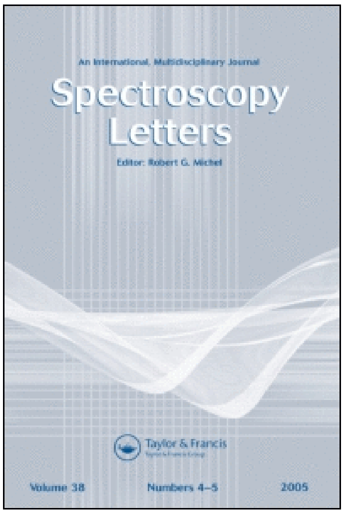

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FIBER OPTIC PROBE APPLICATIONS USING NEAR-INFRARED COMPOUNDS: DETERMINATION OF NaOH

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ABSTRACT

The development of a near-infrared fiber optic probe (FOPP) for the determination of caustic soda will be reported. The utility of the FOPP is demonstrated using an NIR non-commercially available tetrasubstituted chloroaluminum naphthalocyanine NIR dye which is susceptible to pH changes in solutions. The naphthalocyanine derivatives have been synthesized in our laboratories. The spectral characteristics of these NIR dyes are above 700 nm and the absorbance maximum is a perfect match for the use of semiconductor laser diodes as the excitation source. The response of the Q-band against solution pHs and caustic soda will be reported. The FOPP consists of a semiconductor laser diode (λ_{max}=780 nm), an NIR dye and a detector. The probe made of poly(methyl methacrylate) serves as a support for a permeable polymer that is used for the entrapment of the NIR dye (about < 2mm in diameter). The permeable polymers used were Nafion and Gelatin. As hydrogen ions diffuse through the permeable polymer, a complex is formed with the dye, accompanied by changes in the spectral characteristics of the dye. Excitation of the complex is induced with a semiconductor laser diode at 780 nm. The probe shows good reproducibility of the data in NaOH measurements. Also the molar absorption coefficient of these dyes at different concentrations of polymer will be reported. The effect of Nafion on the spectral characteristics of the NIR dyes will be presented. The general procedure used for the attachment of the dye to the distal end of the probe can be used to construct compact non-invasive detectors. The use of photodiodes as detertors will be discussed. Use of smaller components shown here will allow for the manufacturing of portable instruments for applications in medicine and industry.

INTRODUCTION

Many of the pH sensitive chromophores that have been used in fiber optic applications and reported in the literature have spectral characteristics in the visible region [1,2]. The most common fluorescent dyes that have been used are fluorescein and its

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derivatives [3-5]. Other indicators such as p-nitrophenol [6], congo red [7], bromophenol blue [8], and several others [9-12] have also been used successfully in pH investigations. One of the reasons for the continuous development of pH probes using Fiber Optic Technology is the importance of pH determination in the physiological range (pH 6-8) which is critical in medicine and life sciences. In addition, the on-line detection of pH in many industries has increase the demand of alternate detection techniques.

Sensitive fluorophores for the physiological range have been reported in the literature [3,5,13,14]. Peterson reported a pH probe for the physiological range of 7.0 to 7.4 [15]. The pH response was by monitoring two different wavelengths. Seitz, et al., [16] reported a fluorescent sensor for the pH range of 6.5 to 8.5 using a trisodium salt derivative as the indicator. Anion-exchange membranes are alternative matrices for the physiological pH range [16,17].

These dyes absorb and fluoresce between 300 and 650 nm, making them susceptible to extensive interference, especially from biomolecules and causing many obstacles in the development of probes that can be used in industry and especially in medicine. Fluorescence quenching has been reported by the presence of biomolecules such as thiamine (vitamin B_1) [18], nicotinamide [19], nucleosides/nucleotides [20], and pyruvate [21]. To circumvent the obvious limitations associated with the use of UV or visible fluorophores in fiber optic applications, the potential use of near-infrared fluorogenic dyes as molecular probes with alternative methods of detection is under investigation and the successful synthesis of several new NIR dyes has been reported [22-26].

Only few a NIR fluorophores have been reported for pH measurements [10,27]. The changes on the absorption spectra was attributed to the protonation of the oxygen atom which induced a shift to the cationic enol form of the dye [10]. Another NIR pH sensitive dye showed changes in the spectra following Beer's law [27]. A shift in the useful pH range from the range expected was reported the pK_a value of the dye due to the extremely acidic environment in the Nafion membrane. If a lower pH range is desired, the probe should be built using a different polymer support that will allow pH ranges comparable to the lower values observed in solution [28]. The entrapment of these dyes in a permeable polymer has been reported [29]. Nafion was used successfully in the development of several pH probes. The use of thin films of Nafion impregnated with a dye for humidity measurements has been reported by Bright, et al.,[30] and by Patonay [27] for pH determination [31]. An optical sensor for sodium hydroxide measurements was reported by Burgess using bromothymol blue entrapped in Nafion [32].

One of the advantages of using NIR dyes and fluorescence detection is that the signal-to-noise is much greater than absorbance or reflectance with respect to the background [31]. Since fluorescence is measured against background the sensitivity of the technique is dictated by the background fluorescence. Additionally, fluorescence spectroscopy introduces more selectivity into the method because fewer molecules exhibit spectral properties in this region [33]. The advance in laser diode technology in recent years has the ability to increase the utility of many NIR applications [29]. These small laser diodes may be beneficial for the production of small portable units. As compared to the uv-vis region, laser excitation can be performed in fluorescein (and derivatives). The absorbance maximum of this uv-vis dye coincides with the output wavelength emitted from an argon laser (499 nm). Unfortunately, argon lasers are costly and bulky, thus limiting the practicality for the development of small portable units.

