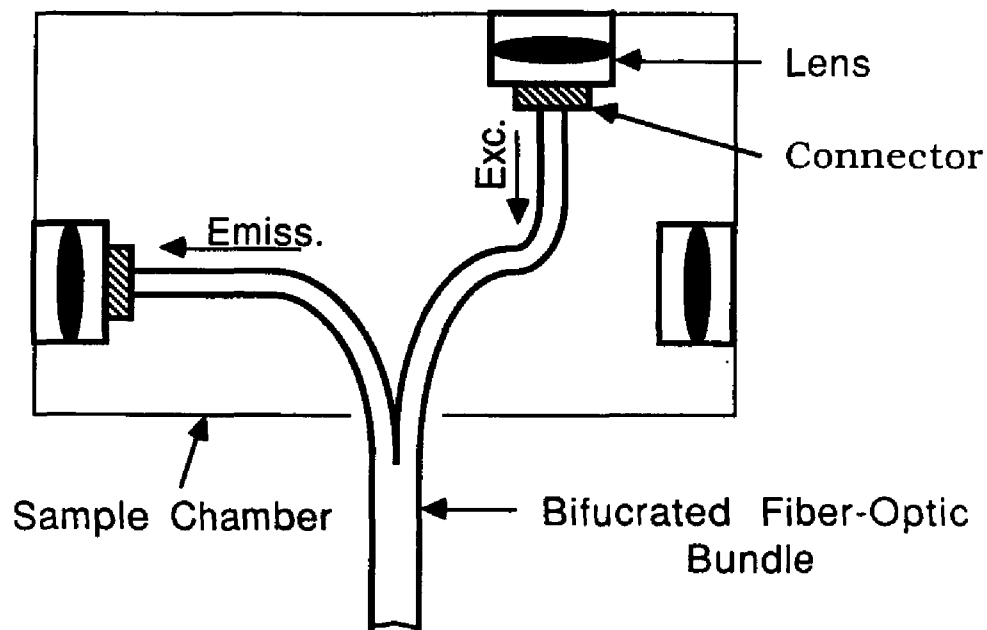
Figure 2.2 : Modification of SLM 8000 sample chamber for use with the bifurcated fiber optic.

TECHNIQUES :

Immobilization Techniques :

Different methods were used to prepare the sensing elements. DA^+,5-dimethylaminonaphthalene-1-sulfonamidtrimethyl ammonium ion, was immobilized on silica surfaces by a combination of electrostatic and hydrophobic interactions. AEANS, 5-(2-amino-ethyl)aminonaphthalenesulfonate ion, was immobilized covalently on controlled pore glass by formation of a Schiff base, and on both cellulose and poly(vinyl alcohol) using cyanuric chloride as a coupling agent. ET(30), 2,6-Diphenyl-4-(2,4,6-triphenyl-N-pyidino) phenolate, was mechanically immobilized in the silicone rubber membrane.

Both PVOH and silicone rubber membranes were attached to the common end of the fiber optic bundle as illustrated in figure 2.3.

Preparation of Solutions :

All chemicals used were reagent grade. The water used to prepare solutions was doubly deionized by passing house-deionized water through a mixed-bed ion exchange resin (Ultrapure, Fisher) and then distilled in an all glass still (Mega Pure, Corning).

Buffer solutions were prepared by adding drops of concentrated acid or base (NaOH or HCl) to the buffer solution until the desired pH was measured using a glass electrode. The solution

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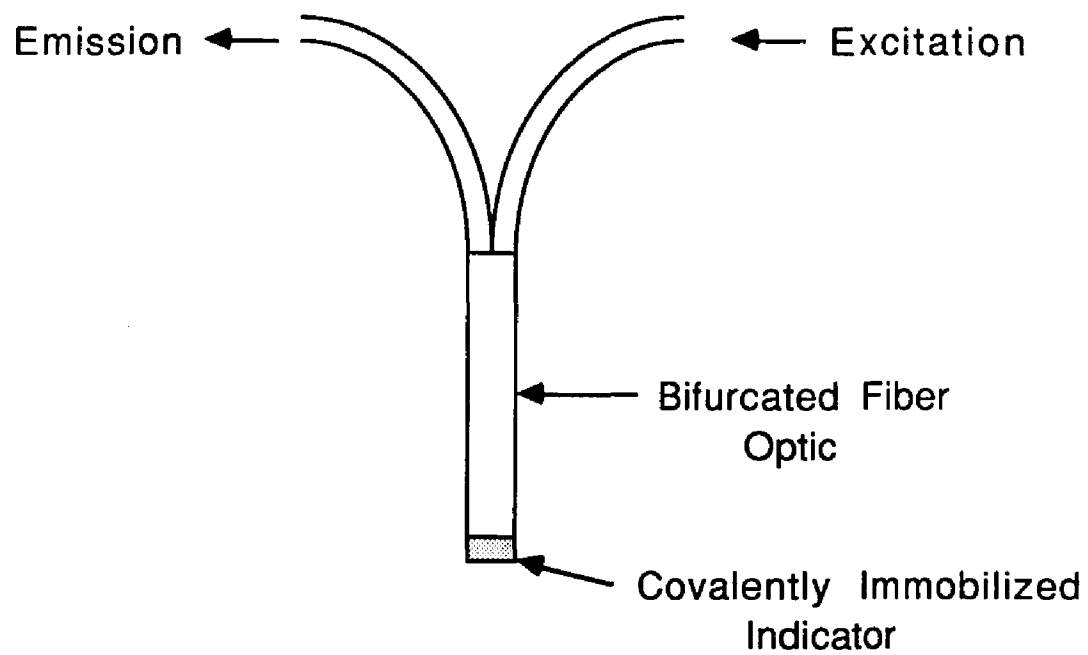
Figure 2.3 : Schematic of covalently immobilized indicator
on the common end of bifurcated fiber optic.

was then brought to volume, and the pH was again measured.

Analyte solutions were prepared in two ways. Solvent mixtures of different compositions were prepared by mixing known volumes of organic solvent with deionized distilled water. Surfactant solutions of different concentations were prepared by dilution from concentrated surfactant standard solutions using buffer or water.

Fluorescence Measurments :

The optical behavior of immobilized indicators on solid substrates was initially observed visually using a UV hand lamp for excitation. The colors of the fluorescence in the presence and the absence of analyte were compared.

Fluorescence spectra of immobilized fluorophors were obtained by two different methods depending on the nature of the immobilization substrate. For substrates such as controlled pore glass and cellulose which are particles, spectra were obtained for immobilized fluorophor packed into glass melting-point capillary tubes. These were placed into solutions of various analyte concentrations in sealed containers. Solutions were drawn into the tubes by capillary action.

Spectra were obtained with the capillary tube centered in a quartz cuvet, as shown in figure 2.4. This was accomplished by placing the tube through a small hole in the center of a cuvet cap. The fluorescence intensity was maximized by centering the packed
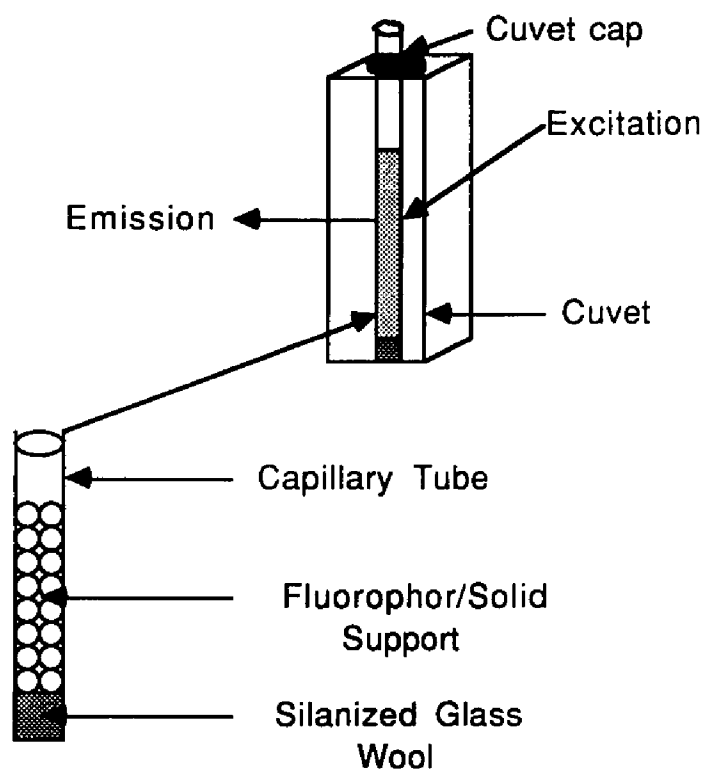
Figure 2.4 : Schematic of optical system used to obtain emission
spectra of immobilized fluorophors on solid supports.

tube in the cuvet. When measuring spectral shifts as a function of sample composition, the maximum output signal was normalized to the same value for each sample by adjusting the voltage to the photomultiplier tube. After recording the emission spectrum, the wavelength of maximum emission was identified by manually varying the wavelength at 1 or 2 nm intervals.

The response of the non-immobilized indicator in solution as a function of solvent composition, surfactant concentration or pH was measured with the SLM 8000 spectrofluorometer. A quartz cuvet was used to contain the analyte solution.

The other method of obtaining fluorescence spectra was using the fiber optic/fluorometer system with the immobilized indicator on the common end of a bifurcated fiber optic bundle. The response of the immobilized indicator to solvent composition or surfactant concentration was obtained by immersing the immobilized indicator phase into the appropriate solution.

Absorption Measurments :

Absorption spectra of immobilized ET30 in a polymeric membrane were measured using a Spectronic 200 spectrophotometer. A small piece of the immobilized membrane was attached to the side of a cuvet containing the analyte solution as illustrated in figure 2.5.
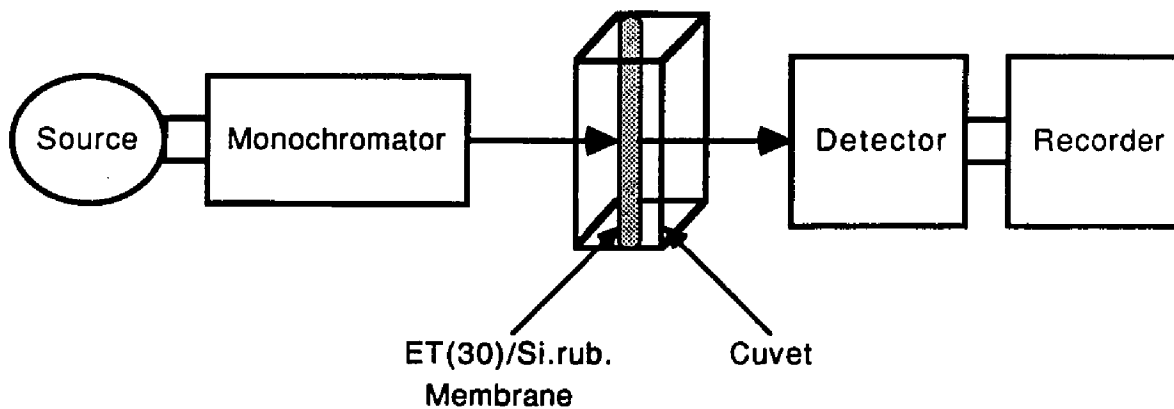
Figure 2.5 : Schematic of spectronic 200 spectrophotometer used to obtain the absorption spectrum of immobilized ET30 dye immobilized in a silicon rubber membrane.

CHAPTER III

ORGANIC CATIONIC BINDING TO HYDROCARBON
BONDED SILICA SURFACE

INTRODUCTION:

Fluorescence characteristics of organic cations bonded to silica are described in this chapter. Fluorescence is dependent upon the microenvironment polarity which is a function of solvent composition and surface structure.

The organic cationic indicator used was 5-dimethylamino-naphthalene-1-sulfonamidoethyltrimethylammonium iodide (DA$^+$I$^-$). The structure is shown in figure 3.1. DA$^+$ is one of a large number of fluorescent derivatives of 5-dimethylamino-1-naphthalenesulfonic acid collectiveley known as dansyl compounds. When dansyl compounds are electronically excited, there is a transfer of charge from the amine substituent to the sulfonate. Because the resulting polar excited state is stabilized by dipole and electrostatic interactions with the solvent as illustrated in figure 1.5, the emission spectrum shifts to longer wavelength as the excited state environment becomes more polar.

In addition to being sensitive to environmental polarity, DA$^+$ was chosen because it is a hydrophobic cation and, therefore, has a strong affinity for negatively charged solid surfaces and can be

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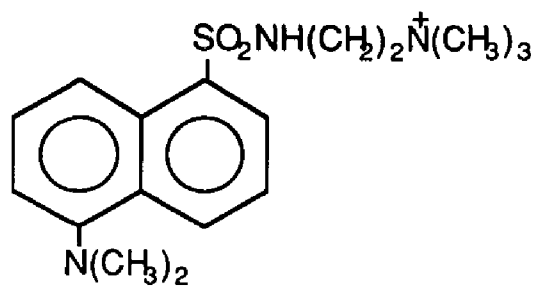
Figure 3.1 : 5-dimethylamino naphthalene-1-sulfonamidotrimethyl-ammonium ion (DA+).

conveniently immobilized by ion exchange.

DA$^+$ was immobilized on both underivatized silica and silicas bonded to ethyl, octyl and octadecyl groups, designated RP2, RP8 and RP18, respectively. Their structures are shown in figure 3.2. This immobilization is a result of a combination of hydrophobic and electrostatic interactions.

Underivatized silica surfaces are covered by silanol groups, which interact strongly with polar solvents (69-71). Previous studies showed that a monolayer of water strongly binds to the surface as a result of direct interaction with the silanol groups. The monolayer is covered by two more weakly bound layers of water which interact with the surface through the primary water layer(69).

The silanol group on the silica surface is weakly acidic with a pKa around 9.5 (72). Transfer of a proton from silanol groups to amines often results in tailing when amines are separated by liquid chromatography (73). In some separation techniques, long chain aliphatic amines are used to shield the silanol groups by selectively binding with them so that they can not interact with the solute (70).

Hydrocarbon bonded surfaces are prepared by reacting silanes with silanol groups on the silica suface (71). Because this reaction does not go to completion, some silanol groups are left which can interact strongly with organic cations. In order to minimize the effects of unreacted silanol groups on hydrocarbon bonded phases the hydrocarbon bonded phases are reacted with trimethyl

a) RP2 (ethyl-)

b) RP8 (octyl-)

c) RP18 (octadecyl-)
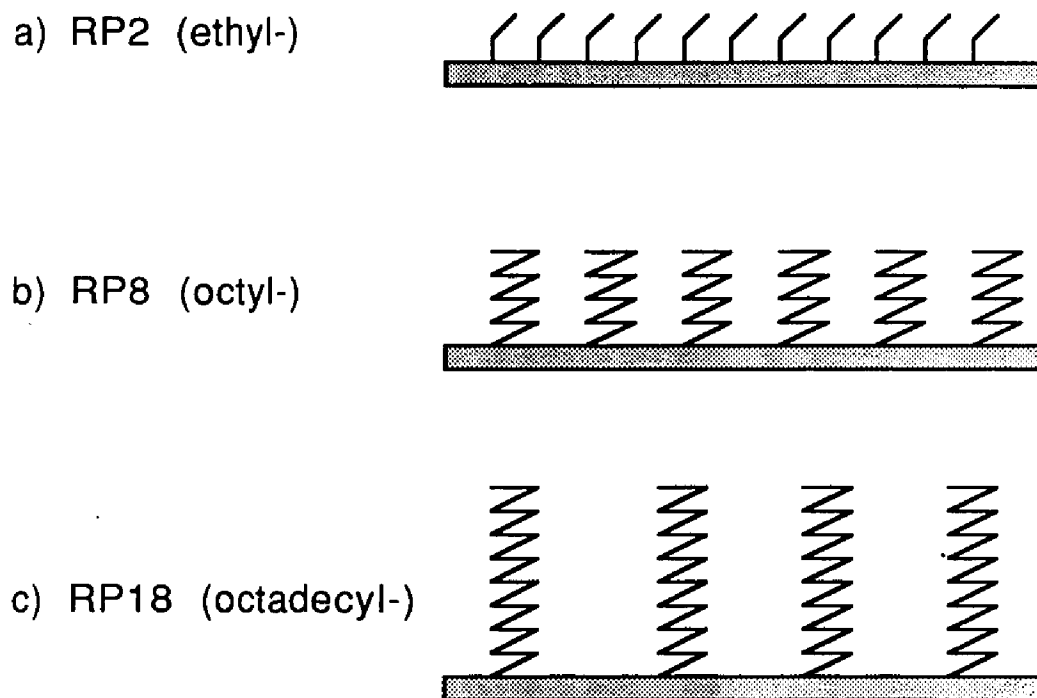
Figure 3.2 : Schematic of relative coverage of RP surface.

chlorosilane, a procedure called endcapping.

Because fluorescence measurements are quite sensitive, a very small percentage of remaining unreacted silanols is sufficient to bind enough fluorophor to give a strong signal.

Recently, Harris and Carr pointed out, based on fluorescent studies (51), that the interaction of organic modifiers with the reversed phase produces an inverse relationship between the polarity at the surface and the organic solvent-aqueous compositions. In water, the emission spectrum of the indicator has the shortest wavelengths indicating that the hydrocarbon chains shield the indicator from the aqueous environment providing a less polar environment. But as the organic solvent percentage increases, the emission spectrum shifts to longer wavelengths. This indicates that in organic solvents, hydrocarbon chains are solvated and open up. As a result the indicator environment becomes more polar as it becomes more exposed to solvent.

Since the extent of the interaction between the hydrocarbon chains and organic solvent depends on the percentage of organic solvent, DA^+ can serve as an indicator of the level of this interaction as a function of solvent composition.

This chapter presents the results of experiments to evaluate the microenvironment polarity of underivatized silica and silicas monomerically bonded to ethyl, octyl and octadecyl groups, and the effects of various solvent compositions. Results of preliminary investigation have been reported in the literature (13,14).

EXPERIMENTAL:

<u>Reagents</u> :

Silica gel with an average pore diameter of 60 Angstroms and an average particle size of 30 micrometers was obtained from Sigma. Ethyl-, octyl- and octadecyl bonded silicas were also obtained from Sigma. These materials are designated RP2, RP8, RP18, respectively. The structures are shown in figure 3.2. They are prepared by reacting monochlorosilane with silica. Manufacturer's data supplied with these materials indicates the following. The average particle size of RP2 is 30 micrometers with a 60 Angstrom average pore diameter and 5.6% carbon content which corresponds to 2.3 mmol/g. Both RP8 and RP18 have a 40-63 micrometer average particle size and a 60 Angstrom average pore diameter. The carbon content of RP8 is 14% coresponding to 1.5 mmol/g. The carbon content of RP18 is 22% corresponding to 1.0 mmol/g. After the hydrocarbon was bonded to the surface, unreacted chloro groups were hydrolyzed, and hydroxyl groups on the surface were blocked by reacting them with trimethylchlorosilane.

The fluorescent probe, 5-dimethylaminonaphthalene-1-sulfonamidoethyltrimethylammonium iodide (DA$^+$), was obtained from Molecular Probes, Inc. Its structure is shown in figure 3.1. Methanol (99.9% from Fisher), acetonitrile (100% from J.T.Baker) and tetrahydrofuran (99.0% minimum assay by GLC, 0.1% water content, from Eastman Kodak) were used without further purification. Water

was distilled and deionized.

Apparatus :

Emission spectra were measured on an SLM 8000 spectrofluorometer which is described in chapter 2. They were not corrected for instrumental effects. The excitation wavelength was 360 nm for all spectra.

Procedure :

Preliminary Testing : The first experiments were to prepare 0.01 millimolar DA^+ in different solvents such as methanol, acetonitrile and tetrahydrofuran, and visually observe the color of fluorescence excited by a UV lamp.

The effects of mixed solvent composition on the emission spectrum of DA+ were investigated using 0.1 mM DA^+ solutions.

Immobilization of DA^+ on Solid Substrates : DA^+ was immobilized on solid substrates as follows : 1.0 gram of substrate was combined with 20.0 ml of 10.0 micromolar DA^+. After being shaken for five minutes, the substrate was separated by filtration, washed extensively with distilled water, allowed to dry and then stored in a dessicator. Emission spectra were taken at various points in the drying procedure until they remained constant.

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<u>Solvent Effects on Immobilized DA$^+$</u> : Equal amounts of immobilized DA$^+$, were immersed in different solvents such as water, methanol, acetonitrile and tetrahydrofuran. The difference in the fluorescence color among them was observed visually using a UV hand lamp for excitation.

