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Luminescence probes for the measurement of oxygen

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LUMINESCENCE PROBES FOR
THE MEASUREMENT OF OXYGEN

by

Mary Katherine Carroll
"

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Submitted in partial fulfillment
of the requirements for
Honors in the Department of Chemistry

UNION COLLEGE

June, 1986

ABSTRACT

CARROLL, MARY KATHERINE Luminescence Probes for the
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The goal of this project was to design an optical sensor for oxygen. One type of system suitable for oxygen detection in aqueous solutions is based on two distinct emission bands: one band oxygen-sensitive, the other insensitive to oxygen quenching. By taking the ratio of these bands, a quantitative measurement of the oxygen present could be obtained. This could be accomplished in a system where a fluorescing compound, through triplet state energy transfer, causes a lanthanide ion to luminesce. By choosing a compound which has a triplet energy near the excitation wavelength of terbium(III) or europium(III) and which has a fluorescence that is itself insensitive to oxygen quenching, a suitable system could be developed. The lanthanide luminescence bands would not be subject to direct oxygen quenching. Rather, the triplet state energy transfer to the lanthanide would compete with the rate of quenching of the triplet state by oxygen. As the solution is deaerated, an increase in lanthanide luminescence would be observed. Chemical systems tested for this application include micellar solutions containing a naphthalene- or pyrene-derivative and terbium(III) or europium(III).

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There are many others to whom I am grateful for the past few years. Special thanks must go to Michael Mahony, for many wonderful memories of Union, and to Julie Stone and Roberta Susnow. I am especially grateful for the love and support given to me by my parents, who taught me that I could do whatever I set my mind to.

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INTRODUCTION

Fluorescence is a process by which molecules or atoms, called fluorophores, emit radiation via a photophysical relaxation process. The molecule exists in the singlet ground state before excitation. When a molecule absorbs a photon of appropriate energy, one electron is promoted to a higher energy orbital and the molecule enters a higher energy singlet excited state. Fluorescence is the emission of photons from the singlet excited state to a singlet ground state. Fluorescence is an "allowed" transition, that is, one which is allowed by the selection rules of quantum mechanical theory. The emissive rates of these transitions are high and result in fluorescence lifetimes on the order of $10E-8$ sec (10 nsec). The fluorescence lifetime of a fluorophore is defined as the average time that the fluorophore remains in the excited state (1).

Fluorophores display several general characteristics. First, the light emitted is of lower energy, and therefore longer wavelength, than the light absorbed by the fluorophore. This shift in wavelength is known as the Stokes' shift, and is observed for all systems except for atoms in the vapor phase. Second, as a result of the rapid relaxation of higher energy states to the lowest singlet excited state, the same emission

spectrum is generally observed for all excitation wavelengths. Third, the emission and absorption spectra of a given fluorophore are usually mirror images of each other because electronic transitions occur without the position of the nuclei in the fluorophore changing. Deviations from the mirror rule can result from a change in the geometric arrangement of the nuclei in the excited state, or as a result of excited state dimer (eximer) formation (1).

Most fluorophores used in this study have aromatic ring systems. The compounds used are, therefore, often highly nonpolar and relatively insoluble in water.

Phosphorescence emission results from the transition from the triplet excited state to the singlet ground state of a molecule. Transitions of this sort are not allowed quantum mechanically and have slow emissive rates as well. Phosphorescence lifetimes are much longer than fluorescence lifetimes, ranging from milliseconds to seconds (1).

The overall goal of this research project is to find a suitable system for the luminescence detection and quantification of oxygen present in an aqueous solution and to then design a fiber optic sensor in which this detection

system could be implemented.

Oxygen quenches the luminescence (fluorescence and/or phosphorescence) of many chemical species. As a result of this link between luminescence and oxygen, luminescence can be used to measure oxygen concentration. The Stern-Volmer equation describes collisional quenching of fluorescence, the process by which oxygen quenching occurs:

$$\frac{I^0}{I} = 1 + k_q \tau [Q]$$

where I^0/I is the ratio of the fluorescence intensity of the fluorophore in the absence of oxygen to that in the presence of oxygen, k_q is the bimolecular quenching constant, $[Q]$ is the concentration of the quencher (in our case, the concentration of oxygen), and τ is the lifetime of the fluorophore in the absence of the quencher (1). The greater the value of I^0/I is for a given concentration of oxygen, the more sensitive the fluorescence is to oxygen.