This paper describes the use of NIR dyes for the determination of solution pH and high concentrations of caustic soda. The instrument used utilizes a semiconductor laser diode, a NIR fluorophore and a silicon photodiode [26]. A commercially available dye (IR144) as well as several derivatives of a tetrasubstituted chloroaluminum 2,3-naphthalocyanine dye were synthesized in our laboratories for these investigations. The measurement of NIR fluorescence was achieved by entrapping the dye in a Nafion (or gelatin) matrix. Nafion (or gelatin) was used because it is permeable to hydrogen ions and at the same time prevents the dye from exiting the matrix. The matrix was then attached to the polymer support that held the distal end of two optical fibers. One is used for the excitation of the dye and the second is used for the collection of the fluorescent signal. Fluorescence was induced by a semiconductor laser diode with an output wavelength at λ=780 nm followed by an 820 nm emission filter (10 nm bandwidth filter, OD>5). The fluorescence signal was collected by a commercially available Spectrofluorometer and by using a photodiode detector [34]. This type of NIR probe may be used in biomedicine for the detection of metals and in industry for the determination of saline solutions.

EXPERIMENTAL

Materials

The poly (methyl methacrylate) (PMMA) optical fiber was purchased from Mitsubishi Cable America, Inc. (New York, NY). Spectragrade methanol, methylene chloride (CH_2Cl_2), and sodium chloride were purchased from Fisher Scientific Company (Fair Lawn, NJ). Hydrochloric acid was obtained from J. T. Baker, Inc. (Pillipsburg, NJ). Commercially available IR144 and n-nonyl(phenol) were obtained from the Eastman Kodak Company (Rochester, NY). All chemicals were used without further purification.

Dye synthesis

The 2,3-naphthalocyanine derivatives were prepared by following this procedure [35]: a solution of 28.0 ml (5.0 g) 2,3-dicyanonaphthalene and 100 ml 1,2-dichlorobenzene were refluxed and then 14.0 mmol (14.0 ml) 1 M $AlCl_3$ in nitrobenzene was added dropwise. The reaction was refluxed for 2.5 h and monitored by UV / VIS / NIR every 0.5 hr. The reaction was then cooled to room temperature and poured into methylene chloride (500 mls). The resulting suspension was filtered and washed with more methylene chloride. The dark-green naphthalocyanine product was dried at 125 °C (yield=92%, 5 gm). This product was sulfonated with oleum (150 mls, 15 % free SO_3) at 50 °C, stirring for about 60 min. The reaction mixture was then poured onto crushed ice and the suspension was centrifugated and filtered. The dry solid was dissolved in 10% NaOH solution and filtered. After adjusting the filtrate's pH to 1 with concentrated HBr solution, the filtrate was centrifugated. The final sulfonated aluminum 2,3-naphthalocyanine Dye-I product was dried at 125 °C (yield=23%, 1.62 gm). The precursor naphthalocyanine and the sulfonated naphthalocyanine final product were identified by UV/VIS/NIR, NMR, FT-IR and elementary analysis.

Methods

The stock solutions for all dyes used in these investigations were prepared in methanol. For the molar absorption studies, the dilutions were made by pipetting aliquots of the stock solution into the appropriate solvent (methanol or the polymer solution). The mixtures were sonicated for a minimum of 5 min. (at room temperature). All working solutions were made fresh from stock on the day of the analysis.

A 10^{-2} M stock solution of IR144 NIR Dye (and all other dyes) was prepared freshly on the day of the experiment or stored for a week at 4°C. The dye is very stable under these conditions. For the solution pH controlled experiments, several vials (5 mls) were prepared by slowly adding small amounts of HCl or NaOH, respectively. The vials were sealed until ready for measurements. Stock polymer solutions were prepared by aliquots taken directly from the manufacturer's bottles. No further purification of the materials was performed. All pH measurements were done using a Fischer Accumet pH meter model 805.

For the measurement readings, the probe was allowed to reach equilibrium in distilled water for about 5-10 minutes. Any adjustments of the instrument were carefully done to obtain a constant intensity value and the fluorescence intensity was maximized. Then the probe was removed and rinsed with distilled water (any excess water was carefully removed), the probe was immersed in the vial of lowest solution pH. This process was repeated for all concentrations. After the highest concentration, the probe was immersed in the vial solution previously adjusted to pH 2 or lower but no more than 0.5 M HCl. The probe was dried for several hours or overnight. A deep purple color at the end of the fiber appeared when the probe was completely dry.

Preparation of the probe

Many different polymer matrices have been reported in the literature. The use of polyvinyl alcohol (PVA) [4,36], plasticized polyvinyl chloride (PVC) [37-42], polyacrylamide-methylenebis (acrylamide) copolymer (PAMAC) [43], Ethylene-vinyl acetate polymers [44], Rigid rod-type polymers such as poly (phenylquinoline) and poly (biphenylquinoline) has been reported [45]. All of these polymers have shown different degrees of success, however, these polymers need additional additives for a good indicator response. Polyvinyl chloride was used with little success in this investigation because of its viscosity at higher concentration. Of the polymers that we used, poly (methyl methacrylate) and gelatin gave the best results.