The effect of solvent composition on the emission spectrum of immobilized DA$^+$ was then studied instrumentally. The immobilized DA$^+$ was packed into glass melting point capillary tubes. These were then placed into various solvent mixtures (0-100%) in sealed containers so that evaporation would not alter the solvent composition. Solvents were drawn into the tubes by capillary action. Spectra were taken at various times to confirm that the solvent had equilibrated with the substrate.

Emission spectra and the wavelength of maximum emission were obtained as described in Chapter 2.

RESULTS AND DISCUSSION :

<u>Nature of Bonding to Surface</u> :

DA$^+$ binds strongly to the silica substrate, since there was no evidence of significant leaching from the various surfaces when they were washed vigorously with water and organic solvents. No fluorescence could be observed from the solvent mixture after equilibration with DA$^+$ immobilized on various substrates. Also, there was no observable change in the shape of the spectrum with

added organic solutes. This indicates that in all samples the DA^+ is in a single environment. If it was in multiple environments each with a different polarity, then the observed spectrum would be expected to be broad since it would be the sum of several DA^+ spectra each with a different maximum emission wavelength.

The amount of immobilized DA^+ was not determined. However, it was sufficient to give a strong fluorescent signal. Significant amounts of DA^+ remain immobilized on all silica substrates even after extensive washing with water, indicating that DA^+ binds strongly to all surface even after endcapping. This indicates that unreacted silanols remain on the surface. Deprotonation of these unreacted silanol groups leaves negative sites on the surface which can interact strongly with the positive site of hydrophobic organic cations such as DA^+. If there were not an electrostatic interaction between DA^+ and the surface, the washing procedure would remove all of the fluorophor. Because fluorescence measurements are quite sensitive, even a small percentage of remaining unreacted silanols is sufficient to bind enough DA^+ fluorophore to give a strong signal.

The intensity increases as a function of the percent of organic solvent as expected for dansyl derivatives. However, because it is impossible to reproducibly position samples in the fluorometer, it was not practical to try to derive accurate information from intensity data.

<u>Free DA$^+$ in Solution</u> :

Our preliminary investigation showed that the color of DA$^+$ fluorescence depends on environmental polarity. Fluorescence color was green to yellowish when DA$^+$ was dissolved in water, but in less polar organic solvents such as methanol, acetonitrile and tetrahydrofuran the fluorescence became blue to greenish. This indicates that fluorescence of DA$^+$ shifted to shorter wavelengths as the polarity of the solution surrounding the fluorophor decreased.

The results in table 3.1 show that the emission wavelength maximum for DA$^+$ dissolved in pure solvents depends of the polarity of those solvents. The maximum emission wavelengths were 561, 526, 525 and 509 nm for water, acetonitrile, methanol and tetrahydrofuran respectively.

Figure 3.3 shows that solvent composition has a significant effect on the fluorescence of DA$^+$. The fluorescence of DA$^+$ dissolved in water/organic solvent mixtures with methanol, acetonitrile or tetrahydrofuran, shifts to shorter wavelengths as the percentage of organic solvent increases.

The magnitude of the spectral shift for DA$^+$ in solvent mixtures depends on the relative polarity of the organic solvents. As shown in figure 3.3, the spectral shifts are greatest for tetrahydrofuran, the least polar solvent. The shifts are smallest for acetonitrile, the most polar of the three organic solvents tested.

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Table 3.1: Values for wavelength of maximum emission of DA+ in solution, silica Gel, RP2, RP8 and RP18 exposed to H2O, ACN, MeOH and THF.

DA+ Emission wavelength (nm)

Solvent	solution	Silica gel	RP2	RP8	RP18
-------	-------	--------	----	----	-----
Water	561	543	488	494	493
Acetonitrile	526	531	522	520	515
Methanol	525	526	519	516	508
Tetrahydrofuran	509	512	508	506	500
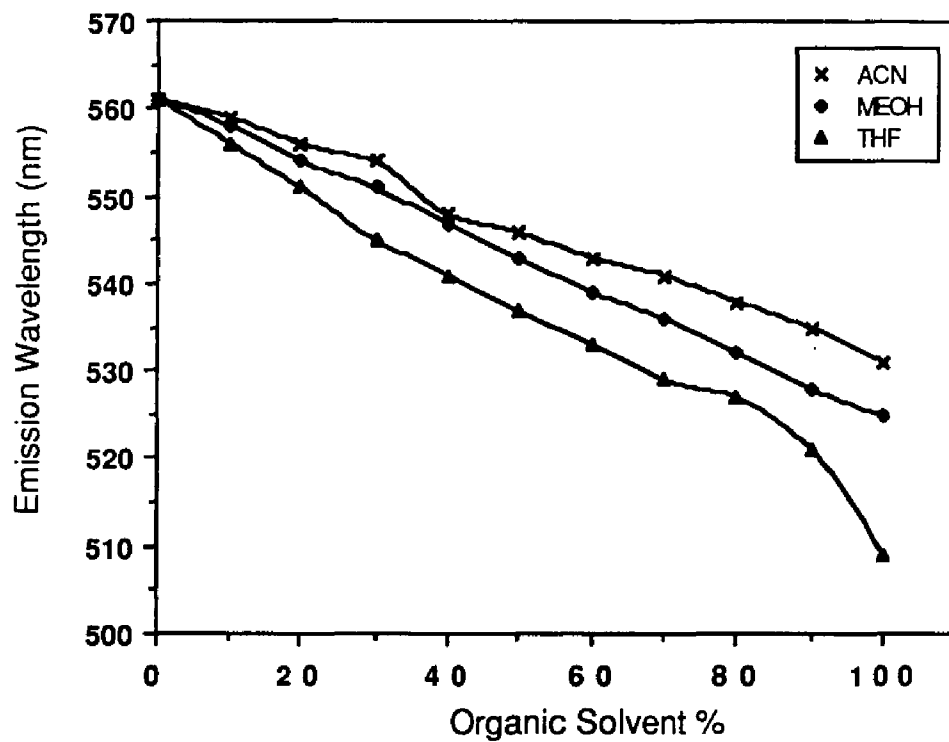
Figure 3.3 : Wavelength of maximum emission of DA+ in solution as
a function of the percentage of, ACN, MeOH and THF in water.

DA$^+$ on Underivatized Silica :

The emission wavelength of immobilized DA$^+$ on underivatized silica depends on the composition of organic solvent. Figures from 3.4 to 3.6 show the effect of adding acetonitrile, methanol and tetrahydrofuran to water on the spectral shifts of DA$^+$ on silica. The emission spectrum shifts to longer wavelengths as the percentage of organic solvent increases. The results show the total spectral red shifts for 0-10% tetrahydrofuran, 0-20% acetonitrile and 0-40% methanol are 4, 10, 4 nm, respectively. The spectrum shifts to shorter wavelengths as the percentage of organic solvent increases above these limits.

Figures from 3.4 to 3.6 show that the main difference between dissolved DA$^+$ and DA$^+$ on underivatized silica was observed in pure water. The emission wavelength for DA$^+$ on silica in pure water is actually shorter than that observed for 10% organic solvent. This suggests that in pure water, DA$^+$ is not seeing a purely solvent environment. Instead, it is being excluded from the solvent and experiencing what is partially a surface environment.

It is not possible to establish where the fluorophor is on the silica surface. However, because the primary layer of water is held so much more strongly to the surface than the above layers (74), it is suspected that the DA$^+$ molecule is resting on this primary layer. This weak interaction must contribute to the binding of DA$^+$ to the surface, otherwise one would expect DA$^+$ to be displaced from the surface when extensively washed with water.
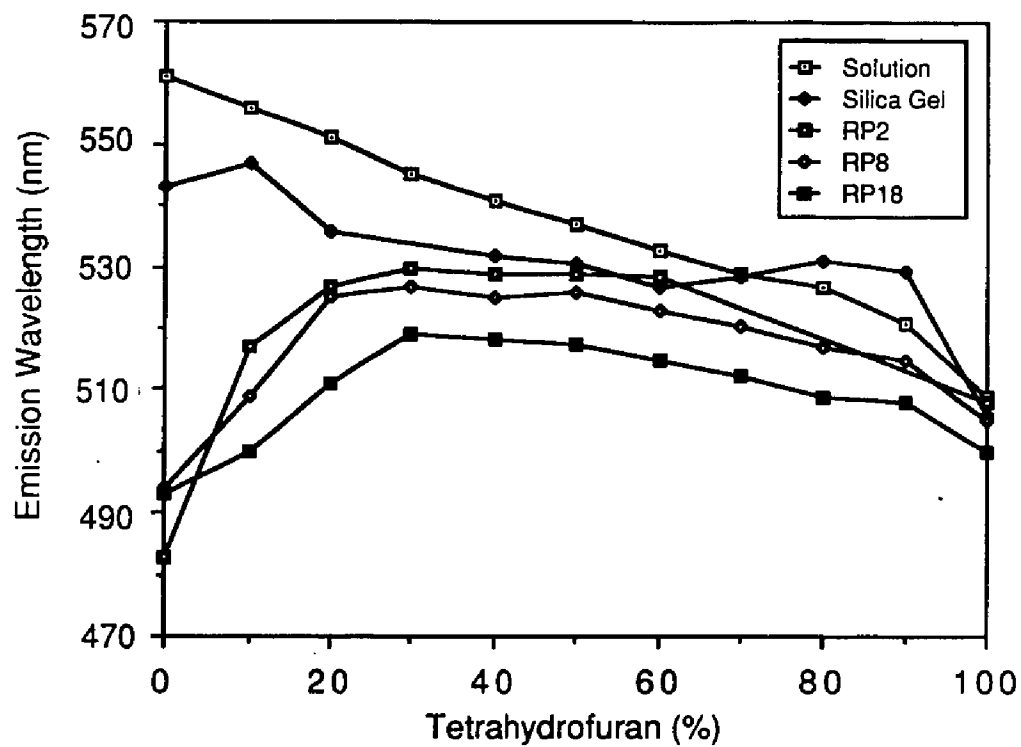
Figure 3.4 : Wavelength of maximum emission as a function of the
percentage of THF in water for DA+ in solution and
immobilized on silica gel, RP2, RP8 and RP18.
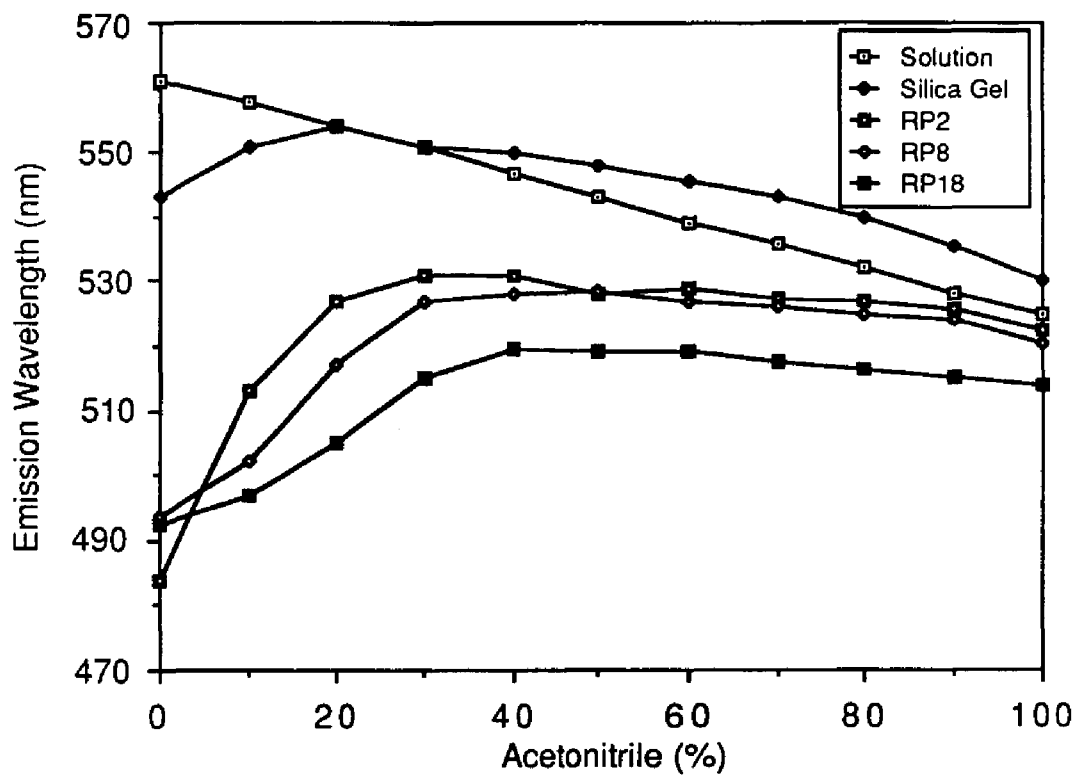
Figure 3.5 : Wavelength of maximum emission as a function of
percentage of ACN in water for DA+ in solution and
immobilized on silica gel, RP2, RP8 and RP18.
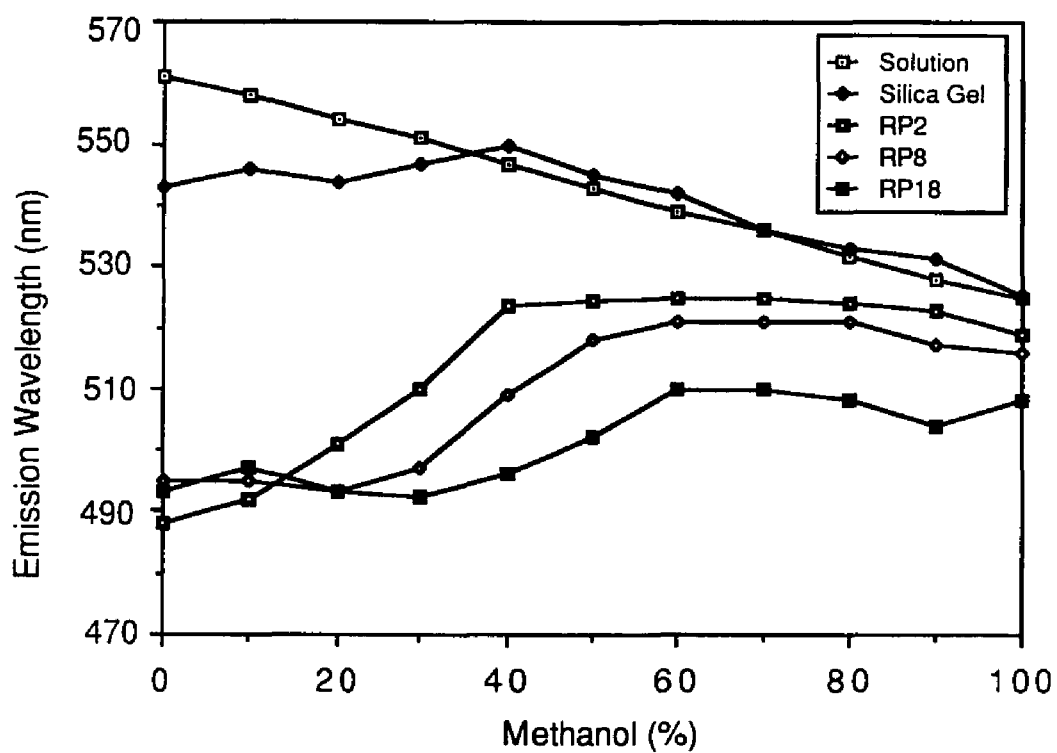
Figure 3.6 : Wavelength of maximum emission as a function of
pecentage of MeOH in water for DA+ in solution and
immobilized on silica gel, RP2, RP8 and RP18.

From table 3.1, it is obvious that the emission wavelength of immobilized DA^+ on underivatized silica depends on solvent polarity. For pure in water, acetonitrile, methanol and tetrahydrofuran, the emission wavelengths were 543, 531, 526 and 512 nanometers, respectively. Spectral shifts are consistent with the solvent polarity of water, acetonitrile, methanol and tetrahydrofuran which decreases respectively.

Emission wavelengths for DA^+ on underivatized silica in pure organic solvents are longer than those of DA^+ dissolved in the same solvents. This suggests that the fluorophor is partially in a surface environment, and is still exposed to the primary layer of water on the silica surface. On the other hand, the emission wavelength of DA^+ on silica in pure water is shorter than that of DA^+ dissolved in pure water. This indicates that the silica surface provides a less polar environment than water.

Overall, the variation in emission wavelength with solvent composition is similar to that observed for dissolved DA^+ indicating that DA^+ is exposed to the solvent environment under all conditions.

DA<u>$^+$ on Hydrocarbon Bonded Silicas</u> :

On the hydrocarbon bonded surfaces, behavior is dramatically different than on underivatized silica or in solution. DA^+ immobilized on hydrocarbon bonded silicas emits at the shortest wavelengths in water, unlike DA^+ dissolved in solution or immobilized on underivatized silica which emits at much longer

wavelengths. The addition of organic solvent causes the emission spectrum to shift to longer wavelengths. This behavior is related to the arrangment of the hydrocarbon surface which depends on the solvent environment as illustrated in figure 3.7. In water, the hydrocarbon chains in the bonded phase assumed a "folded" configuration in which they preferentially associate with each other rather than the aqueous medium. In water, hydrocarbon chains can fold over the DA^+ molecules isolating them from the high polar aqueous medium and providing a non-polar hydrophobic environment. However, in less polar organic solvents such as methanol, acetonitrile and tetrahydrofuran, the hydrocarbon chains are solvated and adopt a "bristle" configuration in which they extend out into the solvent as shown in figure 3.7. As a result, solvent molecules can get into contact with immobilized DA^+ and provide a more polar environment thus causing a spectral shift to longer wavelengths. But, in all cases, even in pure organic solvents, the maximum emission wavelength for DA^+ on hydrocarbon bonded surfaces is still shorter than for dissolved DA^+ in the same solvents. This means that the non-polar hydrocarbon surface still has an effect on the fluorophor environment.

The emission wavelength for DA^+ on RP2 is shorter (488 nm) than on RP8 (494 nm) and on RP18 (493 nm). This indicates that the surface coverage by the hydrocarbon chains in terms of micromoles per gram of substrate is more important for promoting maximum hydrophobic interaction than either high surface coverage in terms of grams of carbon per gram of substrate or the presence of long

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A) In Water
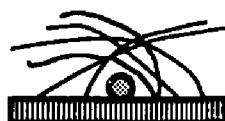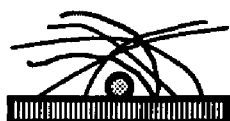
Slica

B) In Solvent/H2O
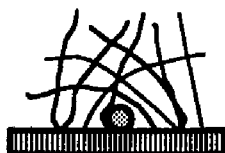
C) In Solvent
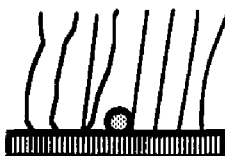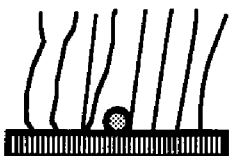
Figure 3.7 : Postulated solvent effect on alkyl bonded phase orientation.

⊙ : DA+ fluorophor.

alkyl chains on the surface. It was expected that RP18 would provide the most hydrophobic environment for DA^+ because of the ability of octadecyl chains to wrap around and fold over the fluorophor. However, the flexibility of octadecyl chains may enable them to interact more strongly with each other than with the aromatic fluorophor. The results on RP8 are almost the same as for RP18.

The Effect of Solvent Composition on DA^+/RP :

In water, DA^+ on hydrocarbon bonded silicas is excluded from the solvent and emits at the shortest wavelengths. The addition of organic solvent causes the spectrum to shift to longer wavelength indicating an increase in the polarity of DA^+'s environment. As the percentage of the organic solvent increases, DA^+ is exposed to an environment that includes more of the solvent and less of the hydrocarbon surface. The absence of an increase in the width of the emission spectrum suggests that DA^+ exists in a single homogenous environment.

Figures from 3.8 to 3.10 show that for each system there is a range of solvent compositions where there is a large shift to longer wavelength with increasing added organic solvent. Above this range, emission wavelength is either constant with further added organic solvent or decreases slightly.