For these investigations, optical fibers (two) were carefully polished with sandpaper to obtain a clean surface. The distal ends were then connected using a solution of PMMA in methylene chloride (CH_2Cl_2). The amount of CH_2Cl_2 used to dissolve the PMMA polymer was minimum because complete evaporation from the binding site is necessary. After the solvent evaporates, the two fibers are held together and a solid crystalline phase is obtained. The binding site was dried in about 2 hours or overnight in a room kept at 20°C. No attempts were made to use the probe before it dried out, since the polymer is not well adhered to the optical fibers unless is dried first. Enough PMMA solution was applied to form an even binding site about 2 mm in diameter. At the beginning of this investigation a smaller binding site volume was used but it was not as efficient in the following steps of the preparation process.

The polymer support was then coated with the NIR entrapped in Nafion. Different concentrations of dye were investigated ranging from 10^{-5} to 10^{-2}M (in Methanol); however, probes made with a 1×10^{-2}M concentration gave the best signal-to-noise ratio. The ratios of Nafion were 80/20%, 60/40%, 50/50% and 40/60%.

Apparatus

A Perkin-Elmer Lambda 2 UV/vis/near-IR spectrophotometer was used for the absorption measurements. The spectrometer was interfaced to a Zenith 286 computer equipped with a PECSS program used to store data and control the spectrophotometer. The excitation wavelength was determined from the absorption spectrum, using the maximum absorbance wavelength. Fluorescence data was taken with an SLM Aminco 8000. Integration times of 0.1 sec were used for low concentrations of analyte. A slit width of 4 mm was used throughout the experiments. A PS/2 PC controlled data acquisition and stored the data for later analysis. Quartz cuvettes (Fisher Brand) were used in all these investigations.

An NIR optical fiber pH probe has been developed in our laboratories from commercially available optical components. The instrument shown here is similar to the one used previously for similar investigations [34]. The FOPP instrument's main components are a semiconductor laser diode, optical fibers, an NIR dye and a detector. Semiconductor laser diodes (30 mW) with output wavelength between 750-780 nm (Toshiba or Sharp) were used. The laser beam was directed to a 10:1 microscope objective lens (numerical aperture 0.30) (Rolyn Gau, Germany). A density filter wheel (Newport, CA) and a manual shutter (Newport, CA) were positioned in front of the fiber to control the laser intensity. A density filter also minimized continuous light that may result in bleaching of the dye. The fluorescence signal was collected by the second optical fiber routed to the detector housing. The detector has a bandpass filter, a lock-in amplifier, and an avalanche photodiode. The lock-in amplifier was used to achieve minimum sensitivity. A Tenma digital multimeter model 72-050 was used for the readout. The 820 bandpass filter (Corion Corp., Holliston, MA) with a 50% transmittance was a good match for the fluorescence band signal from the dyes. Raman shift had no significant effect on the signal. To avoid possible room light interference all the data was taken in a darkroom. The room temperature was kept constant at 20°C.

RESULTS and DISCUSSION

Near-infrared (NIR) fluorogenic dyes possess spectral properties above 700 nm. But in order to be successful fluorophores for fiber optic applications some standard criteria should be met. These dyes should have adequate response to analytes. The spectra may increase, decrease or complete elimination of the absorbance maximum may occur. Also, a high lipophilicity and/or reactive functional groups may be desirable. These will permit good complexation or bonding to the analyte or to the polymer matrix. In addition, the absorbance maximum should be compatible with available laser diodes. Some dyes may require high photostability if laser excitation is used. Other important requirements are high fluorescence quantum yield and molar absorptivity [46].

Of the NIR absorbing chromophores, carbocyanines, and naphthalocyanines have been investigated in our laboratory. Naphthalocyanine dyes have been shown to be stable to light, heat and oxidation degradation compared to other NIR dyes [47]. The absorbance maxima of many naphthalocyanine dyes (and derivatives) are a good match to the output wavelength of small semiconductor laser diodes (λ = 690, 780,750, 830 nm). A representative absorption spectra for the tetrasubstituted chloroaluminum 2,3-naphthalocyanine dyes (in methanol) used in this investigation is shown in Figure 1. Only molecules of this nature (with extensive conjugated system) are able to absorb NIR energy. Table 1 shows the chloroaluminum 2,3-naphthalocyanine (2,3-Nc) derivatives used in this investigation. The absorption spectra of these 2,3-NC derivatives are consistent with $\pi \rightarrow \pi^*$ transitions in the energy region above 700 nm. It is obvious that a bathochromic shift of the Q-band occurs with the addition of different moieties. This particular characteristic may be beneficial since the dyes can be modified for an excitation wavelength to suit a particular experiment. An added advantage is their low aqueous solubility which makes them ideal candidates for water investigations. In most cases, the

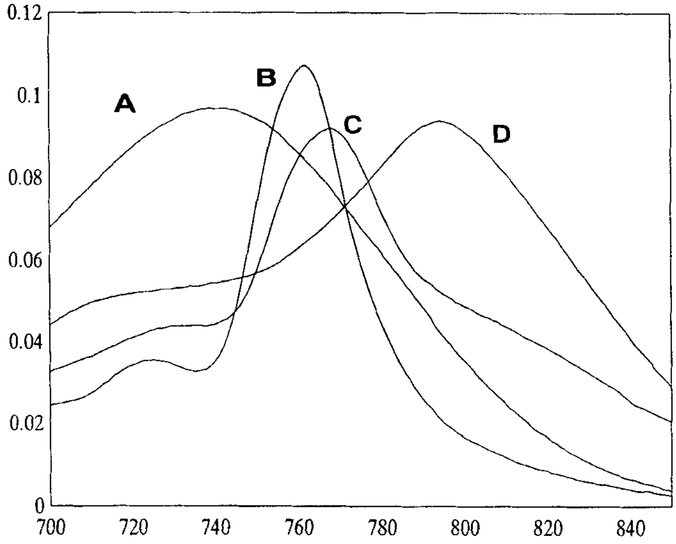

Figure 1.
Representative absorption spectra for the tetrasubstituted chloroaluminum 2,3-naphthalocyanine dyes (in methanol) used in this investigations: IR144 (745 nm) (A), CNC126 (762 nm) (B), CNC140 (765nm) (C) and for CNC169 (793 nm) (D).

Table 1. Data of the NIR compounds compatible with the output wavelength of semiconductor laser diodes (λ=780 nm).

Name	(Group)	Fluorescence		log (ε)[a]	log (ε)[b]
		λ_{EXC}	λ_{EM}		
CNC126	$(5\text{-}NC)_4$-	762	766	4.15	6.54
CNC140	$(5\text{-}O_2N)_4$	765	773	4.31	4.26
CNC169	$(5\text{-}HOOC)_4$	793	803	3.76	3.81
IR144[c]		745	825	5.09	2.63

[a] All dyes dissolved in methanol [b] All dyes dissolved in 30% nafion (v/v) in methanol [c] in DMSO

molar absorption of these NIR dyes is constant in the presence of Nafion (30% in methanol). These results suggest that the polymer has little effect on the stability of the NIR dye. However, a very drastic change in the molar absorption coefficient is observed for IR144.

The stability of the NIR dye at several concentrations of polymer is shown in Table 2. The effect of the polymer concentration on the absorbance intensity produces a 40% decrease on CNC126 while more than a 79% decrease is observed with IR144. Also there is a bathochromic shift in the Q-band for CNC 126 with polymer concentration. This may suggest that the strong negativity of the Nafion polymer is acting in the central Nitrogen atom of the NIR dye. Additional studies of this effect are underway. Different solution pHs have been shown to have a similar effect on other investigations especially when NaOH is used. The excess OH^- forms a complex with the central hydrogen ions producing a distortion of the molecular structure splitting the $\pi \rightarrow \pi^*$ transition energy.

Due to the nature of the polymer, the OH- groups are able to permeate through the polymer. Once the probe has been placed in the controlled solution, osmotic pressure dominates the permeation of the hydroxyl ions inside the polymer. Once inside the polymer, the hydroxyl ions are able to complex with the NIR dye. Other OH- groups will exit the probe. At this point, chemical potential equilibrium takes over and the probe reaches a steady state of equilibrium. The complexation of the OH- ions with the dye can be detected either by absorbance or fluorescence. Figure 2 shows the absorbance of the NIR dye changes in different NaOH concentrations. These changes are accompanied by changes in the fluorescence spectra as well.

Degree of complexation

The color change of acid/base indicators is routinely utilized to determined solution pH and these color changes always parallel spectral changes in the indicator. Spectral changes such as absorbance or fluorescence can then be used for the development of fiber optic pH probes as well as for higher NaOH concentration measurements. Of these, fluorescence has proven to be the most valuable detection method in fiber optic applications [48,49]. It is advantageous to use sensitive indicators that show a sharp absorbance band away from the interference of other molecules that may be present. To expand the utility of the probe even further, the dye must undergo a reversible change

Table 2 Spectroscopic data for the NIR compounds at several Nafion concentrations

dye	%Nafion[a] (v/v)	Absorbance Int.	Absorbance λ_{max} (nm)	Absorbance % change	Fluorescence Int	Fluorescence λ_{max} (nm)	Fluorescence % change
CNC126	0	.1072	762	0	7985	766	0
	10	.0568	762	47	2191	766	73
	20	.0640	762	40	2642	766	67
	30	.0609	762	43	2702	766	66
	40	.0558	762	48	2266	766	72
	50	.0523	762	51	2039	766	62
CNC140	0	.0719	768	0	154	776	0
	10	.0916	777	27	438	784	184
	20	.1143	775	59	503	782	227
	30	.1291	775	78	488	781	217
	40	.1335	775	86	438	784	184
	50	.1359	776	89	407	783	164
CNC169	0	.0938	794	0	35	802	0
	10	.0312	794	67	23	802	34
	20	.0335	794	64	28	802	20
	30	.0397	794	58	96	802	174
	40	.0491	794	48	47	802	34
	50	.0547	794	42	240	802	586
IR144	0	1.25	742	0	1,150	819	0
	10	.2586	742	79	183	819	84
	20	.0287	742	98	12	819	99
	30	.0169	742	99	14	819	99
	40	.0260	742	98	28	819	98
	50	.0456	742	96	14	819	99

[a] all solutions dissolved in Methanol.

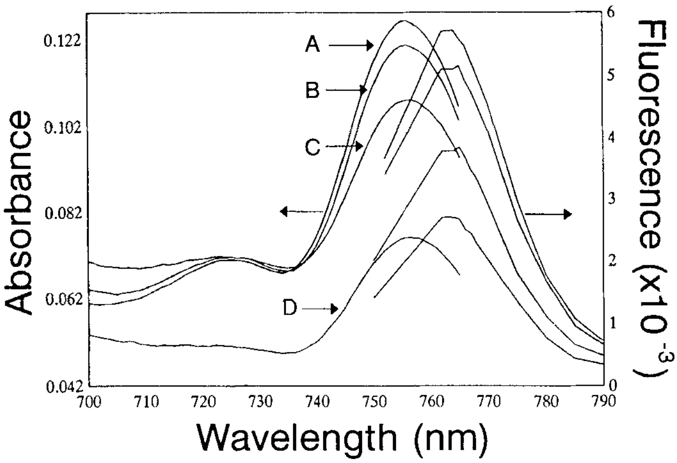

Figure 2.
Typical intensity chanes of absorbance and fluorescence spectra for the CNC169 NIR dye in different NaOH molar concentrations. Experimental spectral data for 2M NaOH (A), 4M NaOH (B), 6M NaOH (C) and 7M NaOH (D) shown.

during the complexation process with a concomitant change in the degree of conjugation of the molecule.