As illustrated in figure 3.7, an increase in the percentage of the organic solvent around the hydrocarbon surface enhances the interaction between the hydrocarbon chains and solvent molecules,
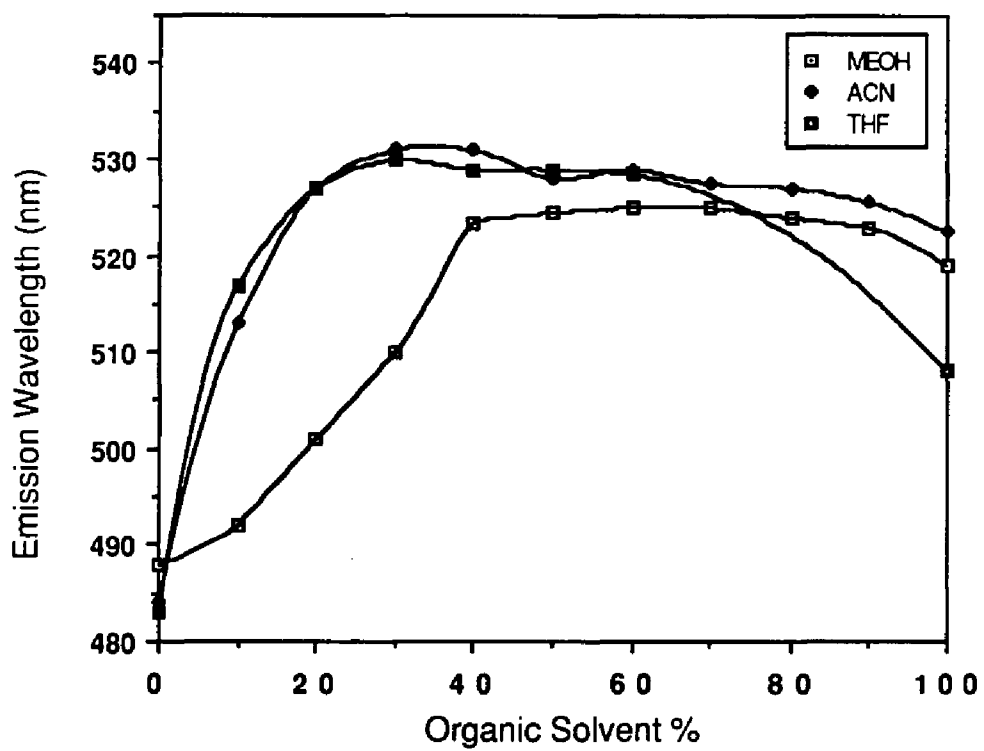
Figure 3.8 : Wavelength of maximum emission of DA+ on RP2
as a function of the percentage of MEOH, ACN
and THF in water.
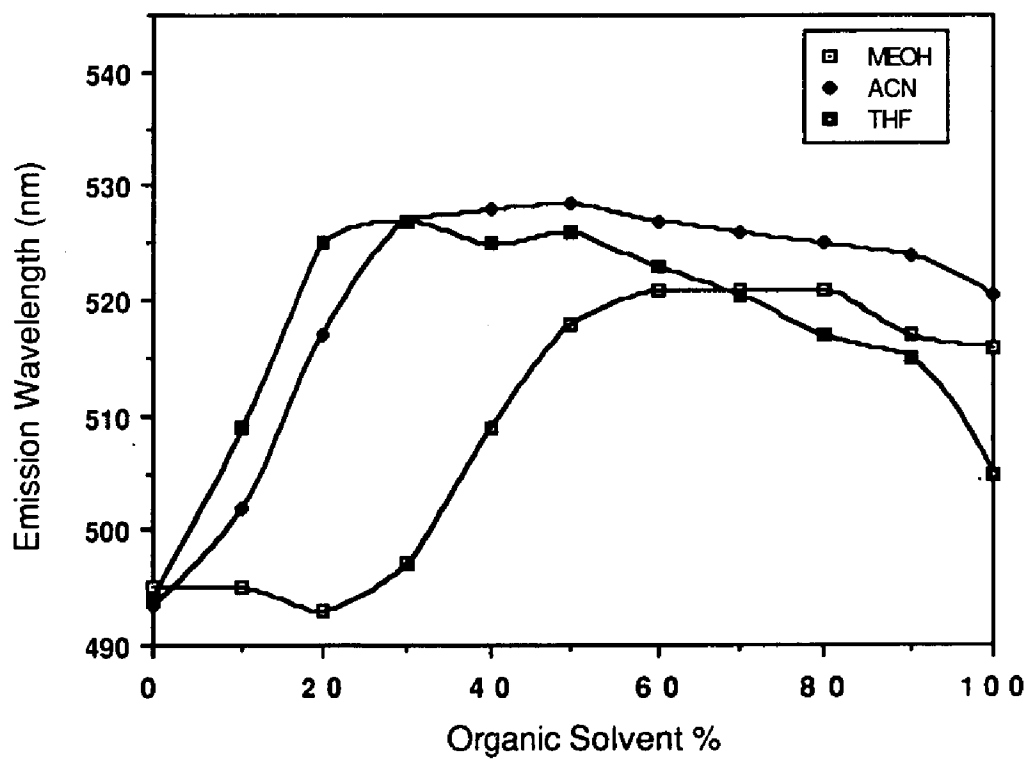
Figure 3.9 : Wavelength of maximum emission of DA+ on RP8
as a function of the percentage of MEOH, ACN
and THF in water.
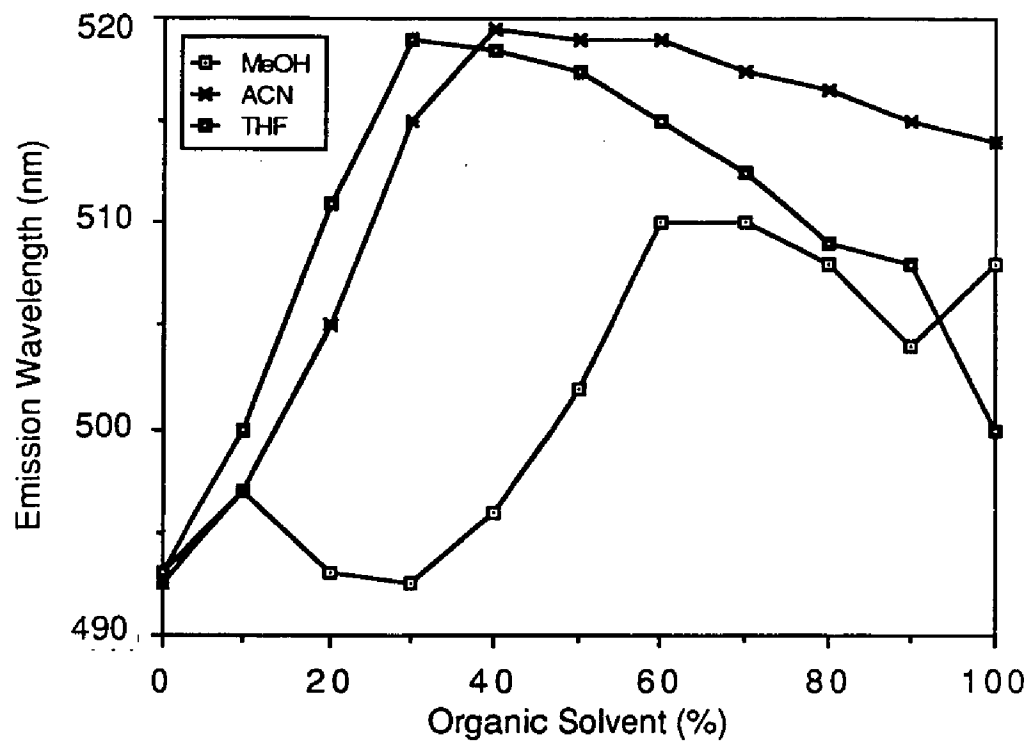
Figure 3.10 : Wavelength of maximum emission of DA+ on RP18
as a function of the percentage of MEOH, ACN
and THF in water.

and disrupts the self interaction between the hydrocarbon chains. The extent of this effect increases with the percentage of organic solvent. At certain percentage the hydrocarbon chains are completely solvated and opened up so that immobilized DA^+ experiences an environment more characteristic of the solvent and less characteristic of the substrate surface. Then, above this percentage the emission spectrum shifts gradually to shorter wavelengths with added organic solvent. In this region, DA^+ is primarily experiencing the bulk solvent rather than the surface environment in which the increases in the percentage of organic solvent decreases the net polarity of solvent mixture and causes the emission spectrum to shift to shorter wavelengths.

Figures from 3.8 to 3.10 show that the percentage of organic solvent required to solvate the hydrocarbon chains depends on the nature of organic solvent. The higher the solvent strength the stronger the interaction produced between the solvent and the hydrocarbon.

The percentage of methanol required to produce a significant interaction with the hydrocarbon chains is high , about 30%. This is related to the low solvent strength of methanol.

The emission spectrum starts to shift to longer wavelengths at lower percentages in tetrahydrofuran and acetonitrile than in methanol. The data indicate that the interaction between the solvent and the hydrocarbon chains is greatest for tetrahydrofuran and weakest for methanol. This is consistent with the relative solvent strengths of tetrahydrofuran, acetonitrile and methanol as described

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in the literature (51).

The composition of organic solvent required to solvate the hydrocarbon chains depends not only on the nature of the organic solvent but also on the structure of the solid substrate. Results show that the emission spectrum starts to shift at a lower percentage of organic solvent for the shorter hydrocarbon chain . For example, figures from 3.8 to 3.10 show that RP2, RP8 and RP18 respond to methanol at different percentages, about 10, 20 and 30%, respectively. This indicates that longer hydrocarbon chains produce more interaction among themselves. It is anticipitated that the hydrocarbon chain interaction increases for ethyl, octyl and octadecyl groups respectively. This means that a greater percentage of organic solvent is required in order to overcome the self interaction between the hydrocarbon chains.

The maximum spectral shift of DA^+ on hydrocarbon bonded surfaces depends on the nature of the organic solvent and the hydrocarbon layer. Octadecyl groups on RP18 require a higher percentage of organic solvent to be solvated. At a high percentage of organic solvent the net polarity of the solvent mixture is lower. As a result, the longest emission wavelength for DA^+ is only 518 nm on RP18 while it is 530 nm for RP2 in tetrahydrofuran.

Application to Optical Sensing :

These studies show the potential of these systems for optically sensing organic in water based on spectral shifts as a

function of composition. DA$^+$ on RP2 shows the largest wavelength shift as a function of added organic solvent even for a small amounts. Therefore this system has the greatest potential for optical sensing.

CHAPTER IV

SURFACTANT DETERMINATION BASED ON AN ENVIRONMENT
SENSITIVE FLUOROPHORE BONDED TO A SOLID SUPPORT

INTRODUCTION :

This chapter describes the characteristics of an indicator for cationic surfactants and pH based on an environment sensitive fluorophore immobilized on solid supports including controlled pore glass and cellulose and on a poly(vinyl alcohol) gel which can be conveniently coupled to fiber optics. The indicator is based on an anionic derivative of 5-dimethylamino-1-naphthalenesulfonic acid that is highly sensitive to environment polarity. As the indicator environment becomes less polar, fluorescence increases in intensity and shifts to shorter wavelength. Cationic surfactants with hydrophobic alkyl chain can modify the polarity of the indicator environment causing these changes. Because there is currently no optical method for sensing surfactants on a continuous basis, the indicator system could have wide applicability.

The immobilized indicator responds to surfactant as a result of the following types of interactions between the indicator and surfactant: A) The electrostatic attraction between the positive charge on the surfactant and the negative charge on the immobilized fluorescent indicator (54,75), and B) The hydrophobic attraction

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between the hydrocarbon tail of the surfactant and the aromatic fluorophore. This is analogous to hydrophobic interactions in reversed phase HPLC. The actual energy comes not from the hydrophobic interaction itself but from the fact that the polar solvent molecules can interact with each other rather than having to interact with the nonpolar parts of the surfactant and the fluorescent probe. The combination of electrostatic and hydrophobic interactions leads to the formation of an ion pair. As a result of these interactions, the surfactant modifies the polarity of the immobilized indicator environment causing a shift in optical properties.

The fluorescent indicator AEANS, 5-(2-aminoethyl)amino-naphthalene-1-sulfonate ion (Figure 4.2), belongs to the large class of dansyl derivatives. These compounds are highly sensitive to environment polarity. AEANS in water, a highly polar environment, fluoresces green (505 nm). In a less polar environment the fluorescent color is blue. The sulfonate group of AEANS can interact electrostatically with the cationic end of a surfactant (54,75). This interaction does not directly perturb the chromophore of dye (76).

Cyanuric chloride, 2,4,6-trichloro-1,3,5-triazine, has been used as reagent in immobilizing indicators on solid substrates for fiber optic chemical sensors (22,77). Cyanuric chloride is a versatile reagent that reacts with aromatic and aliphatic amines, and alcohols (78,79). Many authors have shown that, in water, individual chlorines of cyanuric chloride can be replaced depending upon the temperature. The first chlorine reacts readily at 0 C^0; the second reacts at room temperature.
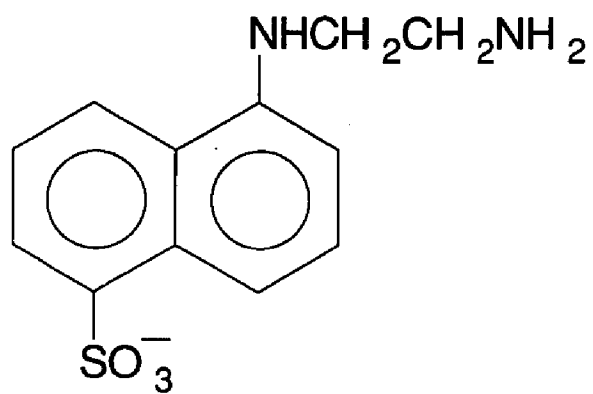
Figure 4.2 : Structure of 5-(2-aminoethyl)aminonaphthalenesulfonate ion (AEANS).

EXPERIMENTAL

Reagents :

Glycophase-controlled pore glasses (G-CPG) with an average pore diameter of 460 Angstroms and particle sizes of 37 -74 micrometers and with an average pore diameter of 550 Angstroms and particle sizes of 5-10 micrometers were obtained from Pierce Chemical Co. Powdered cellulose (microcrystalline for TLC through 60 mesh sieve) was purchased from Baker. Poly(vinyl alcohol) (PVOH), 100% hydrolyzed, with an average molecular weight of 14,000 and cyanuric chloride were purchased from Aldrich. Glutaraldehyde solution, 50% (W/W), was obtained from Fisher Scientific. Dodecyl-, tetradecyl- and cetyl- trimethylammonium bromide, sodium dodecyl sulfate and polyoxyethylene-23-lauryl ether were obtained from Aldrich. They are designated as DDTMA, TDTMA, CTMA, DDS and PELE, respectively. Their structures are shown in figure 4.1.

The fluorophor, 5-(2-aminoethyl) aminonaphthalenesulfonate (AEANS), was obtained from Molecular Probes, Inc. Its structure is shown in figure 4.2. Water was distilled and deionized.

Apparatus :

Emission spectra were measured on an SLM 8000 Spectrofluorometer as described in chapter 2 (SLM-Aminco.

A) Cationic Surfactants :

$$CH_3(CH_2)nCH_2 - \overset{+}{N}(CH_3)_3$$

Where;

n = 10 : Dodecyltrimethylammonium ion (DDTMA).
    12 : Tetradecyltrimethylammonium ion (TDTMA).
    14 : Cetyltrimethylammonium ion (CTMA).

B) Anionic surfactants :

$$CH_3(CH_2)_{11}OSO_3^-$$
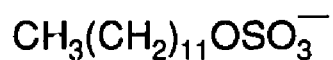
Dodecylsulfate ion (DDS)

C) Nonionic surfactants :

$$C_{12}H_{23}(OCH_2)_{23}OH$$

Polyoxyethylene-23-lauryl ether (PELE)

Figure 4.1 : Structures of cationic, anionic and nonionic surfactants.

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Instruments Inc., Urbana, IL.). The excitation wavelength was 370 nm for all spectra. The photomultiplier voltage was not the same for all experiments; therefore, the scales for fluorescence intensities were not identical. The excitation and emission arms of a bifurcated fiber optic bundle were attached to the source and the detector lens housings in the sample chamber by means of light-tight aluminum fittings and O-rings as described in chapter 2.

Procedures :

AEANS Immobilization on CPG : AEANS was immobilized on the CPG solid support by the reactions shown in figure 4.3. Aldehyde phase-controlled pore glass (A/CPG) was prepared as follows ; 1.0 g of Glycophase-CPG was mixed with 20.0 mL of 0.010 M sodium metaperiodate solution and stirred for approximately one hour. The solid particles were filtered and washed vigorously with distilled water. The A-CPG solid particles were mixed with 30.0 mL of 0.90 mM AEANS in pH 8.5 borate buffer. After two hours of stirring , 200 mg of sodium borohydride were added in 50 mg portions every 20-30 minutes. The immobilized AEANS/A-CPG particles were filtered and extensively washed with distilled water until all of the free AEANS was removed.

AEANS Immobilization on Cellulose : AEANS was immobilized on cellulose as follows; The powdered cellulose was soaked in a solution of 1M KOH for 15 min. After washing away the excess base,

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$$\stackrel{\displaystyle\xi}{\text{Si}}-\text{CH}_2\text{CH}_2\text{CH}_2\text{OCH}_2\text{CHOHCH}_2\text{OH} \quad \text{(CPG)}$$

$$\downarrow \text{NaIO}_4$$

$$\stackrel{\displaystyle\xi}{\text{Si}}-\text{CH}_2\text{CH}_2\text{CH}_2\text{OCH}_2\text{CHO} + \text{H}_2\text{NCH}_2\text{CH}_2\text{NH} - \text{naphthalene-SO}_3^-$$

(AEANS)

$$\downarrow \begin{array}{c}\text{Borate Buffer}\\ \text{pH 8.8}\end{array}$$

$$\stackrel{\displaystyle\xi}{\text{Si}}-\text{CH}_2\text{CH}_2\text{CH}_2\text{OCH}_2\text{CH}=\text{NCH}_2\text{CH}_2\text{NH} - \text{naphthalene-SO}_3^-$$

$$\downarrow \text{NaBH}_4$$

$$\stackrel{\displaystyle\xi}{\text{Si}}-\text{CH}_2\text{CH}_2\text{CH}_2\text{OCH}_2\text{CH}_2\,\text{NHCH}_2\text{CH}_2\text{NH} - \text{naphthalene-SO}_3^-$$
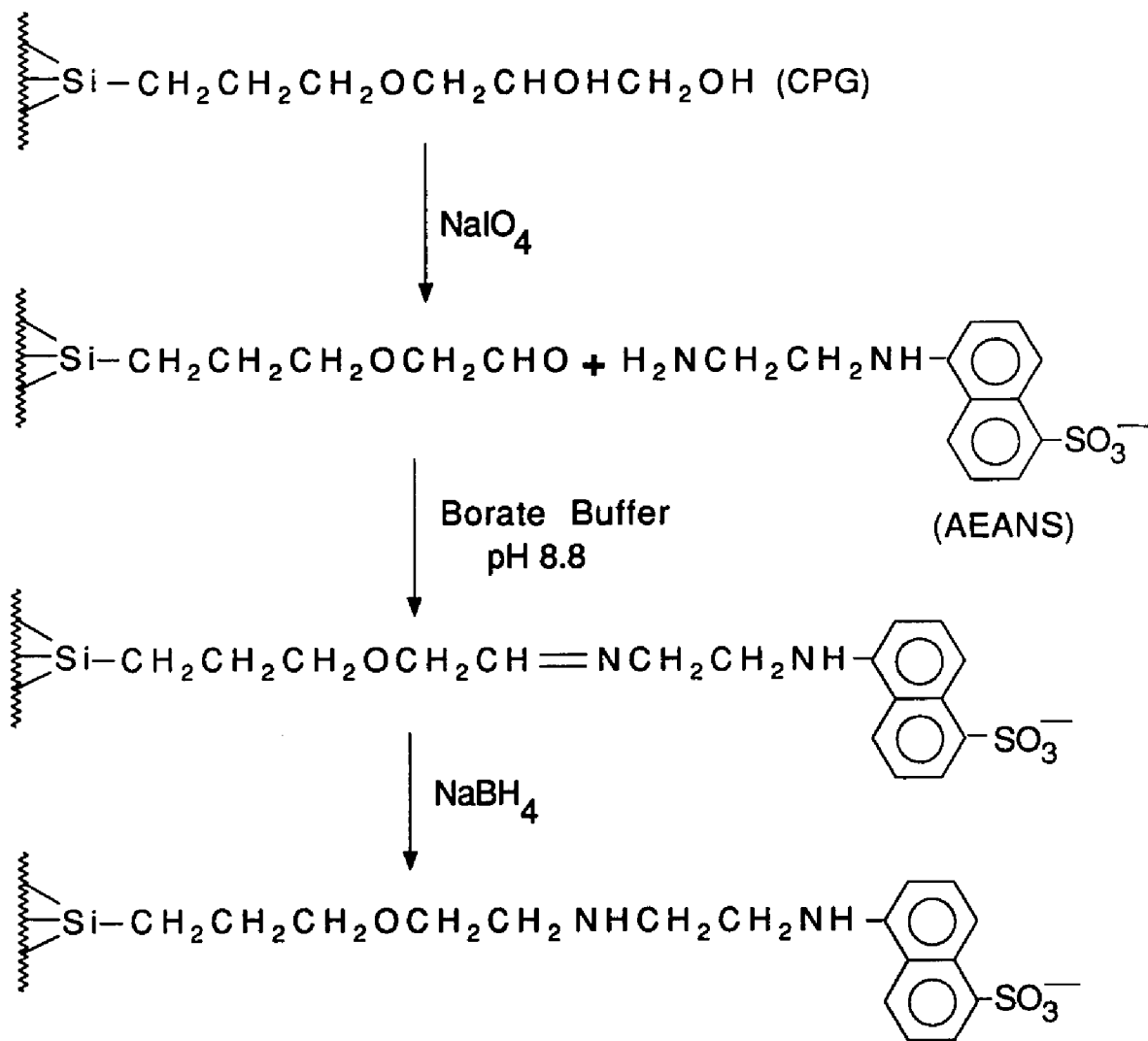
Figure 4.3 : Reaction scheme for AEANS immobilization on CPG.