Usually these spectral changes are associated with concomitant changes in the degree of conjugation upon ionization or shifts in the absorbance maximum equilibrium. For this NIR dye is observed a decrease in intensity of the Q-band followed by the appearance of a second absorbance band with a decrease in OH^- solution.

These changes can then be represented by the following equation

$$D_l = \frac{A - A_0}{A_1 - A_0} \qquad (1)$$

where A is the absorbance of NIR dye during analysis, A_0 is the absorbance of NIR dye in the absence of ions, and A_1 is the absorbance of the reacted NIR dye. A plot of the degree of complexation of these dyes against NaOH molar concentration is shown in Figure 3. The dynamic range of the dye can be determined from this plot.

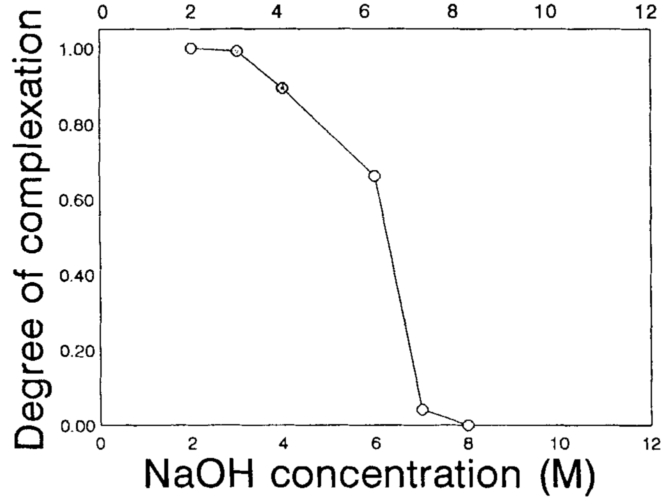

Figure 3.
A plot of the degree of complexation against NaOH concentrations.

A possible trend in the energy shift can be attributed to the distortion of the central cavity. Uncomplexed naphthalocyanine dyes have a square planar structure according to D_{4h} symmetry. An increase in ion concentration may be complexing with the central aluminum producing bond distortions by forcing the nitrogen atoms further away from the geometric center of the dye. An increase in hole cavity changes the dyes' symmetry to D_{2h} and may be attributed to some extent to the energy shift observed.

Probe

The association of H^+ to the dye, assuming monofunctional dye and that no other ions are present, can be represented as $[H^+ + D^- \rightleftharpoons HD]$. The fiber optic response, determined following the same steps as in the case for the ion chromatography and the equilibrium constant, K_{op}, is written as

$$K_{op} = \frac{[HD]}{[D^-][H^+]} \quad (2)$$

where D is the dye immobilized in the matrix. The total concentration of dye is the sum of the complexed (HD) and uncomplexed forms (D^-).

The concentration of dye that forms a complex with the ions can be determined from $[D_t = D^- + HD]$ (where D_t is the total dye concentration, D^- id the uncomplex dye and HD is the complex dye). Then the equilibrium constant can be rewritten as

$$K_{op} = \frac{[HD]}{([D]_T - [HD])\ [H]^+} \qquad (3)$$

solving for the complex dye HD and rearranging, we obtain

$$[HD] = \frac{K_{op}\ [D]_T\ H^+}{(1 + K_{op}\ H^+)} \qquad (4)$$

The intensity of fluorescence can be defined by the following equation

$$I_f = c\ d\ k\ I_1\ Q \qquad (5)$$

where c is the dye's concentration that has complexed, d is the cuvette's path length, k is ln (ε, molar absorptivity), I_1 is the laser intensity and Q is the quantum yield. However, because only the concentration varies, all other parameters are considered constant during the analysis and the equation is written as

$$I_f = k\ [HD] \qquad (6)$$

where k (= c d I_1 Q) is a fluorescence proportionality constant [6] and [HD] is the complexed dye. Arranging and substituting Eq. 6 we obtain

$$I_f = \frac{k\ K_{op}\ [D]_T\ H+}{(1 + K_{op}\ H+)} \qquad (7)$$

which can be rearranged. Then substituting 10^{-pH} for H^+ we obtain [50].

$$\frac{1}{I_f} = \frac{1}{k\ [D]_T} + \left(\frac{1}{k\ [D]_T\ K_{op}}\right)\frac{1}{10^{-pH}} \qquad (8)$$

A plot of $1/I_f$ vs pH produces a straight line. The fiber optic pH probe equilibrium constant, K_{op}, can be calculated from the slope. A similar derivation can be done for the

NaOH concentration. Even though some of the NIR dyes have more than one functional group for interaction with the analytes, the fluorescence intensity is still a continuous function of the pH; therefore, a calibration curve, albeit a nonlinear one could be constructed. The Equilibrium constant for the probe in NaOH was calculated as 29.98 with a correlation coefficient of 0.9839. By using a 1:1 complex of dye with OH^- the equilibrium constant was calculated to be 25.

Association constant determination

The spectral changes of the NIR dyes may be described as similar to a ion-pair solvent extraction. The equilbrium constant can be written as

$$K_{EQ} = \frac{[\,DNa\,]}{[Na^+]\ [D^-]} \qquad (9)$$

where D^+ represents the dye and OH^- is the concentration of hydrogen ions present in solution; the square bracket represents the molar concentration of the species in solution.

The changes in the absorbance spectra that have been observed are due to changes in solution pH. If no overlap caused by other biomolecules that may be present in solution is observed, these changes can be represented by the Beer-Lambert equation as follows [17]

$$A_{\max} = \epsilon\ b \left(\frac{[DNa]}{K_{EQ}}\right)\left(\frac{1}{Na^+}\right) \qquad (10)$$

Using this equation a relationship between OH^- ion concentration and the dye-complex absorbance can be established. A Fourier series relationship can be obtained from this relationship from which only the first item is of importance. Linearity due to Na^+ concentrations may be used in many applications where the detection of caustic soda is important. At high concentrations of OH-, Eq. 10 becomes non-linear because the dye becomes insensitive due to saturation and the lack of open pores into which the ions can permeate.

The equilibrium constant of the dye-hydrogen ion complex calculated from the slope of the curve from Eq. 3 for the NIR dye in solution was calculated as $5.69\text{x}10^2$ with a correlation coefficient of 0.909. The magnitude of the dyes' association constants and their ability to complex to the dye, dictates the value of K_{EQ}, and the dynamic response of the dye. As anticipated, in solution the K_{EQ} high compared to the values obtained in the probe where the dye has certain limitations on the amount of complexation.

Matrix stability

The effect of Nafion on the dyes is shown in Table 2. All of the dyes are stable in the polymer matrix. A drastic change in the measured values indicates that care must be excercised when Nafion is used as the support matrix in probe applications.

Matrix thickness

The response of the probe can be controlled by the thickness of the polymer membrane [51]. In this investigation, three different coatings were applied to the polymer support in order to determine the best response. The coatings used were 5, 10 and 15 µl polymer-dye solution. More volume can be applied but the time for solvent evaporation is longer without gaining any sensitivity in the probes response. It was also observed that thicker membranes are slower to produce a steady response. One disadvantage of using a large probe (larger than 5 mm) is that the tip may be too large for certain applications. The fluorescence signal response for the probe coated with 5 µl was weak. Accordingly all other coatings were made using 10 µl.

The thickness of the membrane can be calculated by Fick's law of diffusion:

$$L = (\tau D_c)^{1/2}$$

where L is the thickness of the membrane, τ is the time for the response to reach a 95% steady state signal and D is the diffusion of the analytes which range from 10^{-8} (large molecules) to 10^{-10} cm_2/sec (typical values for ions) [52]. The response time required for a probe made of Nafion at pH 7.00 was less than 5.0 seconds. Similar results were obtained for all successive readings. This may suggest that the size of the probe was not affected by the amount of analytes.

The ability of the hydrogen ions to diffuse in and out of the polymer matrix depends on the pore size of the permeable polymer and the free space available for the ions to complex with the dye. In this case, only small ions can penetrate the matrix and larger molecules will be excluded from the matrix. As the dye-analyte complexes increase, the amount of free space into which other analytes can diffuse decreases. This principle can be used to make more selective fiber optic probes suited to a particular analyte. However, the selectivity will be determined primarily by the functional group in the dye and secondly by the nature of the polymer matrix entrapping the dye (pore size).

Probe response

The ability of an optical fiber to accept light is represented by its numerical aperture (NA). Large NA values indicate that the fiber can accept light from very wide angles, and the position of the light source (including fluorescence of the dye) does not necessarily have to be at the center of the core. Hence high NA values would permit the fiber to accept the fluorescent signal from a wider illuminated region which would afford a larger detected signal. A diagram of the fiber as it receives the fluorescent signal is shown in Figure 4.

As the analyte concentration changes, the refractive index (RI) of the sample medium also changes. A large change in the RI may interfere with the fluorescent signal because the numerical aperture of the fiber is proportional to the RI as shown in the relation below:

$$n_o \sin \phi = \left(n_1^2 - n_2^2\right)^{1/2} \qquad (12)$$

where n_o is the RI of the solution, ϕ is the numerical aperture (collection angle), n_1 is the RI of the fiber's core and n_2 is the RI of the fiber cladding.

For example, the RI of water at 25°C changes from 1.3325 to 1.3358 when 1% (by wt.) of NaOH is present in solution. For less concentrated NaOH solutions, the RI change is less significant. The presence of NaOH produced a change of less than 0.1% in the NA of the fiber [53]. This change in the NA has no significant effect. Hence, the signal that is measured is solely from the changes of the dye as it complexes with the ions in solution. The FOPP has a dynamic response range from pH 6.80 to pH 12. The response of the probe to changes in analyte concentration was less than 5 seconds. Longer response times were observed as the concentration of NaOH increased, but it was no longer than 10 seconds. The longer response may be attributed to the limited diffusion of NaOH through the Nafion matrix as the limiting rate of the response or may be caused