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the cellulose was immersed in a solution of cyanuric chloride in acetone (0.5 g/20 mL) and 20 mL of water were added immediately and was left for one hour at room temperature. The cellulose was then washed with 100 mL each of water and acetone and soaked in a solution of AEANS (10mg/20mL) over night. The product was washed first with acetone and then several times with water until no fluorescence was visible when the washings were exposed to UV light. Immobilization of AEANS on CPG using cyanuric chloride as a coupling agent was attempted using the same conditions.

AEANS Immobilization on Poly(vinyl alcohol) : Ten mg of AEANS were dissolved in basic solution (0.5 g each of sodium hydroxide and sodium carbonate dissolved in 20 mL distilled water), then 20 ml of 25 g/L of cyanuric chloride in acetone was added. The reactivity of cyanuric chloride subsitution reaction was controlled by varying the temperature as shown in figure 4.4. The mixture was stirred and allowed to react for one hour at a temperature between 0-5 C^O, and for another one hour at a temperature between 35-45 C^O. Then, 2.0 g of PVOH was added and the temperature was raised to between 55-65 C^O. This mixture was stirred for about 6 hr. The product was filtered and washed, first with acetone and then with water, until no futher unreacted indicator could be observed in the washings. The resulting material was dissolved in 20 mL of distilled water at temperatures above 85 C^O.

An AEANS-PVOH gel membrane was formed by combining 0.3 mL of AEANS-PVOH solution with 0.01 mL of glutaraldehyde solution

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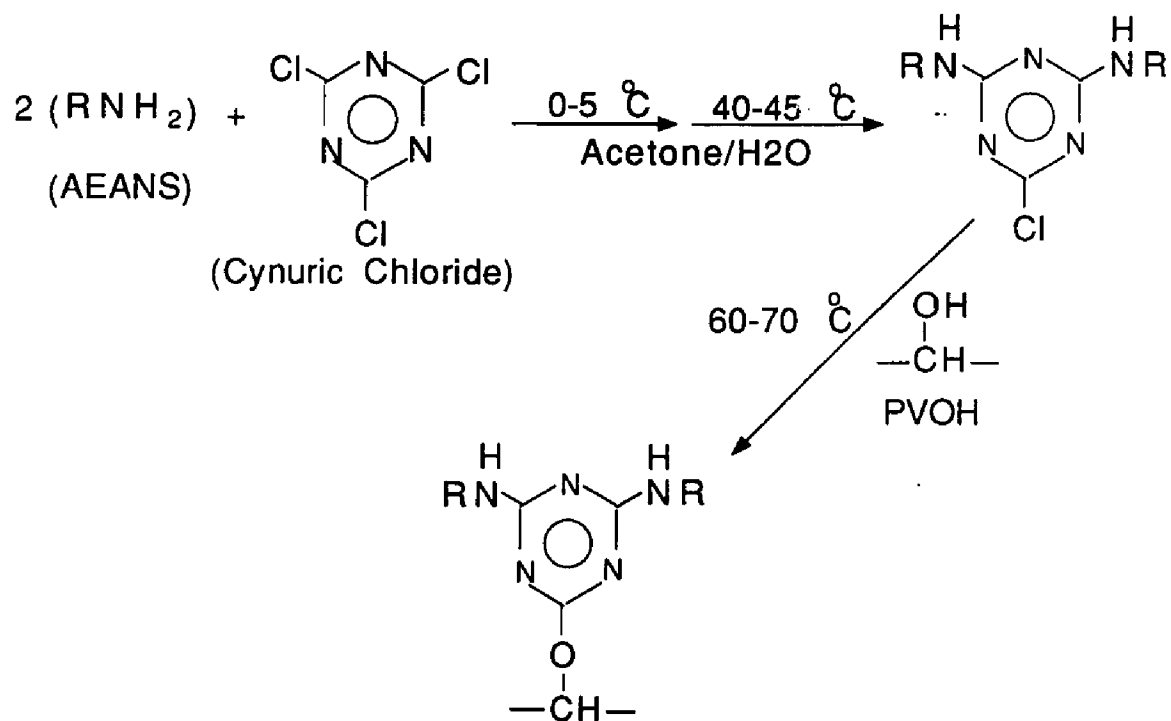
Figure 4.4 : Reaction scheme for AEANS immobilization using cyanuric chloride as a coupling reagent.

(5% W/W) and 0.01 mL of 3 M HCl. The crosslinking reaction is shown in figure 4.5. Immediately a known volume of the mixture was transferred onto the end of a bifurcated fiber optic bundle using a micropipet and left for a couple of minutes to solidify as described in chapter 2. The crosslinked gel was then inserted into distilled water to wash out excess acid and crosslinking reagent.

AEANS/CPG Response to Cationic Surfactant : Surfactant solutions of varying concentrations were prepared by dilution from concentrated standard solutions. AEANS-CPG packed in a capillary tube was immersed in a small bottle containing surfactant solution. Then it was left overnight before the spectrum was measured so that the ion pair interaction between the surfactant and the sensor surface would reach equilibrium. Emission spectra were obtained at different periods of time to confirm that the interaction had reached equilibrium. The spectra were measured as described in chapter 2.

Reversibility was studied by replacing the surfactant solution with distilled water after the response to surfactant had reached equilibrium. Emission spectra were then measured as a function of time.

Emission spectra were measured for 0.10 mM AEANS dissolved in cationic surfactant solutions of various concentrations for comparison with the immobilized fluorophore.

The effect of pH on response to cationic surfactants was studied using carbonate buffers of varying pH values (8.8-10.2). Cationic surfactant solutions (0.10 M) were prepared in carbonate
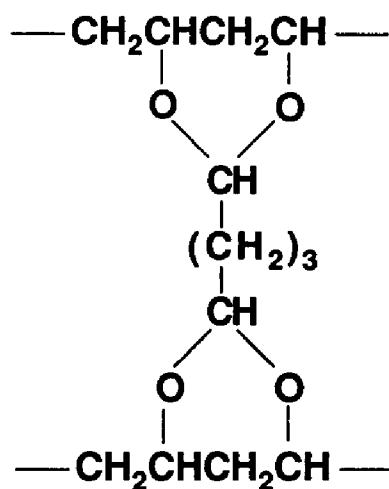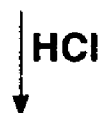
Figure 4.5 :
Reaction scheme for PVOH/Glutaraldehyde crosslinking.

buffer. Lower surfactant concentrations were prepared by dilution with the same buffer.

AEANS/CPG Response to pH : The response of the AEANS-CPG to pH was studied using citrate, phosphate and carbonate buffers to cover the pH ranges, 3.0-6.0, 5.5-8 and 8.8-12 respectively. AEANS-CPG was packed into a capillary tube and immersed in a buffer solution having a specific pH value.

The response of dissolved AEANS to pH was tested for comparison to the immobilized fluorophore. Solutions of AEANS in buffers at different pH values were prepared by mixing 1.0 mL of 1.0 mM AEANS fluorophore in distilled water with 9.0 mL of borate buffer. The pH was adjusted with NaOH using a pH meter.

The emission spectra of the solutions were obtained with the SLM 8000 spectrofluorometer. The excitation wavelength was set at 366 nm.

AEANS/CPG Response to Noncationic Surfactants : The response of immobilized AEANS to non-cationic surfactants was studied using an anionic surfactant, dodecyl sulfonate at concentrations up to 0.10 M, and nonionic surfactant, polyoxyethelene-23-lauryl ether, at concentrations up to 0.050 M.

The emission spectra of immobilized AEANS in these non-cationic surfactants were obtained using the same procedure used for testing response to cationic surfactants.

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CPG Substrate Effects on Response : These studies were made using two different batches of glycophase-controlled pore glass as a solid substrate. Both batches were purchased from the same source (Pierce Chem. Co.). The first one was Glycophase-G/CPG-550, Batch number 1028719, with 550 Angstroms pore diameter and 5-10 microns particle size. The other was Glycophase-G/CPG-460, batch number 87050508, with 460 Angstroms pore diameter and 37-74 microns particle size. The same procedure was used to immobilize AEANS fluorophore on both batches. The response to surfactant was tested for both batches under the same conditions.

AEANS-Cy/Cellulose Response to pH and Surfactant : The response of AEANS-Cy/Cellulose to pH was tested using borate and phosphate buffers to cover the pH ranges, 5.65-8.70 and 7.07-11.60, respectively. The AEANS-Cy/Cellulose was packed into glass melting point capillary tubes and placed into the buffer.

The response of AEANS-Cy/Cellulose as a function of cationic surfactant concentration was studied. AEANS-Cy/Cellulose was packed into capillary tubes and immersed in small bottles containing TDTMA solutions of varying concentrations (0.010-100 mM). They were left over night before the spectrum was measured so that the ion pair interaction between the surfactant and the sensor surface would reach equilibrium. The excitation wavelength was set at 360 nm.

AEANS-Cy/Poly(vinyl alcohol) Response : AEANS-PVOH response to cationic surfactant was intially investigated by placing pieces of gel in water and 0.10 M of aqueous DDTMAB. The color of fluorescence was observed visually, while exciting with a UV hand lamp.

Spectral shifts as a function of surfactant concentration were determined by immersing small pieces of AEANS-PVOH gel in separate bottles, each containing a different concentration of surfactant. The bottles were sealed and left for about one hour to allow the response to reach a steady state. Emission spectra were then obtained by placing the membrane and surfactant solution in a cuvet. The SLM 8000 spectrofluorometer was used to obtain the emission spectra. The excitation wavelength was set at 370 nm. In order to obtain the spectral shift as a function of surfactant concentration. The peak heights of the emission spectra were kept constant by adjusting the photomultiplier voltage.

AEANS-PVOH was applied to the fiber optic system as described in Chapter 2. After the gel formed on the tip of the optical fiber, it was immersed in distilled water and emission spectra were taken repeatedly until there was no further increase in fluorescence intensity. Then the water was replaced with surfactant solution and emission spectra were taken at different periods of times until no further change in fluorescence intensity was observed. The ser or response to surfactant concentration was done in sequence starting with low concentration and ending with high concentration.

Reversibility of sensor response was tested by replacing the

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surfactant solution with distilled water and measuring the emission spectra at different periods of time until there was no further decrease in fluorescence intensity. The immobilized AEANS-PVOH membrane on the tip of the bifurcated fiber optic bundle was always stored in distilled water.

The membrane stability on the tip of the fiber optic was tested by using the sensor at different periods of time.

The effect of pH on AEANS/PVOH response was studied by immersing pieces of gel in buffers of various pH and visually observing the color of fluorescence excited with a UV hand lamp.

AEANS/PVOH response to CTMA based on intensity ratios at two wavelengths was accomplished by measuring the ratio of the peak heights at wavelengths of 450 and 520 nm as a function of CTMA concentration.

RESULTS AND DISCUSSION:

AEANS/CPG Characteristics :

Table 4.1 presents the wavelengths of maximum emission for AEANS dissolved in water and immobilized on various substrates. The shortest wavelength is observed for glyceryl-CPG indicating that the glyceryl surface is providing a non-polar environment for AEANS. The wavelengths of maximum emission for AEANS on cellulose and PVOH are shorter than for AEANS on glyceryl-CPG indicating that cellulose and PVOH provide an environment of intermediate polarity.

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Table 4.1 : Values for wavelength of maximum emission
of AEANS in water, cellulose, PVOH and CPG.

emission wavelength

1) In water   494 nm

2) On cellulose   489 nm

3) In PVOH   488 nm

4) On CPG   482 nm

<u>General Observations</u> : Initially, AEANS was immobilized on controlled pore glass (CPG), a substrate with good mechanical stability under a wide variety of operating conditions (77,78). The immobilized fluorophor responds to cationic surfactants as result of ion pair interaction between the cationic end of the surfactant and the anionic sulfonate group of the immobilized fluorophore. Other studies have shown that the ion-association complexes between ionic surfactants and oppositely charged indicators are electrically neutral, and often poorly soluble in water but readily extractable by low polarity solvents (79-81). Thus, we chose to immobilize AEANS on glyceryl-controlled pore glass which is coated with a organic layer. Extraction of the ion-pair complex by the organic layer promotes ion pair formation and increases the change in the polarity of the indicator environment as a function of added surfactant.

Initial studies showed a significant change in the color of immobilized AEANS fluorescence when it was exposed to cationic surfactant instead of water. When immobilized AEANS immersed in water was exposed to a UV lamp, green fluorescence was observed. When the water was replaced with a solution with a high concentration of cationic surfactant, the fluorescent color changed to blue and the fluorescence intensity increased.

<u>Response to Cationic Surfactants</u> : The emission spectra of both dissolved and immobilized AEANS shift to shorter wavelength with increasing cationic surfactant concentration as shown in figure 4.6. This shift indicates that interaction with the non-polar hydrocarbon

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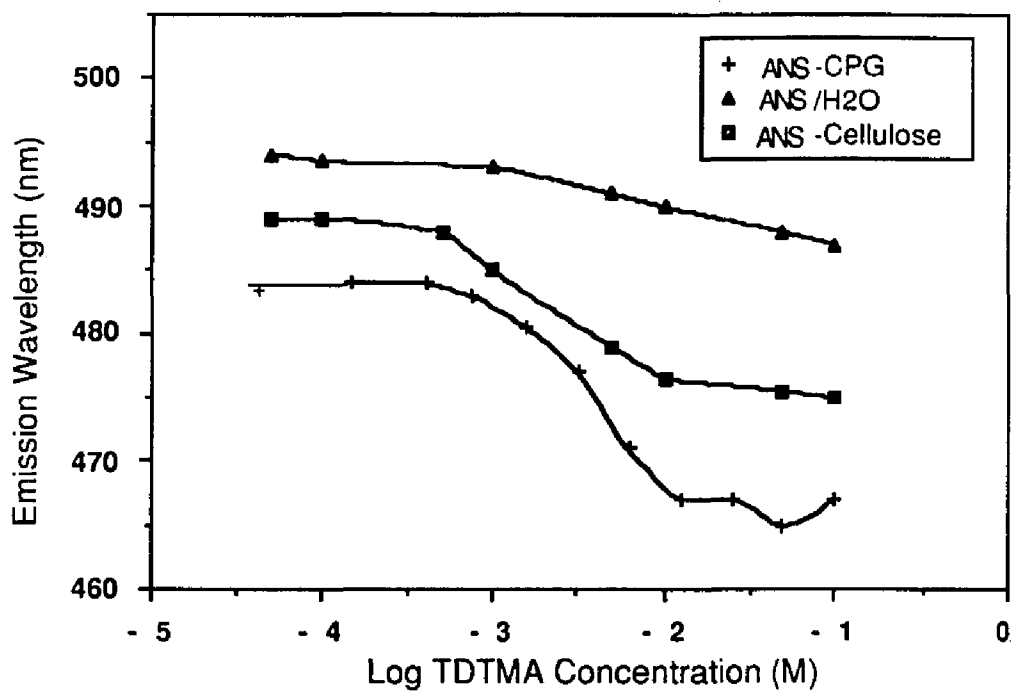
Figure 4.6 : Wavelength of maximum emission as a function of
TDTMA concentration for AEANS in solution and
immobilized on CPG and cellulose.

chains of the surfactant causes a decrease in the polarity of the indicator environment. At concentrations below 0.20 mM the emission spectrum does not change indicating that there is no significant interaction between the surfactant and indicator. At high concentrations the emission spectrum of the immobilized indicator on controlled pore glass levels off and then shifts to slightly longer wavelength. This may be related to the micelle formation in the surfactant solution. At high concentration, just before micelles start to form, surfactant almost covers the substrate surface as a result of the combination of the electrostatic and hydrophobic interaction between the surfactant and the anionic indicator. But at the point where the micelles start to form, some of the surfactant may be extracted from the surface. Therefore, the environment around the indicator becomes less hydrophobic and the emission spectrum shifts to longer wavelength. This indicates that the interaction between the surfactant and the sensor surface is reversible.

In addition, the graph shows that immobilized indicator on controlled pore glass is more sensitive than the dissolved indicator to surfactant. The total shift in the emission spectrum of the dissolved indicator is about 8 nm as the TDTMA concentration changes from 0.10 to 100 mM, while for the immobilized indicator the spectral shift is about 20 nm as the concentration changes from 0.10 to10 mM. This suggests that hydrophobic interaction between the ion pair complex of surfactant and indicator and the organic layer on the CPG surface is enhancing the hydrophobic environment

around the indicator.

AEANS/CPG response takes about 14 hours, before it reaches a steady state. Response is reversible, but it is very slow, taking several days to get back to its initial value.

Effect of Surfactant on Fluorescence Intensity : Surfactant interaction with the fluorophor enhances the fluorescence quantum yield. Figure 4.7 shows how fluorescence intensity of dissolved AEANS increases as a function of surfactant concentration. AEANS fluorescence is more sensitive to the longer hydrocarbon chain surfactant. The change in fluorescence intensity starts to become significant at a certain concentration for each surfactant, 0.30 mM for CTMA, 1.0 mM for TDTMA and 10.0 mM for DDTMA. The upper response limit is established by surfactant solubility which decreases as the hydrocarbon chain length increases.

Figure 4.8 shows that fluorescence intensity of immobilized AEANS on controlled pore glass increases with surfactant concentration. Surfactant concentrations as low as 0.010 mM DDTMA yielded detectable changes in intensity. Immobilized AEANS is more sensitive to surfactant than the free fluorophor. At concentrations above 0.030 M the fluorescence intensity decreases significantly. This may be due to the high concentration of bromide ion acting as a quencher. There was no observable leaching of the immobilized fluorophor from the sensor surface even at high surfactant concentration. Also, no spectral broading was observed, indicating that the indicator is still in a uniform environment polarity.
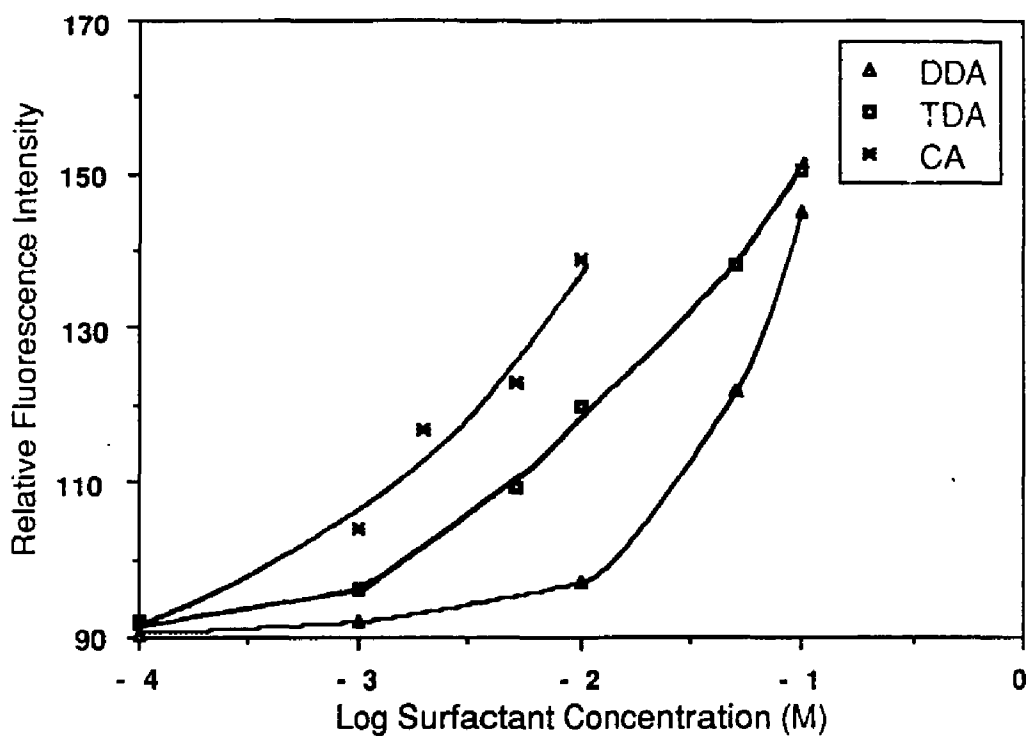
Figure 4.7 : Change in fluorescence intensity for AEANS in solution as a function of the concentration of DDTMA, TDTMA and CTMA.
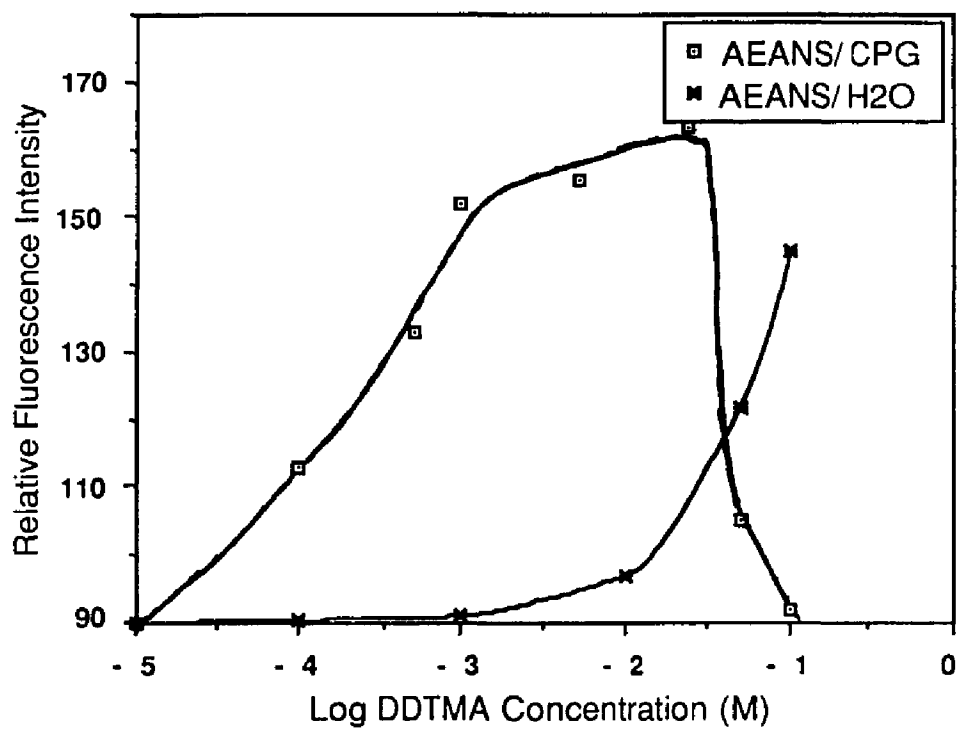
Figure 4.8 : Change in fluorescence intensity as a function of
DDTMA concentration for AEANS in solution and
immobilized on CPG.