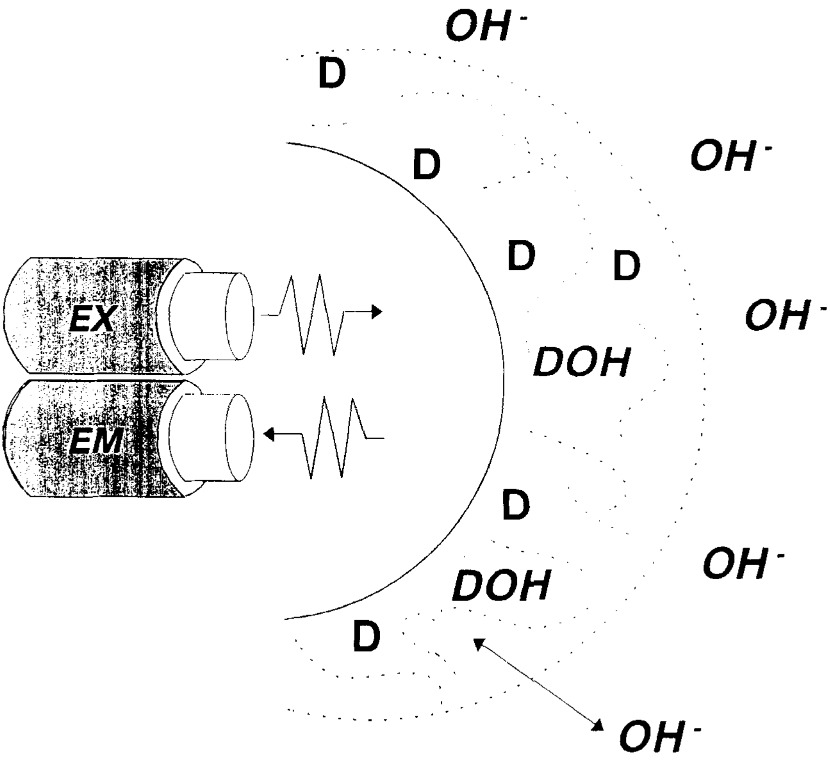

Figure 4.
Distal end of the Fiber Optic pH Probe that represents the response mechanism of the analyte as it complexes with the NIR dye. The smaller analytes can move in and out of the polymer matrix whereas the bigger NIR dye is entrapped in the matrix.

by the saturation of the dye. This probe is very stable and showed good stability and reproducibility.

The probe made with CNC169/Nafion showed a linear response to the NaOH changes in concentration. The complexation of the NIR dye to Na^+ allows this dye to be used where the determination of high concentrations of NaOH is necessary and can be used in qualitative analysis. The complexation of the dye in basic conditions is supported by IR data. The NIR region is characterized by first and second overtones, a combination of high frequency stretching bands due to hydrogen bonding. The first overtones are usually OH- stretching vibrations; the second overtones represent CH-stretches. These overtones (and similar combinations) are caused by the anharmonic nature of the molecular vibration. Most of these vibrations originate with hydrogen stretching vibrations, usually CH, OH, and NH bonds because of the light mass of the hydrogen atom [54]. Therefore, the hydrogen stretching affects different functional groups by causing a band broadening and a shift to the lower frequency as shown in Figure 5. The stretching vibrations of some of the overtones may vary from 1100 nm for a single bond, to approximately 2500 nm for a double bond. Under neutral conditions the peak of the carbonyl group at 1735 cm^{-1} decreases with an increase of a sharp peak at 1680 cm^{-1} (see Figure 6).

Probe stability

The reproducibility and durability of the probe was determined by comparing the intensity data signal of the same probe stored at different time periods. The probe was stored at room temperatures to emulate possible remote testing. Figure 7 shows the response measured using the probe-I indicating a fully reversible mechanism. The probe was reversed by immersing it in an acidic solution for less than 2 minutes and then immersing it in the controlled vials. The response of the probe in the two runs is almost identical. The same procedure can be performed several times. Probes made with Nafion showed better storage durability and reproducibility as compared to the gelatin probes. Nafion probes can be soaked in HCl (0.5 M) to regenerate the baseline and then stored without any further preparation for up to a month.

The FOPP probe was tested at different temperatures. The stability of the response ranging from 30°C to 70°C was investigated. At low temperatures the probes havebeen shown to be stable and the response is linear. This is typically the case for many optical fiber systems. Low temperature has no effect on the mechanism of the probe. Osmotic equilibrium is rapidly achieved and the dye can complex with the analyte. At higher temperatures (above 50°C) the system suffers mechanical problems. The sensitivity of the developed FOPP was investigated by using KOH (or KCl) and LiOH (or LiCl). This indicates that the sensor response is not affected by the presence of other ions.

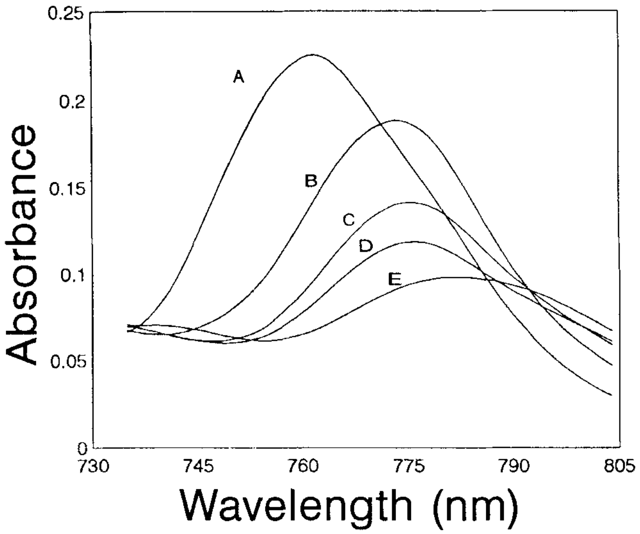

Figure 5.
Experimental absorbance data for different pH solutions. The absorbance maxima shifts at different solution pH. Experimental data for pH 10.8 (A), pH 10.2 (B), pH 9.4 (C), pH 8.0 (D) and pH 5.6 (E) are shown.