The increase in intensity from a fluorescent dansyl derivative as a function of added surfactant has been observed previously, when Nafion solution and sodium poly(styrene sulfonate) were added to a solution of 1-dimethylammoniumaminonaphthalene-5-sulfonamidoethyltrimethylammonium perchlorate (68). Davies has reported similar results, when cetyltrimethylammonium bromide was added to the anionic indicator, 1-dimethylaminonaphthalene-5-sulfonyl glycine, (67). Both studies show that emission shifts to shorter wavelength as surfactants are added to the indicator solution.

The Effect of Hydrocarbon Chain Length on the Response : Figures 4.9 and 4.10 show the effect of hydrocarbon chain length on response. As the hydrocarbon chain length increases, the degree of hydrophobic interaction between the surfactant and the sensor surface increases. Therefore, the surfactant with longer hydrocarbon tails cause a larger change in the polarity of the immobilized indicator and a larger spectral shift.

Figures 4.9 and 4.10 show that the sensitivity increases with the hydrocarbon chain length of the surfactant. The detection limits for cetyl-, tetradecyl- and dodecyl- trimethylammonium ions dissolved in water are 0.39, 0.79 and1.30 mM, respectively. When the same surfactants are dissolved in carbonate buffer at pH 9.5, the detection limits are 0.13, 0.40, 1.0 mM, respectively.

The magnitude of the total spectral shift as a function of surfactant concentration also increases with hydrocarbon chain
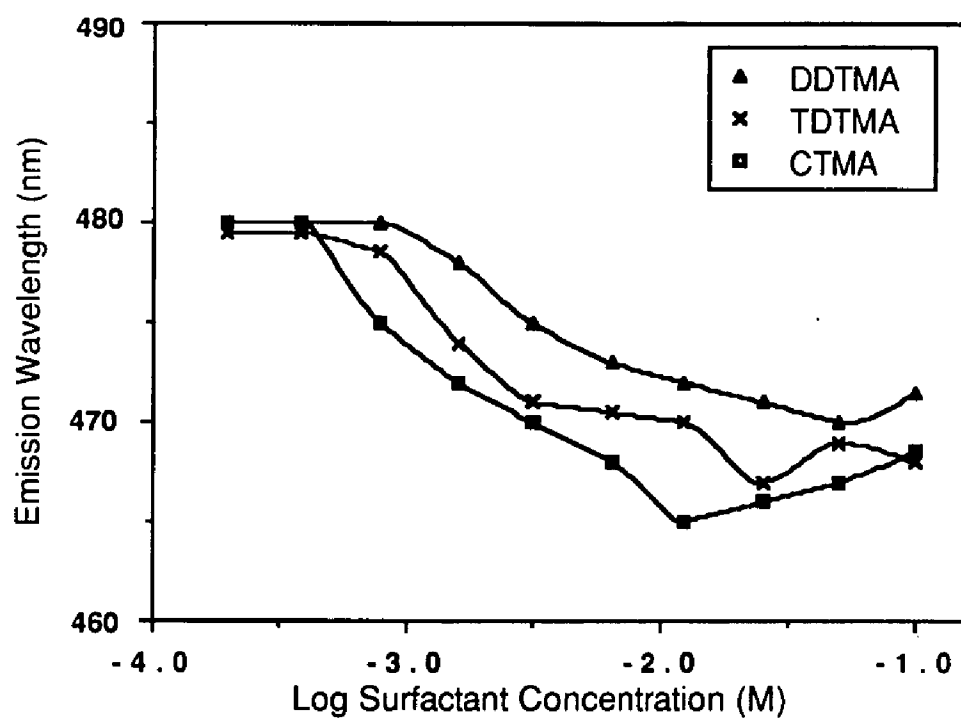
Figure 4.9 : Wavelength of maximum emission of AEANS on CPG
as a function of the concentration of DDTMA, TDTMA
and CTMA in water.
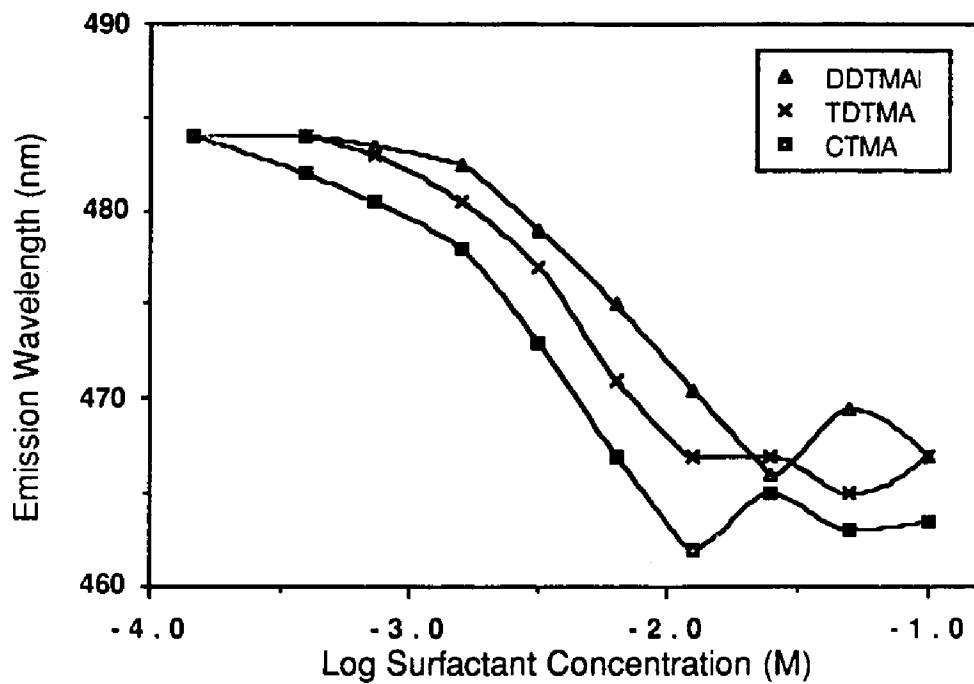
Figure 4.10 : Wavelength of maximum emission of AEANS on CPG
as a function of the concentration of DDTMA, TDTMA
and CTMA at pH 9.5.

length. Figure 4.9 shows that the total spectral shifts for added cetyl-, tetradecyl- and dodecyltrimethylammonium ions are 16, 13 and 10 nm, respectively. The medium also affects the total wavelength shift. Figure 4.10 shows that the total spectral shifts for cetyl-, tetradecyl- and dodecyl- trimethylammonium ions dissolved in pH 9.5, 0.20 M carbonate buffer are 24, 20 and 18 nm, respectively.

The hydrocarbon chain length also determines the upper concentration limit. From the graphs, the highest measurable concentrations of cetyl-, tetradecyl- and dodecyl trimethyl ammonium ions dissolved in 0.20 M carbonate buffer at pH 9.5 are 0.013, .020 and 0.025 M respectively. In pure water the highest measurable concentrations are 0.013, 0.031 and 0.063 M, respectively.

Effect of pH : Figure 4.11 shows that the emission maxima depend on pH. At low surfactant concentration, the emission wavelength maximum is longer at pH 9.5 (484 nm) than in water (480 nm). In addition; the range of linear response to surfactant is larger at high pH than in water. The results show that the AEANS-CPG sensor can respond linearly to DDTMA concentration from 1.0 up to 2.5 mM at pH 9.5, and from 1.0 up to 4.0 mM in water.

Further studies indicate that AEANS-CPG responds to pH. Figure 4.12 shows how the emission maxima shift with pH. As the pH increases the emission spectrum shifts to longer wavelength indicating that the indicator environment is becoming more polar. As

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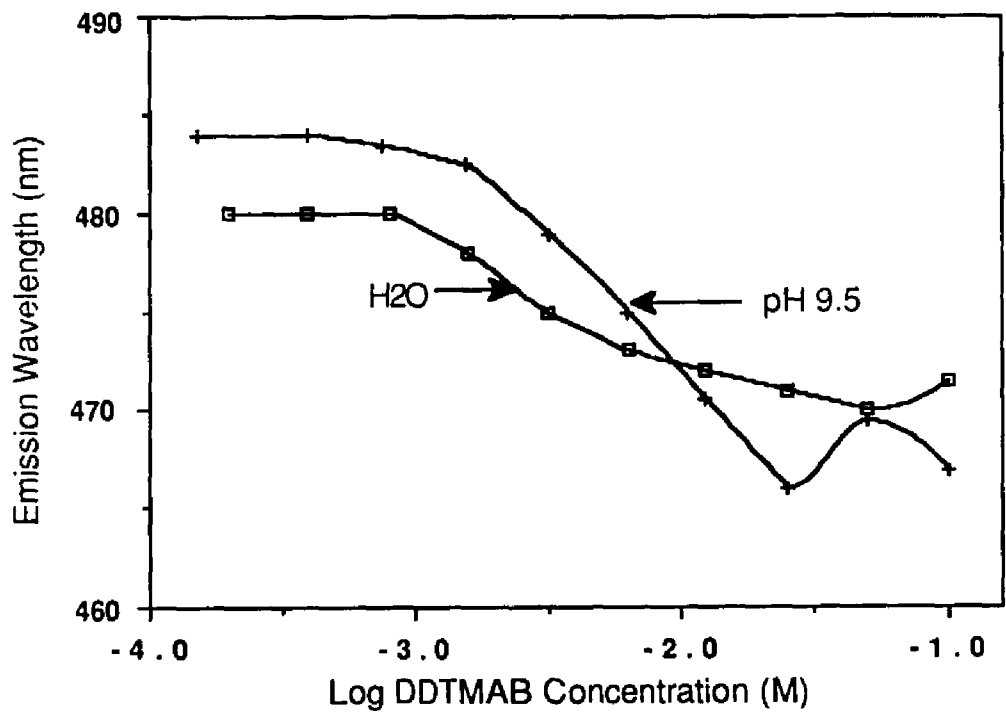
Figure 4.11 : Wavelength of maximum emission of AEANS on CPG
as a function of DDTMA concentration in water and pH 9.5.
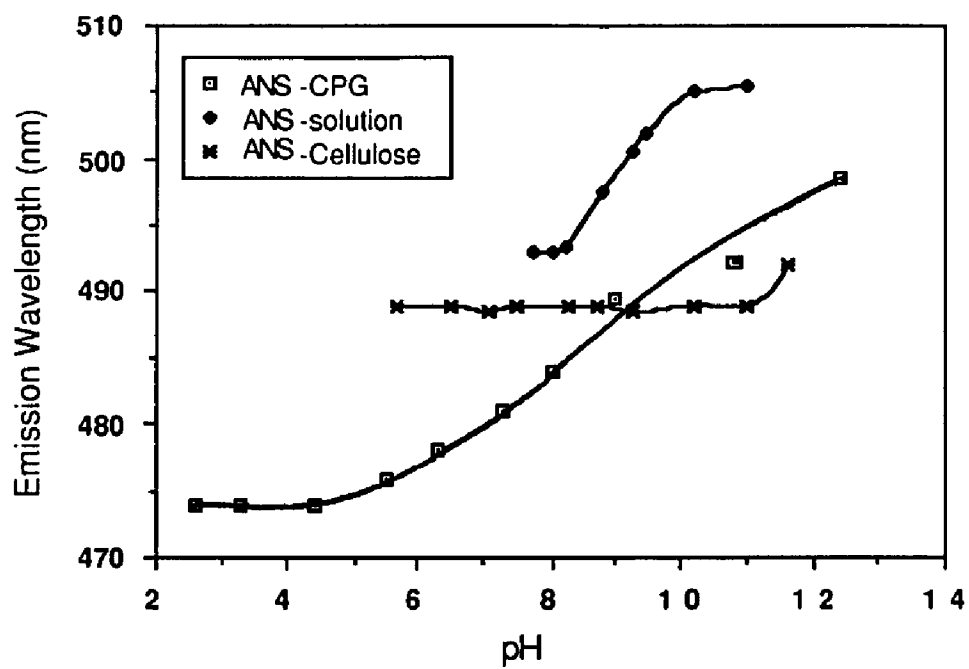
Figure 4.12 : Wavelength of maximum emission as a function
of pH for AEANS in solution, CPG and cellulose.

the pH increases from 5.5 to around 12 the emission spectrum shifts from 474 up to 498 nm, and the fluorescence changes from blue to green. Other experiments show that at high pH AEANS remains immobilized on the surface.

Figure 4.12 shows that the emission spectrum of AEANS dissolved in water also shifts to longer wavelength as pH increases. In contrast to the immobilized indicator the emission spectrum of the free indicator shifts from 493 to 504 nm as pH increases from 8 to 10.

Figure 4.13 illustrates our explanation for AEANS-CPG response to pH. In water the secondary alkyl amine group is protonated and therefore it can interact electrostatically with the sulfonate group of immobilized AEANS. This interaction causes the indicator to be extracted from the aqueous medium into the organic layer on the controlled-pore glass surface. The movement of the indicator from the highly polar solvent environment into the less polar organic layer causes the emission spectrum to shift to shorter wavelength. But as the pH increases the amine deprotonates; therefore, it can not interact electrostatically with the sulfonate group of the immobilized indicator. The free sulfonate ion group increases the aqueous solubility of the immobilized indicator, while it is still covalently attached to the sensor surface. Therefore, the indicator is drawn into the more polar aqueous medium causing the emission spectrum to shift to longer wavelength.

The explanation of the pH dependence for the dissolved indicator is similar. At low pH where the amine group is protonated,

a) In water :
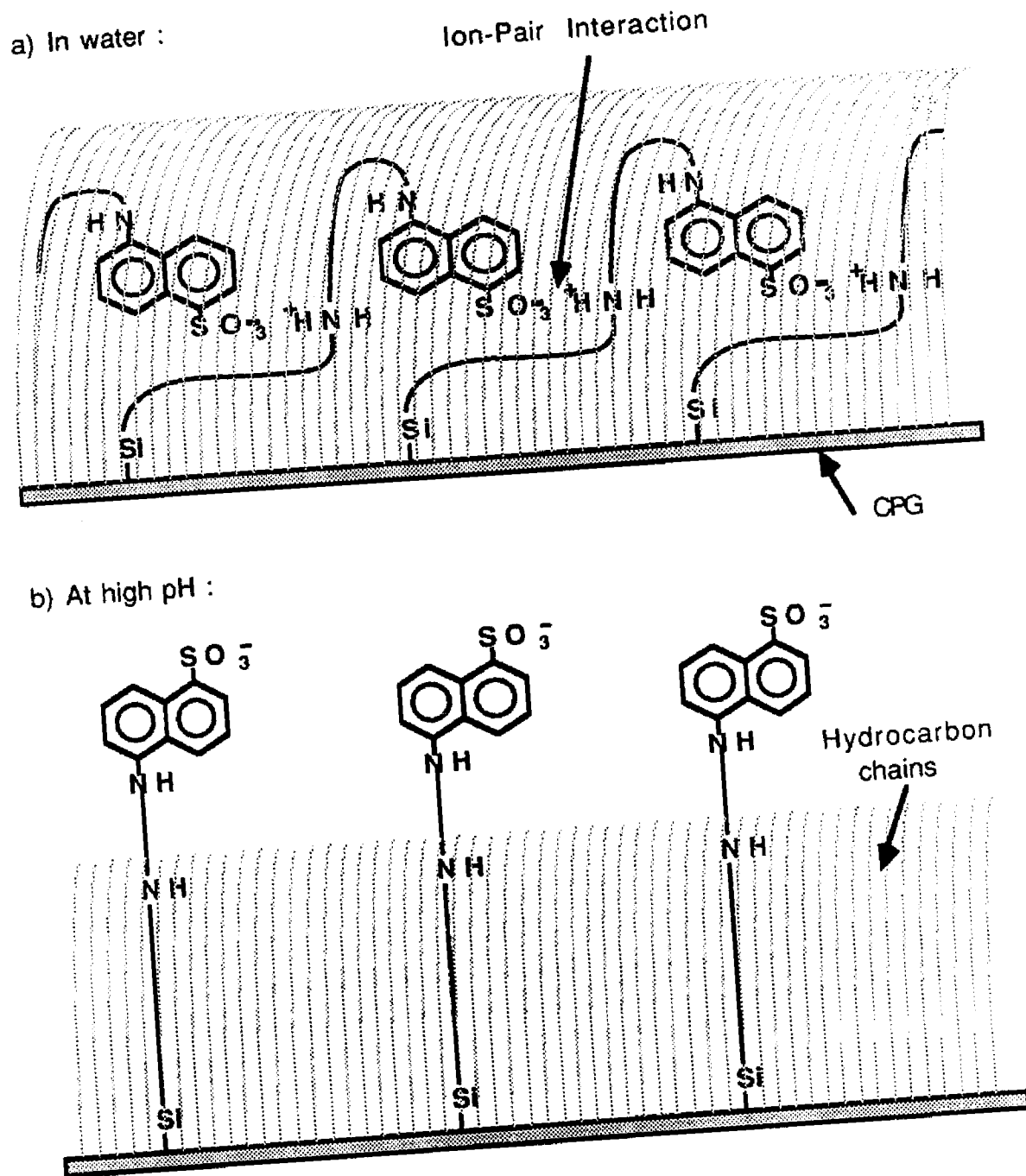
b) At high pH :

Figure 4.13 : Schematic representation of the behavior of AEANS/CPG surface in, a) water, b) at high pH.

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two indicator molecules can form an ion pair with each other. We attribute the shift in emission maximum to shorter wavelength at these pH to the hydrophobic interactions between the two indicator molecules. At high pH values, the amine deprotonates reducing the tendency of two molecules to interact with each other.

The difference in AEANS-CPG response to surfactant dissolved in water and pH 9.5 buffer is illustrated in 4.14 and 4.15. The sensitivity to surfactants in pure water is low because in water the indicator is already extracted from the aqueous medium. To interact with the sulfonate group of the immobilized indicator, the surfactat has to displace the protonated secondary amine as illustrated in figure 4.14.