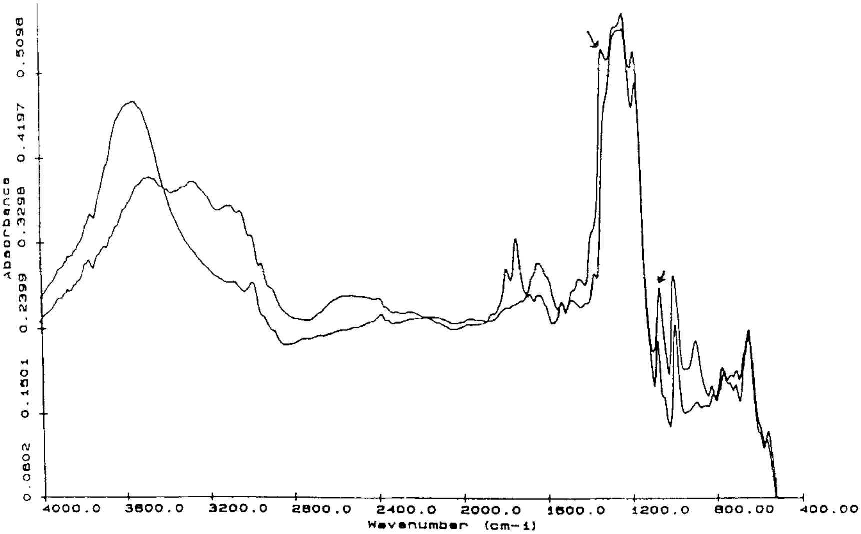

Figure 6.
Representative changes in the IR spetra of the CNC169 dye in the presence of NaOH.

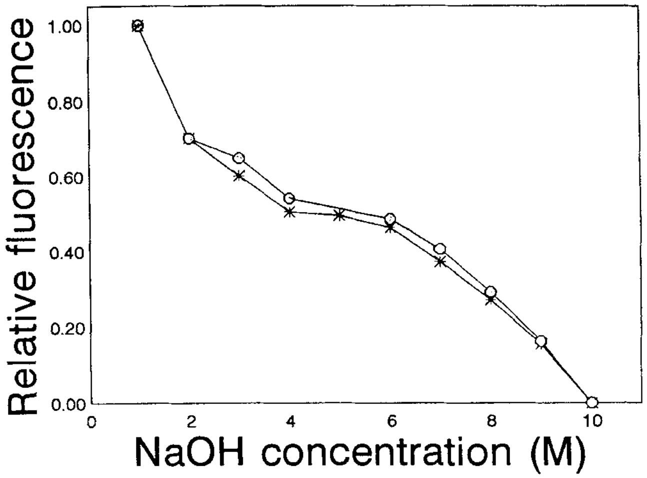

Figure 7.
Typical response of the Fiber Optic pH sensor at different NaOH concentrations. Experimental data for the first run (circles) and reproducibility for the same probe regenerated in a 0.1M HCl solution.

Quantum efficiency in the NIR

In addition, the response of the detector is of particular interest for the development of these probes in the NIR region. The quantum efficiency (QE) of PMT decreases as it approaches the NIR region. Typical QE values for PMT at 800 nm are about 1% compared to 80% using silicon photodiodes; therefore, lower limits of detection may be reached using photodiodes. The main advantage of using SAPD is that low detection limits comparable or better than conventional photo multiplier tubes (PMT) [55] are possible. In addition, they have been shown to have much higher QE values than PMTs in the NIR region [56]. SAPD have been shown to possess a high QE of 7.5% at 633 nm with a minimal dark signal when operated at 3 V above the breakdown voltage at low temperatures [57]. These diodes (made of germanium for long wavelength NIR detection) operate at voltage levels above the breakdown voltage. This is particularly advantageous for detection of samples at low concentration levels. On numerous occasions, we have found that NIR fluorescence results in a high intensity signal. In these cases, the use of a simple Si photodiode, or the operation of a Si avalanche photodiode other than the Geiger-mode may be completely adequate to simplify the detection system function. These detectors are better suited for NIR applications. Recent experiments for single molecule detection using an SPAD detector have reported a QE of about 40% at 830 nm [58] which suggests better detection capabilities.

Conclusion

The data shown here successfully demonstrate the usefulness of NIR FOPP for the determination of NaOH. This FOPP was developed to demonstrate the utility of NIR dyes in fiber optic applications as a possible alternate technique of analysis. These probes can be used for quantitative detection in industry and waste water treatment. The most encouraging results obtained in this investigation is that continous laser excitation apparently does not deactivate or photobleach the NIR dye. The probes made with Nafion had good reproducibility and long storage durability which make them ideal for on-line measurements. Probes made with gelatin can be used in systems were quantitative analysis is not as necessary. Both polymers can be made disposable. Future work in this area will be the construction of an FOPP for the detection of higher concentration acidic solutions. A limitation for the present system is the size of the probe which may be too large for certain applications. This limitation may be overcome in the future by the construction of a smaller probes. Our research group is presently investigating the use of other new NIR dyes for industrial applications. The goal is to develop a sensitive and selective NIR FOPP that could be used on-line. This instrument could also be made portable for field testing. The synthesis of new types of NIR dyes is necessary for the continuous application development of these types of instruments due to the limited commercial availability of NIR dyes. NIR compounds may be synthesized with fluorescence band maxima above 800 nm and with moieties that may be attached to the polymer support directly. The final goal of this group is to fabricate optical fiber pH probes that are portable and economic for medicine and clinical analysis.

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