In contrast, at high pH, the free sulfonate group of the indicator is not associated with deprotonated amine and is more available to surfactant molecules dissolved in solution. Therefore the surfactant can form an ion pair with the sulfonate group which is extracted into the organic layer as illustrated in figure 4.15. AEANS-CPG is more sensitive at high pH because the effect of surfactant on the polarity of the indicator environment is not only dependent on the partition coefficient of surfactant between the two phases as in the case of water medium, but also on the ion pair interaction between the surfactant and sulfonate group of the indicator, which enhances the indicator extraction into the organic layer leading to a larger change in the polarity of indicator environment.
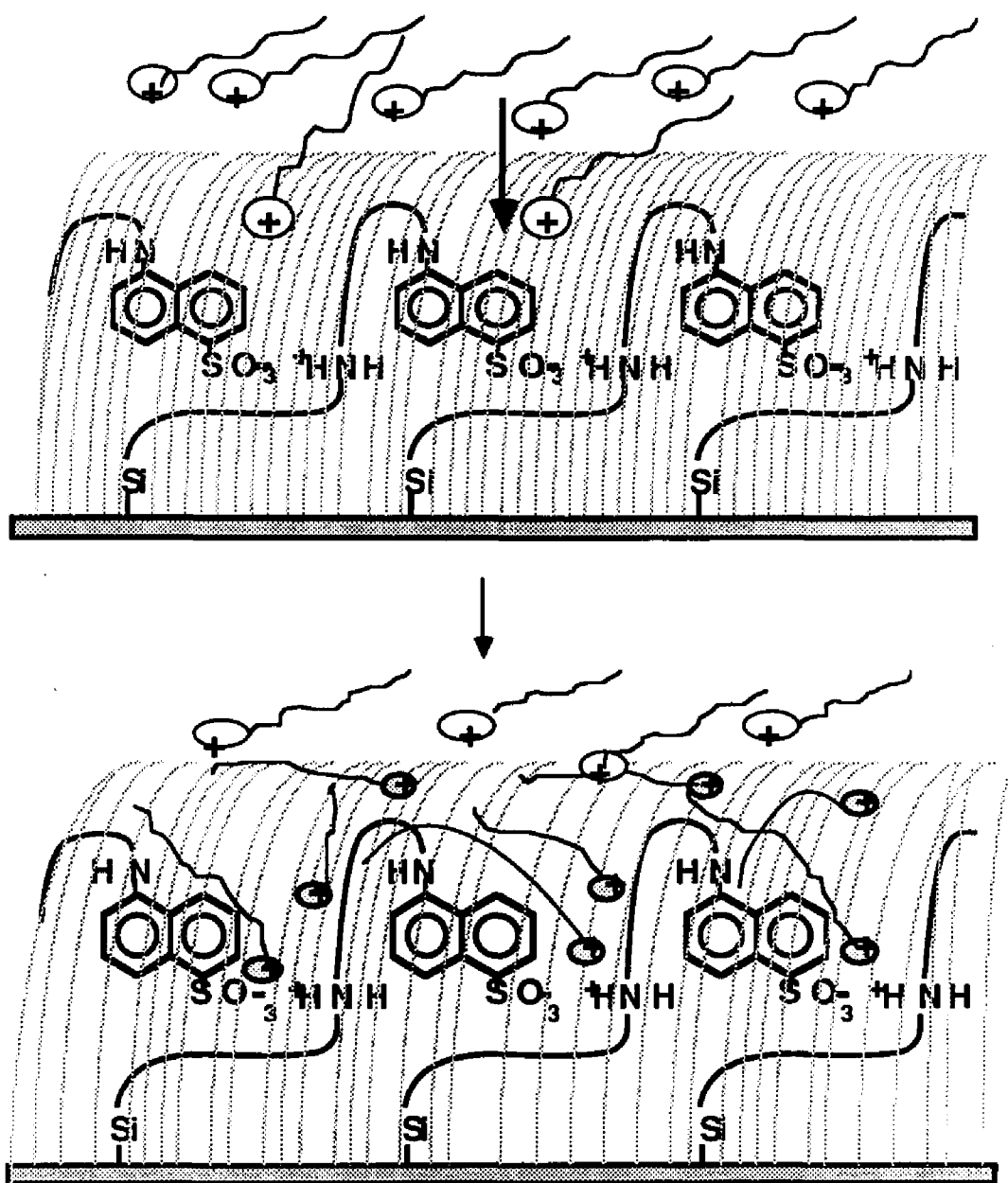
Figure 4.14 : Schematic representation of the interaction of cationic surfactant with AEANS/CPG surface, in water.
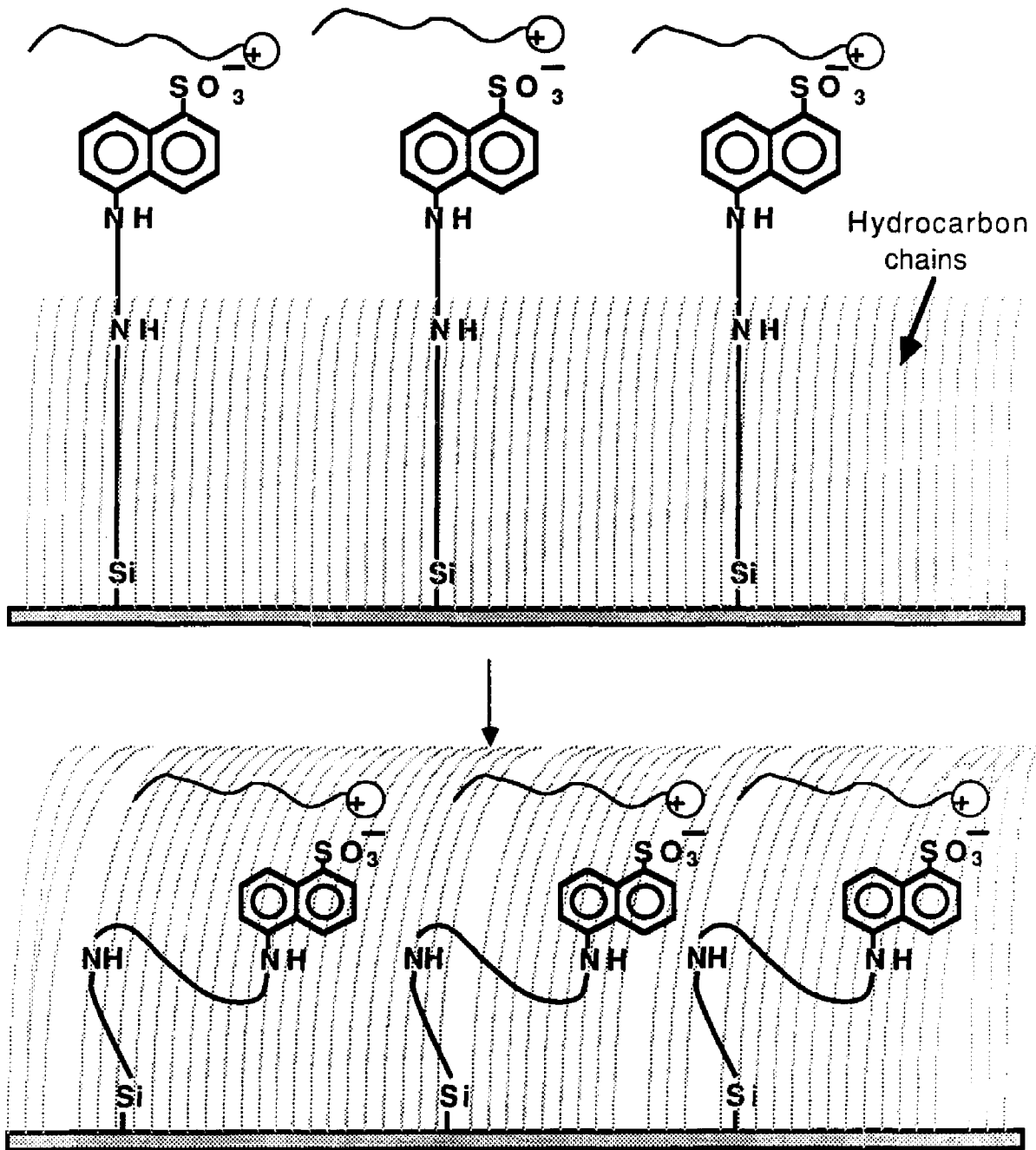
Figure 4.15 : Schematic representation of the interaction of cationic surfactant with AEANS/CPG surface, at high pH.

Response to Non-Cationic Surfactants : Figure 4.16 compares emission spectral shifts as a function of surfactant concentration for dodecyl sulfate (DDS), an anionic surfactant, and polyoxyethylene-23-lauryl ether (PELE), a nonionic surfactant, to that for dodecyltrimetylammonium bromide. Response to DDS and PELE concentration is not significant up to 0.025M, wheras DDTMAB shows a 10 nm spectral shift. Response to PELE becomes significant at concentrations above 0.025 M indicating that it modifies indicator polarity when present at high enough levels. Because the nonionic surfactant can not interact electrostatically with the anionic indicator, it only generates a response at high concentrations.

On the other hand, the emission spectrum shifts to slightly longer wavelength as a function of anionic surfactant concentration. The total shift is about 3 nanometer as shown in figure 4.16. The spectral shift to longer wavelength indicates an increase in the polarity of the immobilized indicator environment caused by the anionic surfactant. This result might be due to partial dissociation of ion pair interaction between the sulfonate ion group of the immobilized indicator and the protonated secondary alkyl amine group formed at low pH.

CPG Substrate Effect : It was found that the use of different batches of controlled pore glass substrate affects sensor response to surfactant as shown in figure 4.17. This might be related to the differences in the organic layer covering the solid support surface,
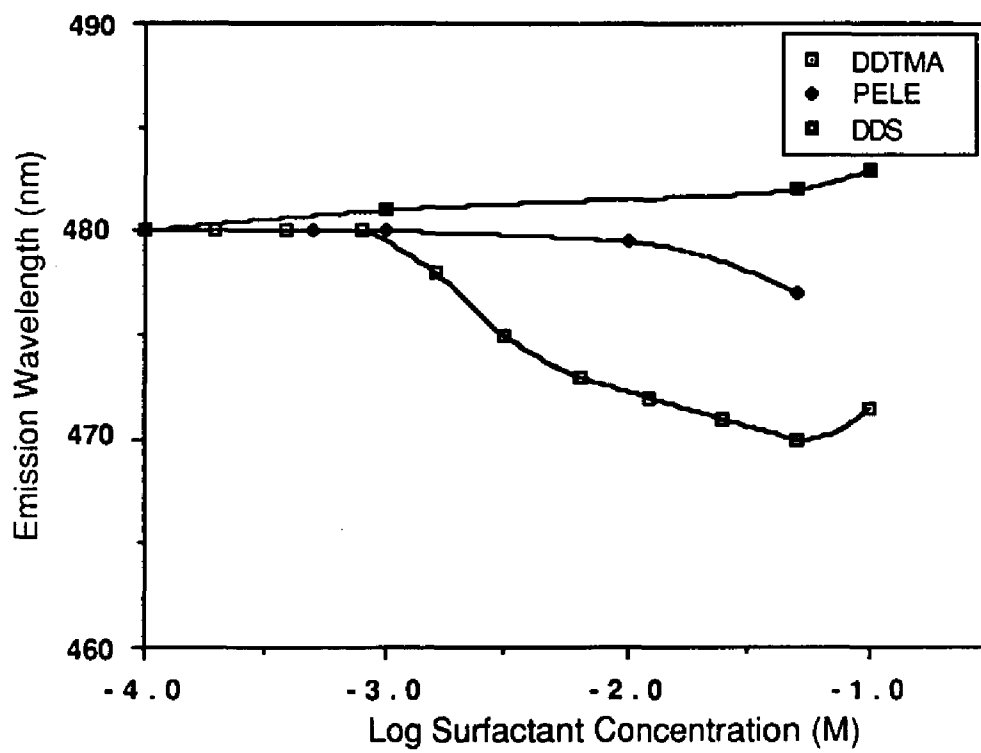
Figure 4.16 : Wavelength of maximum emission of AEANS on CPG
as a function of the concentration of DDTMA, PELE and DDS.
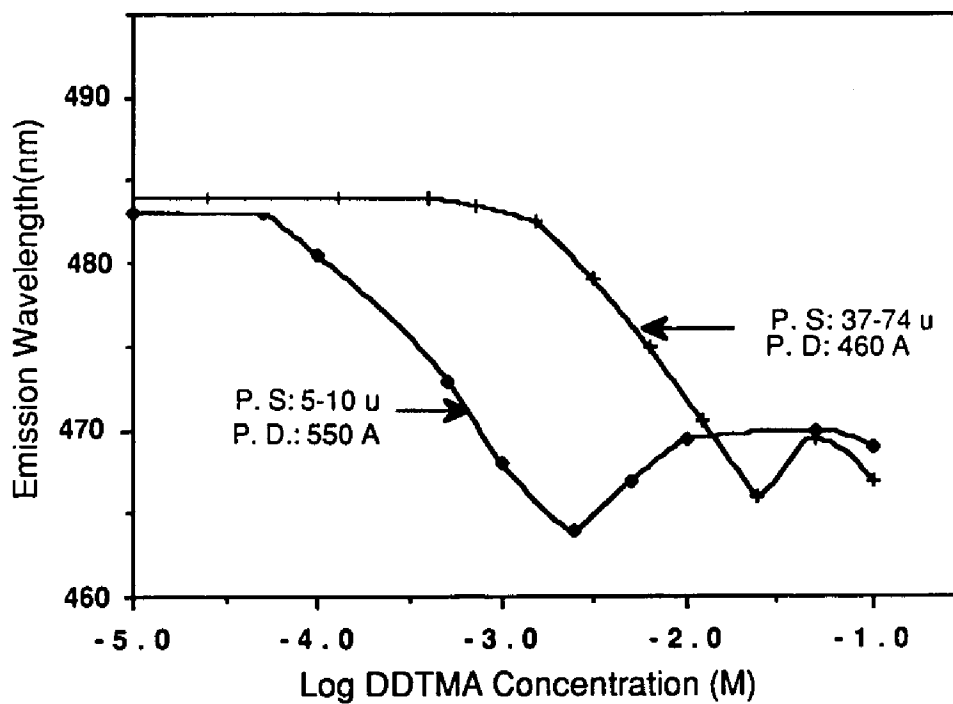
Figure 4.17 : Wavelength of maximum emission as a function
of DDTMA concentration for AEANS on two CPGs.
P.S : Particle size, P.D : Pore diameter.

since the response of AEANS-CPG depends not only on the combination of electrostatic and hydrophobic interactions between the surfactant and the immobilized indicator to form ion pair but also on the hydrophobic interaction between the ion pair and the organic layer on CPG. This latter interaction is highly dependent on the thickness of the organic layer on the CPG. Therefore, the more organic on the CPG, the greater the decrease in the polarity of the immobilized indicator environment as surfactant is added.

Figure 4.17 shows that AEANS immobilized on CPG with 5-10 micrometer particle size and 550 Angstrom pore diameter responds to surfactant from 0.10 up to 5.0 mM of DDTMA dissolved in pH 9 carbonate buffer. But when the same fluorophor is immobilized on CPG with 37-74 micrometer particle size and 460 Angstrom pore diameter using, the same immobilization procedure, the dynamic range shifts to higher concentrations.

<u>Characteristics of AEANS-Cy/Cellulose</u> :

<u>General Properties</u> : Because protonation of the aliphatic amine affects the response of AEANS/CPG, an alternative procedure that would not result in an aliphatic amine was sought. Cyanuric chloride was used as a coupling agent to immobilize AEANS on cellulose.

Cellulose has been used as a substrate to immobilize indicators. Saari and Seitz immobilized fluorescenamine on powdered cellulose using cyanuric chloride as a coupling agent (85). Suter et al. studied the luminescence of heterocyclic compounds

adsorbed on a cellulose filter paper substrate and found that the substrate provides a very polar environment with high hydrogen bonding activity for the adsorbed probe molecules (86,87).

Data in table 4.1 shows that the optical characteristics of immobilized AEANS depend on both the method of immobilization and the nature of the substrate. The emission spectrum of AEANS immobilized on cellulose is at longer wavelength (489 nm) than on CPG (484 nm), but lower than in water (493 nm). This indicates that the cellulose surface is more polar than the CPG surface which is covered with an organic layer, but is still less polar than water.

Immobilized AEANS is less stable on cellulose using cyanuric chloride as a coupling agent than on the CPG surface using the Schiff base reaction. AEANS immobilized on CPG using cyanuric chloride as a coupling agent is even less stable. The fluorescence intensity decreases slowly as a function of time. This indicates that indicator is slowly hydrolyzing from the surface.

Response to pH : Figure 4.12 shows that the emission spectrum for AEANS immobilized on cellulose does not shift with pH. The immobilization chemistry converts the aliphatic amine on AEANS to an aromatic amine. Aromatic amines are much weaker bases than aliphatic amines, having pKb values around 10. Therefore, AEANS immobilized on cellulose is not protonated at neutral pH and can not undergo ion pair interactions with other indicator molecules.

Immobilized AEANS is highly exposed to the aqueous medium at pH above 5.6 so that the emission wavelength is higher than for

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AEANS immobilized directly on CPG, at the same pH.

At a pH around 11.5 the emission spectrum shifts to slighty longer wavelength. This indicates that the immobilized indicator hydrolyzes at high pH. The hydrolyzed indicator leaves the surface and experiences a completely aqueous environment.

Response to Surfactant : AEANS-Cy/Cellulose response to surfactant is similar to AEANS in solution and immobilized on CPG. As the concentration of cationic surfactant increases, the emission spectrum shifts to shorter wavelengths indicating a decrease in the polarity of the indicator environment.

Figure 4.6 shows that at TDTMA concentrations below 1.0 mM the emission spectrum does not change indicating that there is no significant change in the polarity of the indicator environment. At concentrations above 1.0 mM, the emission spectrum shifts to shorter wavelengths as a function of concentration.

AEANS-Cy/Cellulose response to surfactant as a function of concentration is more similar to that of dissolved AEANS. The total spectral shift of the sensor as a function of surfactant depends on the nature of the substrate surface. Results show that the total spectral shift for AEANS-Cy/Cellulose as a function of TDTMA concentration is about 14 nm, compared to 19 nm for AEANS/CPG over the same concentration range. The additional spectral shift on CPG is due to the organic layer covering the substrate surface which adds an extra driving force to extract more of the indicator into the less polar hydrophobic environment. This is a result of the

hydrophobic interaction between the ion pair and the organic layer bonded on the surface.

AEANS-Poly(vinyl alcohol)/Fiber Optic Characteristics :

Experiments with AEANS-Cy/Cellulose were discontinued when other work in the laboratory showed that PVOH crosslinked with glutaraldehyde offered the following significant advantages as a substrate for indicator immobilization (22) :

1) The rate of the crosslinking can be controlled by varying the amount of acid catalyst. The acid level can be adjusted so that there is time to transfer an accurately known volume of glutaraldeyde/PVOH to the common end of a bifurcated fiber optic bundle before it starts to solidify into a clear gel.

2) It is clear and optically transparent through the visible and well down into the ultraviolet.

3) PVOH can be covalently bonded directly to glass or fused silica fiber.

4) Indicator can be bound to PVOH prior to crosslinking. This means that a single large preparation of indicator can be used to reproducibly prepare a large number of sensors. Furthermore, because the indicator preparation can be diluted with underivatized PVOH, the amount of indicator in a sensor can be controlled. Another possibility is to combine two different indicators in a known ratio by mixing them prior to gel formation.

General Properties : Gel formed from PVOH with a molecular weight 14,000 was found to be permeable to cationic surfactants with a long hydrocarbon chain. It is also stable on the tip of the fiber optic bundle, since it was used a month after it was formed without any change in characteristics. To obtain this stability, it is necessary to store the sensor in water or a surfactant solution.

No indicator leaching was observed in the presence or absence of surfactant. When the surfactant solution was replaced with distilled water, the emission spectrum returned to the original wavelength observed when the sensor was intially exposed to water indicating that the response is reversible.

The increase in the fluorescence intensity of AEANS-PVOH when it was immersed in water just after it was formed is shown in figure 4.18. This is due to the decrease in HCl concentration in the gel. Fluorescence develops when the pH is high enough so that the fluorophore is no longer protonated. The time needed to wash out excess acid from a 0.1 mm thick immobilized membrane is about 5 minutes.

Figure 4.19 shows the forward and reverse rates of response of AEANS/PVOH. When AEANS/PVOH is exposed to surfactant, it takes 15 minutes to reach equilibrium. When AEANS/PVOH is then reexposed to water it takes about 10 minutes for the intensity to return to its original value.

Sensor Response to Surfactant: The color of the immobilized indicator fluorescence changes from green to blue when

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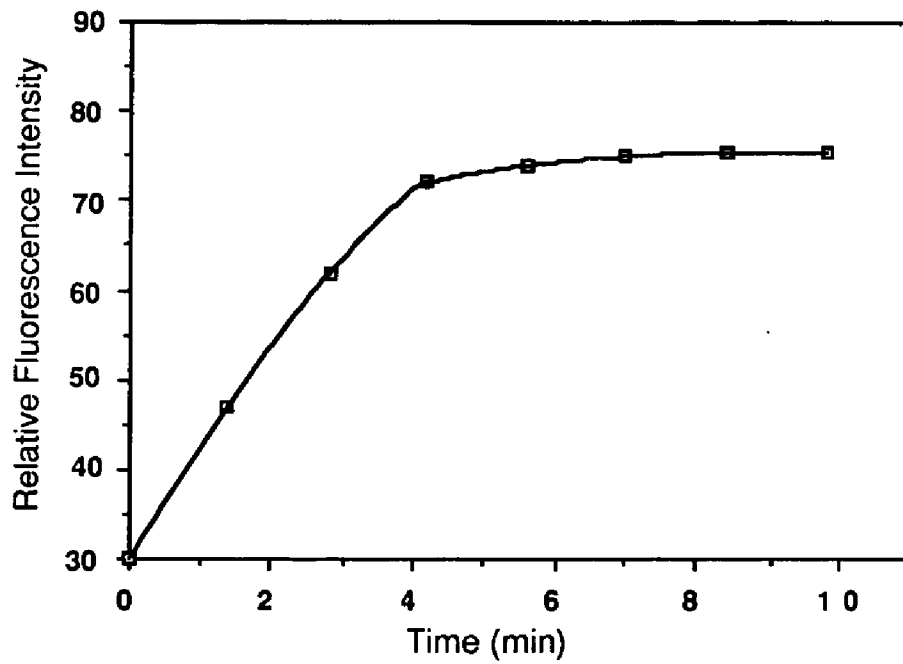
Figure 4.18 : Change in fluorescence intensity of AEANS/PVOH
with time after membrane was formed and
immersed in distilled water.
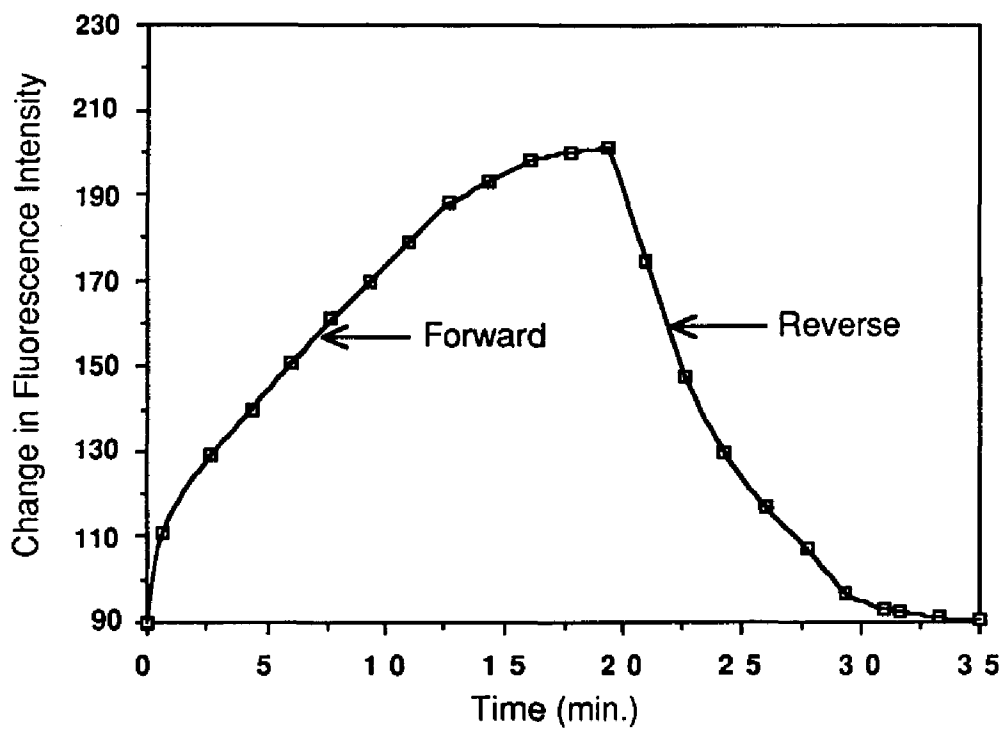
Figure 4.19 : Change in fluorescence intensity of AEANS/PVOH
with time when exposed to 0.10 M DDTMA (Forward)
and then exposed to water (Reverse).

AEANS/PVOH is removed from water and immersed in a surfactant solution. The color of fluorescence returns to green when the surfactant solution is replaced with distilled water. The forward and reverse responses are reasonably fast, occurring within a couple of minutes after the solutions were changed.

Figure 4.20 shows that added surfactants cause an increase in emisson intensity. This means that ion pair formation is taking place as expected. The longer the hydrocarbon tail, the lower the concentration range over which response is observed. This because the longer the hydrocarbon tail, the higher the degree of hydrophobic interaction and the stronger the driving force for ion pair formation. Also, the gel gets saturated at a lower concentration level when the hydrocarbon tail is longer. Saturation is the point where all of the AEANS molecules are associated with surfactant. The immobilized indicator responds to lower concentrations than the dissolved indicator for a given surfactant. This means that on PVOH, the hydrophobic interaction between the ion pair and the PVOH gel provides an additional driving force for ion pair formation.

Figure 4.21 shows that the emission spectra of both dissolved and immobilized AEANS shift to shorter wavelength with increasing concentrations of cationic surfactant. This indicates that the polarity of the indicator environment decreases due to interaction with surfactant. The sensitivity of the immobilized indicator to cationic surfactant is higher than when dissolved in solution. It is noted that the range over which the emission wavelength changes coincides with the range over which intensity changes, as would be
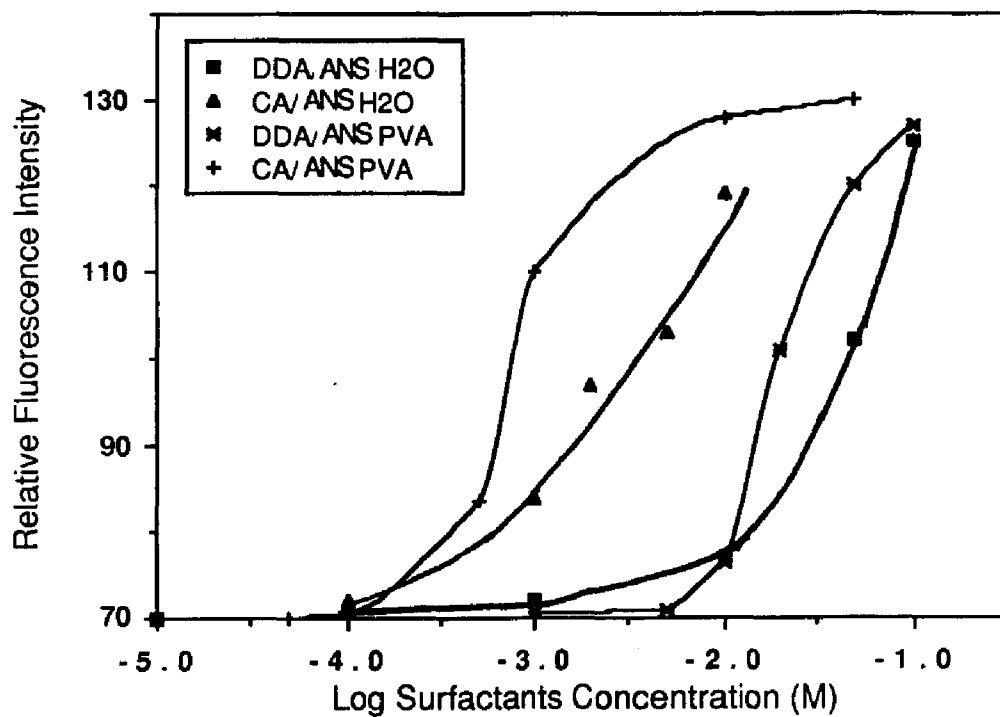
Figure 4.20 : Change in fluorescence intensity of AEANS in solution and
immobilized on PVOH as a function of the concentration
of DDTMA and CTMA.
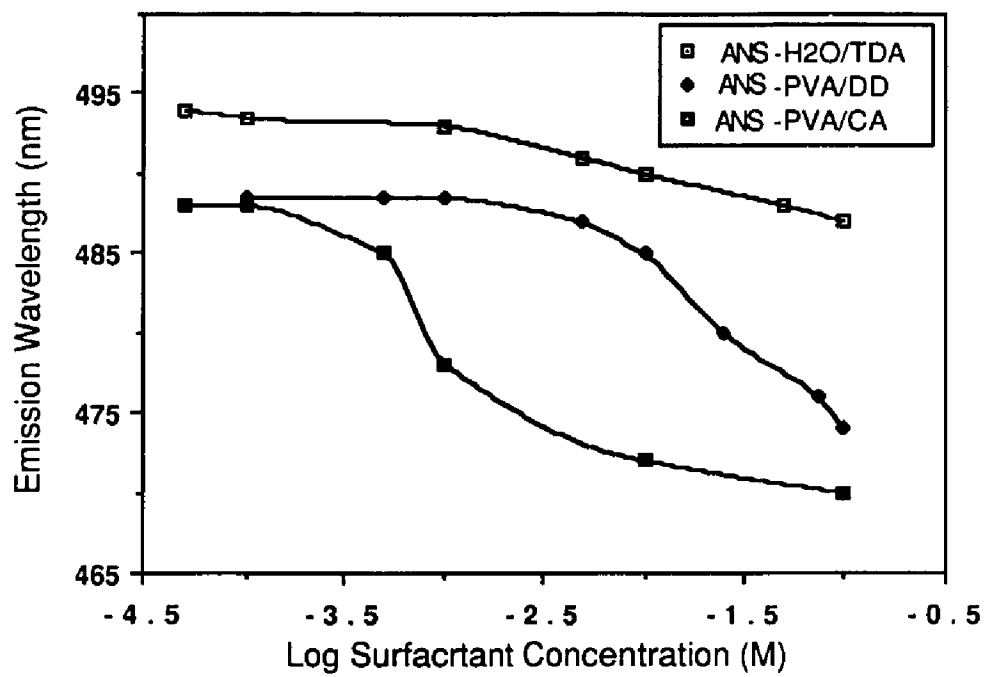
Figure 4.21 : Wavelength of maximum emission of AEANS in
solution and immobilized onPVOH as a function of
the concentration of DDTMA, TDTMA and CTMA.

expected. In addition, the magnitude of the shift in emission wavelength is greater for the surfactant with the longer hydrocarbon tail.

Results show that the total shift in the emission spectrum of dissolved indicator is about 6 nm as the TDTMA concentration changes from 1.0 to 100 mM, while the change for immobilized indicator is about 14 nm as the concentration of DDTMA changes from 1.0 to 100 mM and 18 nm as the concentration changes from 0.1 to 100 mM.

Figure 4.22 shows the response of AEANS/PVOH to CTMA based on intensity ratio measurment at two wavelengths. It is clear that the increase in CTMA concentration leads to an increase in the intensity ratio. This response is similar to the intensity measurement at a single wavelength shown in figure 4.20.
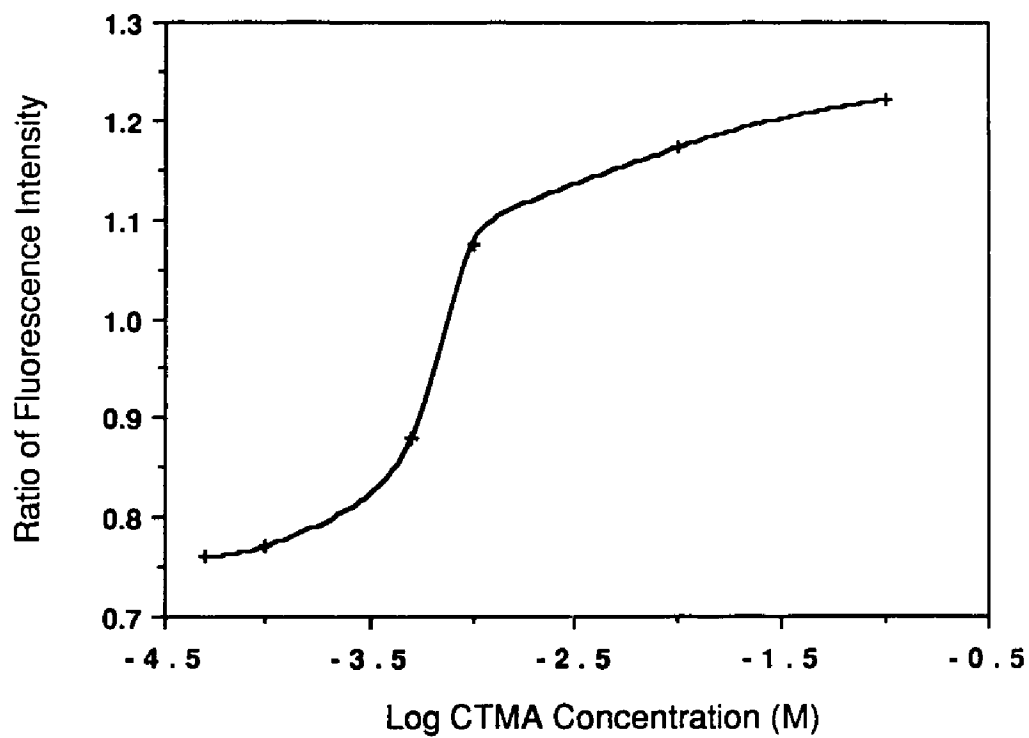
Figure 4.22 : Change of the ratio of fluorescence intensity of
AEANS/PVOH at wavelengths of 450 and 520 nm
as a function of CTMA concentration.

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CONCLUSION

A covalently immobilized anionic environment-sensitive dansyl compound on controlled-pore glass responds to cationic surfactants and pH. The fluorescence spectrum shifts to shorter wavelengths and higher intensity with increasing surfactant concentration. Sensor response is based on the change in the polarity of immobilized indicator environment resulting from hydrophobic interactions between the indicator and the surfactant as well as between the ion pair and the surface of the immobilization substrate. The overall shift is approximately 30 nm. Response is more sensitive to surfactants with long hydrocarbon tails dissolved in high pH solution. In addition, the response takes a long time, about 14 hours, before it reaches a steady state. On the other hand, the response is reversible, but it is very slow which takes days without going back to the original position. There is no significant response to either nonionic or anionic surfactants. This indicator also responds to pH above 6 based on the same principle except that the ion pair complex forms between the sulfonate group and the protonated secondary amine group of indicator molecules which are located on the sensor surface.

AEANS was immobilized on cellulose using cyanuric chloride as a coupling agent. AEANS-Cy/Cellulose responds to cationic surfactants. Unlike AEANS/CPG, AEANS-Cy/Cellulose does not respond to pH especially above 6. However, AEANS on cellulose is not stable, hydrolyzing slowly from the surface.

AEANS covalently bound to a PVOH membrane can be applied onto a fiber optic bundle for cationic surfactant determination. AEANS emission increases in intensity and shifts to shorter wavelengths as the surfactant concentration increases. Response is based on the change in the polarity of immobilized indicator environment as a result of surfactant partitioning between the aqueous phase and the membrane. The response of immobilized indicator to surfactant is similar to that for indicator dissolved in solution, since the PVOH membrane is formed from 85% of water. The overall shift is approximately 18 nm.

CHAPTER V

SOLVENT POLARITY MEASUREMENT BASED ON BETAINE
IMMOBILIZED IN SILICONE RUBBER MEMBRANE

INTRODUCTION:

This chapter describes the immobilization and characterization of a betaine dye indicator in a silicone rubber membrane attached to a fiber optic system as a solvent sensor.

Betaine dyes such as ET(30), 2,6-diphenyl-4-(3,4,6-triphenyl-N-pyidino) phenoxide, generally are polar in the ground state and become less polar in the excited state as shown in figure 5.1 (88-90). Therefore, the ground state of the dye molecule is preferentially stabilized in polar solvents as illustrated in figure 5.2. This effect results in a red shift of the absorption bands of betaine dyes when a change is made from a polar to a non-polar solvent (91,92).

ET(30) dye exhibits the largest observed solvachromic effect of any known organic molecule, amounting to several hundred nm in going from a very polar solvent (water, maximum wavelength = 453 nm) to a non-polar solvent (diphenyl ether, maximum wavelength = 810 nm). In fact, the apparent color of solutions of ET(30) serves as a rough indicator of solvent polarity. In methanol, solutions appear red (maximum wavelength = 515 nm), while in acetonitrile the
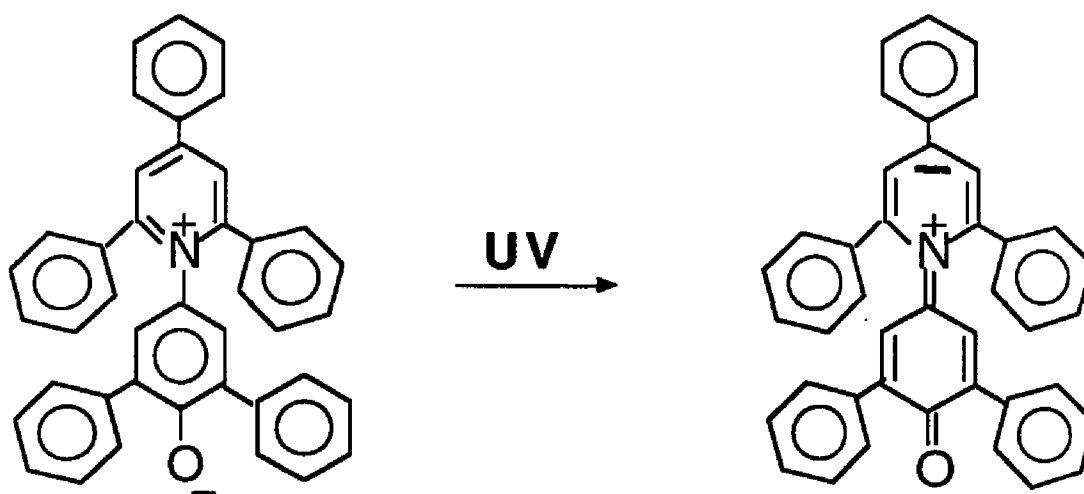
Figure 5.1 : Absorption effect on the electronic structure
of 2,6-Diphenyl-4-(2,4,6-triphenyl-N-pyridino)
phenoxide (ET30).
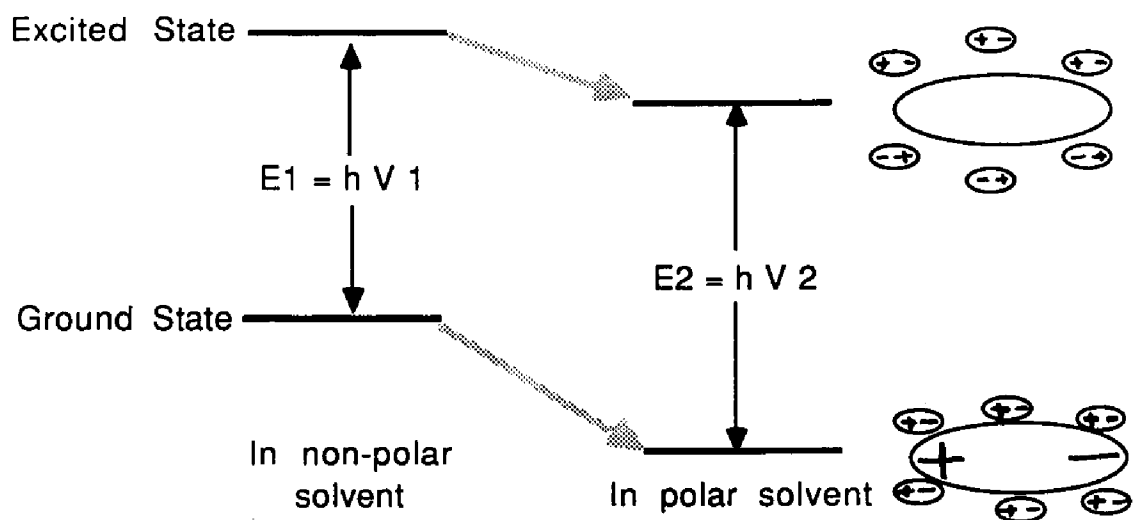
Figure 5.2 : Schematic diagram of solvatochromic effect.
Large ellipse : dye molecule,
Small ellipse : solvent molecule.

apparent color is deep blue (maximum wavelength = 622 nm). ET(30) is only slightly soluble in water (less than 1.0 micromolar), while it is soluble in both surfactant solutions and most organic solvents.

ET(30) has been used to characterize the environment polarity in micelles, microemulsions and phospholipid bilayers. In micellar solutions, ET(30) polarity values have been determined as a function of the surfactant chain length, the nature of the counterion, salt addition, temperature and the surfactant concentration (25-28). Also, ET(30) was used to measure the polarity of binary acetonitrile/water and methanol/water mobile phases used in reversed phase liquid chromatography (29-35). In highly aqueous media, the solvatochromic charge transfer peak of ET(30) merges with that of the aromatic ring and can not be located (93-99).

Multiparameter approaches to solvent effects have also been developed. The most prominent is that developed by Kamlet, Taft and co-workers in which the solvatochromism of various organic molecules is used to define "bi*", "@" and "&" values for a given solvent. "bi*" refers to the " depolarity / polarizability" of a solvent, while @ and & reflect its ability to act as a hydrogen bond donor or acceptor respectively. These parameters tend to differ in sensitivily to various of solvent/solute interactions.

Kominek used silicone rubber to immobilize dyes in a transparent film, which forms rapidly at a low temperatures and is stable during storage (100). This membrane is unlike poly(vinyl alcohol) membranes in that it is not subject to physical changes such as shrinking or swelling. It is insoluble in solvents such as

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water and hexamethylphosphoritriamide (HMPA), but polar organics solvents dissolve readily in it, and are free to diffuse into membrane (101-105). The solvents, water and HMPA permeate slowly as vapors through the membrane. Organic solvent, on the other hand, dissolve in the membrane.

Silicone rubber membranes have been successfully applied to different problems. It has been used to separate permeable organics from the impermeable carrier gas in a gas chromatography/mass spectrometry separator (106). Mason and co-workers used a thin membrane to directly sample various trace pollutants from water and identify them by mass spectrometry (107).

This chapter presents the result of experiments to evaluate the optical characteristics of ET(30) dye immobilized under different conditions as a function of solvent polarity.

EXPERIMENTAL:

Reagents :

ET(30), 2,6-Diphenyl-4-(3,4,6-triphenyl-N-pyidinio)phenolate, was purchased from Aldrich. Its structure is shown in figure 5.1. Siloprene (K1000) and Siloprene crosslinking agent (KA1) were obtained from Fluka.

Apparatus :

Emission spectra were measured with a fiber optic system coupled to the SLM 8000 spectrofluorometer as described in chapter

2. They were not corrected for instrumental effects. The excitation wavelength was set at 370 nm for all spectra. The absorption spectra were obtained by using a Spectronic 200 spectrophotometer as described in chapter 2.

Procedure :

Immobilization : The fluorescent ET(30)/silicone membrane was prepared as follows : Et-30 dye (28mg), was dissolved in 200 microliter of methanol. The solution was mixed well with 200 microliters of siloprene crosslinking agent (KA1) until a homogeneous mixture was obtained. Siloprene(K1000) (1.0 mL) was mixed with 50 microliters of the above mixture. Immediately, the tip of the fiber optic was immersed in the mixture for a few seconds and then removed. It was left for a couples of minutes to allow the crosslinking reaction to go to completion and to form the membrane. The non-fluorescent ET(30)/silicone rubber membrane was prepared using the same procedure with the dye concentrations 10 times higher than that immobilized on the first membrane.

The immobilized membrane was washed extensively with water, acetone and methanol, and allowed to dry. Both membranes were tested for fluorescence using a UV hand lamp.

Solvent Effect on ET(30) Fluorescence : Small pieces of ET(30) immobilized in silicone rubber were immersed in solvents such as water, methanol, ethanol, butanol and hexane. The membrane was

exposed to UV light from a UV hand lamp and differences in the color of fluorescence were observed visually.

When the concentration of the immobilized ET(30) dye in the silicone rubber membrane was very low (3mg/1.0 mL), the color could not be observed visually.

The emission spectra of ET(30)/silicone rubber in different solvents was determined using a fiber optic system attached to the SLM 8000 spectrofluorometer, as described in chapter 2. The immobilized membrane on the tip of fiber optic was immersed in solvent.

Response to changes in the solvent composition was tested by increasing the percentage of organic from zero to 100% using a series of solutions with progressively higher percentages of organic. The emission spectrum was run many times until no further change in intensity was observed.

Solvents Effect on ET(30) Absorption : The effect of solvents on the absorption spectrum of ET(30) was studied qualitatively by dissolving small amounts of ET(30) dye in different solvents (water, methanol and acetonitrile) and visually observing color differences..

The effect of solvent on the color of the immobilized dye was studied by immersing small pieces of the immobilized membrane in different solvents. After a short period of time the color was observed by eye. The absorption wavelength of ET(30) immobilized in silicon membranes was obtained by spectrophotometry. A small

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pieces of immobilized membrane was fixed inside the cuvet as shown in figure 2.4. The cell was filled with solvent and the absorption spectrum was taken at different periods of time until no further change in the absorption spectrum was observed. The maximum wavelength of the immobilized dye was determined.

RESULTS AND DISCUSSION :

ET(30) is stable inside the silicone rubber membrane in the presence or absence of solvents. No significant leaching was observed even when the membrane was in a solvent in which ET(30) is highly soluble.

When the immobilized membrane on the tip of the fiber optic was exposed to different organic solvents, there was no observable shrinking or swelling. It adheres strongly to the tip of the fiber optic and is stable in all solvents. This enhances the lifetime of the sensor.

The concentration of the immobilized dye had to be adjusted to control the optical properties of the sensor. At high immobilized dye concentrations, no fluorescence was observed when the membrane was exposed to UV light. On the other hand, at a low concentration of immobilized dye, strong fluorescence was visible. It develops just after the membrane crosslinking reaction takes place. During the first moments of the membrane formation, no fluorescence was observed, but after couple of minutes, strong fluorescence is observed and increases with time. This may be related to the

formation of a rigid structure during crosslinking and to the evaporation of methanol which was used to dissolve the dye in order to immobilize it in the membrane. Methanol seems to quench the fluorescence of immobilized ET(30). After all of the methanol evaporates from the membrane, there is no further change in the fluorescence intensity.

Solvent Effect :

The absorption spectrum of ET(30) in solution is strongly dependent on solvent polarity. When the immobilized membrane containing a high concentration of ET(30) was immersed in methanol, the dye's color changed from deep blue to red. When the methanol was replaced with acetonitrile, the color changed to blue again. Figure 5.3 shows that the absorption spectrum of ET(30) in the silicone rubber membrane shifts to longer wavelength as the solvent polarity is reduced. In water, the absorption wavelength is short (470 nm), whereas in organic solvents the absorption spectrum shifts to longer wavelengths. The maximum absorption wavelengths for immobilized ET(30) are 500, 535 and 558 nm in acetonitrile, methanol and ethanol, respectively. The same spectral shifts occur for ET(30) in the membrane as for ET(30) in solution.

It was also found that the intensity of fluorescence from membrane prepared to contain a low concentration of ET(30) is highly sensitive to solvent polarity. When the fluorescent ET(30)/silicone rubber membrane was immersed in methanol, the
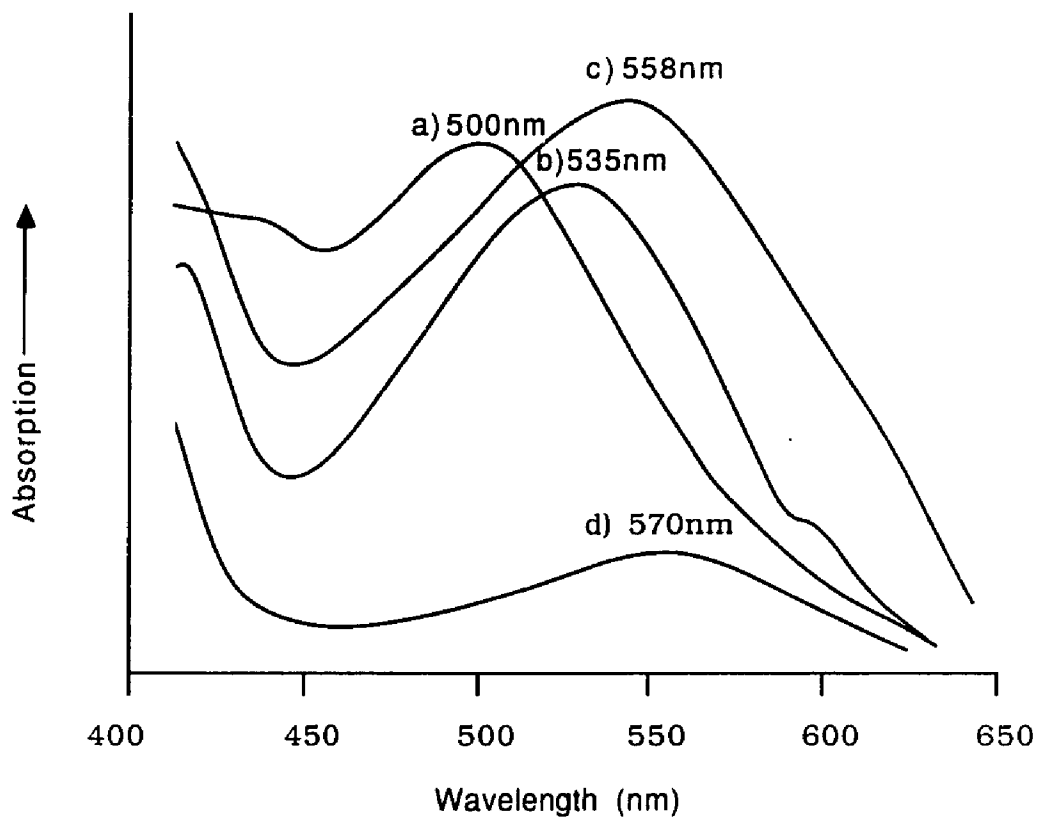
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Figure 5.3 : Absorption spectra of ET30/silicone rubber membrane exposed to a) Acetonitrile, b) Methanol, c) Ethanol. d) H2O.

fluorescence intensity decreased significantly. This can be detected visually within minutes. When the immobilized membrane was removed from the methanol and allowed to dry, the fluorescence intensity increased within a couple of minutes.

Results in table 5.1 show that the maximum emission wavelength for ET(30) in silicone rubber membrane depends on the solvent. In water, the emission spectrum has the longest wavelength (485 nm). Whereas in organic solvents the emission spectrum shifts to shorter wavelengths. The maximum emission wavelengths for immobilized ET(30) are 450, 468, 469 and 477 nm in methanol, isopropanol, butanol and hexane, respectively. The longest wavelength for ET(30) in silicone rubber exposed to water is related to the low water solubility of water in membrane in which the ET(30) is more affected by the hydrophobic silicone rubber environment.

Solvent Composition Effect :

Our studies indicate that the fluorescence intensity from ET(30)/silicone rubber membrane decreases with an increase in the percentage of organic solvent in water.

Figures 5.4 and 5.5 show the decrease in the fluorescence intensity of ET(30) as a function of methanol concentration in water. The relationship between the change in fluorescence intensity and solvent composition is almost linear.

The ET(30)/silicone rubber membrane responds reversibly to

Table 5.1 : Solvent effect on emission spectrum of Et(30) in
Silicone Rubber membrane:

Solvent	Emission wavelength
Water	485 nm
Methanol	450 nm
Isopropanol	468 nm
Butanol	469 nm
Hexane	477 nm
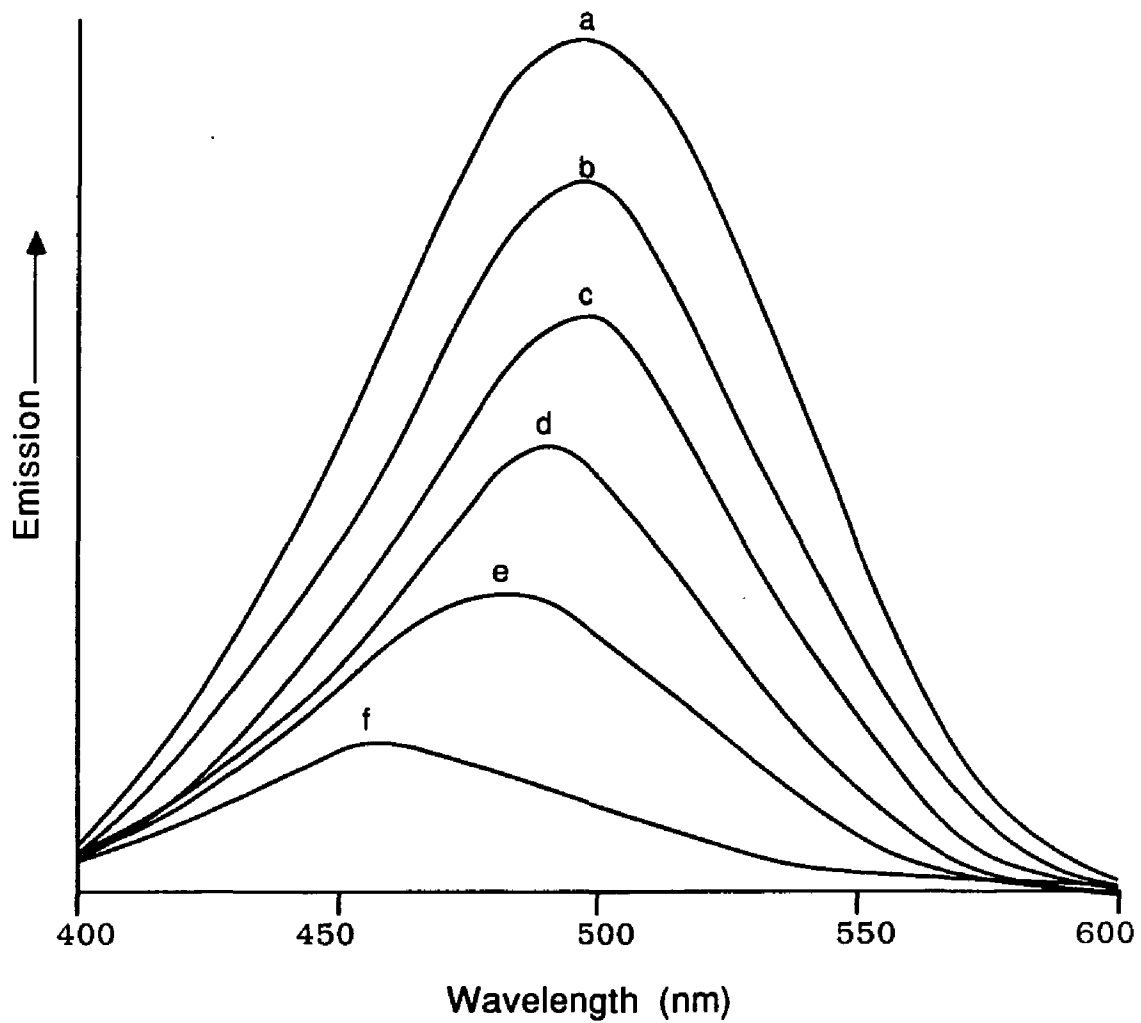
Figure 5.4: Emission spectra of Et30/silicone rubber membrane exposed to methanol/water mixture a) 0%, b) 20%, c) 40%, d) 60%. e) 80%, f) 100%.

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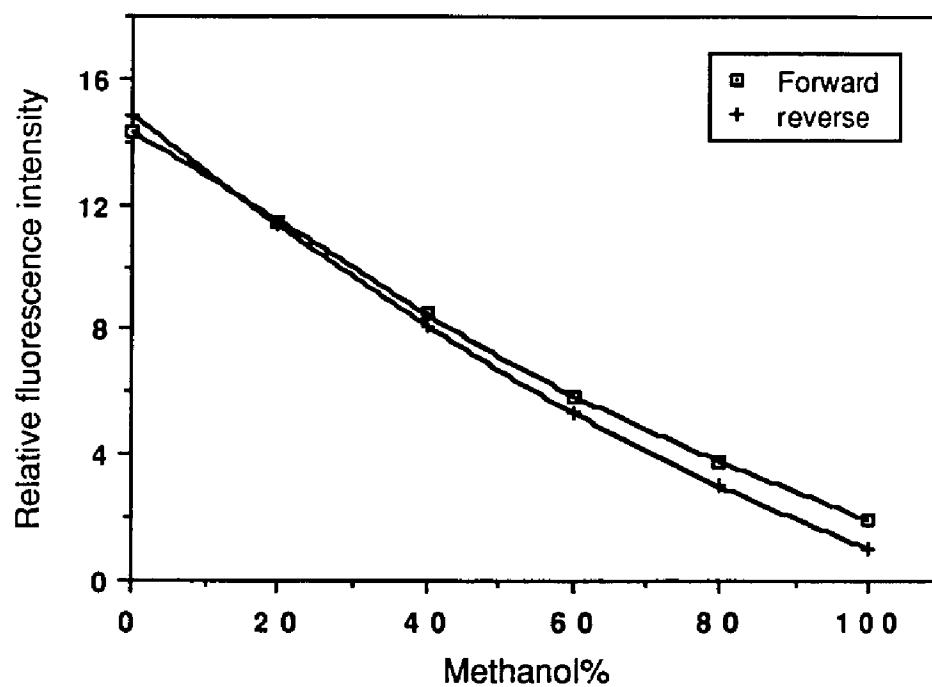
Figure 5.5 : Change in fluorescence intensity of ET30 in silicone
rubber as a function of methanol composition.

solvents. Figure 5.5 shows that the fluorescence intensity almost goes back to its original value when the methanol is evaporated. Also, response to a given concentration of methanol is the same whether the percentage of methanol is increasing or decreasing.

Response Time :

Figure 5.6 shows that the response time when methanol is added is between 7 and 10 minutes for all solvent compositions. Response time is established by the rate at which the solvent molecules diffuse into the membrane.
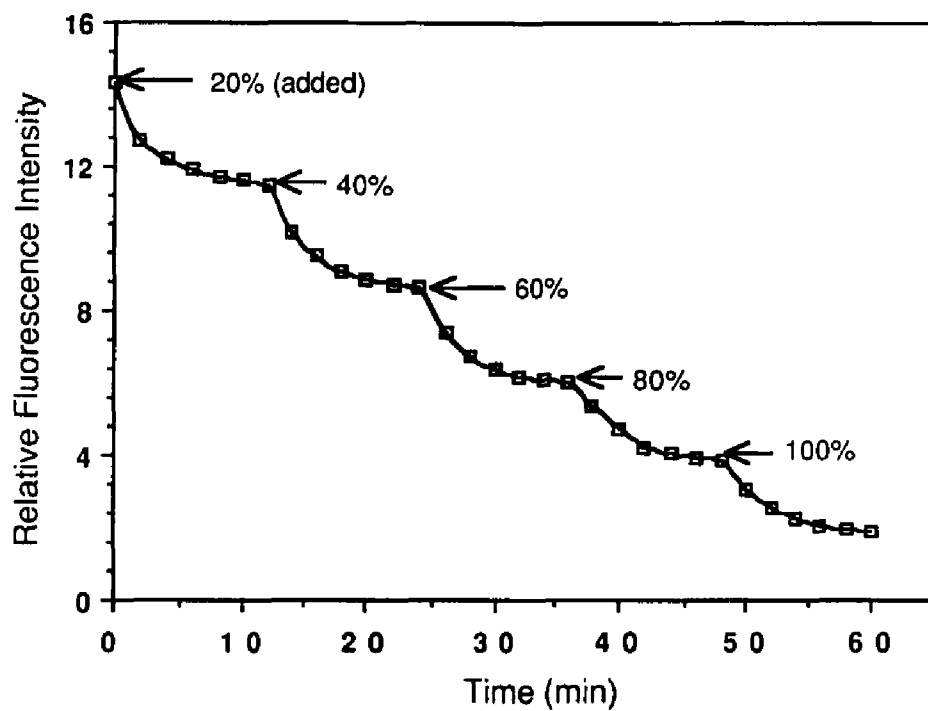
Figure 5.6 : Change in fluorescence intensity of ET30 in silicone
rubber with time as a function of methanol composition.

CONCLUSION

An immobilized environment-sensitive betaine dye in a silicone rubber membrane responds to changes in solvent composition. The absorption spectrum shifts to longer wavelengths as solvent polarity decreases. The optical characteristics of the immobilized dye depend on the concentration of the dye immobilized in the membrane. At a high concentration of immobilized dye, the immobilized dye is non-fluorescent. Its optical behavior is similar to that of the dye in solution. At low concentrations, the immobilized dye is highly fluorescent. In both cases there is a response to solvent, but in the second case the response is more sensitive. The response is reversible and the response time is reasonable. In addition, the immobilized silicone rubber membrane is stable on the tip of the fiber optic bundle and resists physical changes as a function of solvent.

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