The conventional oxygen electrode (Clark electrode, Figure 1) currently used for oxygen measurement has several disadvantages which are not present in a luminescence sensor. The Clark electrode consumes oxygen during measurement (see

electrode reaction, Figure 1) and is, therefore, dependent on a constant rate of mass transfer across the oxygen-sensitive membrane separating the electrode from the solution to be analyzed. Any substance which inhibits this mass transfer will affect the measurement of oxygen. In addition, the Clark electrode is not readily adaptable to very small samples. For example, the electrode used in this research requires a minimum of 40ml of sample and constant stirring of this solution.

The response of luminescence probes is dependent on the equilibrium level of oxygen and is not dependent on mass flow rate. It is possible to miniaturize such a probe, thereby eliminating the need for large samples.

One type of chemical system suitable for oxygen detection is based on two distinct emission bands: one band oxygen-sensitive, the other insensitive to oxygen quenching. The ideal system would be one in which the two luminescence bands were of similar intensities in the visible region. By observing the ratio of the intensity of one band to the other, a quantitative measurement of the oxygen present could be obtained.

This two-band ratio probe has advantages over methods

which measure concentration of oxygen by using the absolute luminescence intensity. These methods (2,3,4,5) require careful calibration and are dependent on changes in the concentration of the luminescing species. The absolute luminescence intensity will also be affected by fluctuations in source intensity. In short, the measurements will have inherent problems in precision and accuracy.

Since the concentration of oxygen is measured as a ratio of the intensities of the two bands in a two emission band sensor, calibration would not be required and the measurements would not be affected by fluctuations in sample concentration or source intensity. Overall, the two band sensor should be a more effective analytical tool than the Clark electrode or a single-band sensor.

Our initial idea for an optical oxygen sensor of this type was based on the knowledge that the oxygen quenching of many molecules was inhibited by performing the measurements in a micellar solution. For example, nonpolar fluorophores in the presence of micelles such as sodium dodecyl sulfate (SDS, above its critical micelle concentration (cmc), the point at which the surfactant molecules form a micelle) have their emission protected from oxygen quenching. These fluorophores

apparently for 1:1 complexes with SDS above the cmc (6). We thought that by using fluorinated surfactants, whose end chain groups are more nonpolar than the hydrocarbon chain of SDS, we might be able to decrease the rate of exchange of the fluorophore in and out of the micelle. If this exchange is rapid, oxygen quenching will occur as the molecule exits the micelle. We hoped that we might be able to develop a system based on two emission bands from the same molecule. One band would be insensitive to oxygen quenching; the molecules responsible for this band would be trapped inside the extremely nonpolar fluorinated micelle interior. The other band would be sensitive to oxygen quenching, as the molecules responsible for this band would be outside of the micelle and, thus, subject to oxygen quenching. The designation "Project I" is used for those systems tested under this theory.

Another possible sensor is based on a system in which a fluorescing compound, through triplet state energy transfer, causes a lanthanide ion to luminesce (Figure 2). By choosing a compound that has a triplet state energy near the excitation wavelength of terbium(III) or europium(III) and a fluorescence that is itself insensitive to oxygen quenching, a suitable system could be obtained. The lanthanide luminescence bands are not subject to direct oxygen quenching (7). Rather, the triplet state energy transfer competes with the oxygen

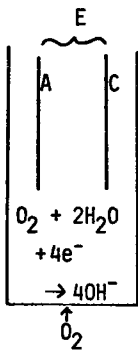
quenching of the triplet state (see Figure 2). In the presence of oxygen, some triplet energy will be quenched, and a decrease in lanthanide luminescence will be observed.

Lanthanide emission can be excited directly, but in order to measure lanthanide emission, large amounts of exciting light intensity are required. Terbium emission can be directly excited in some solutions, using a normal fluorescence spectrophotometer lamp. The intensity of the emission is very weak in this case. A laser is usually necessary to cause direct europium luminescence. Since direct excitation of lanthanides is, at best, difficult, it is not expected to interfere in the oxygen-sensing system described above.

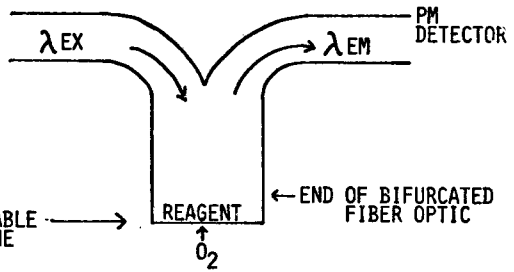
Systems tested for a fluorophore/lanthanide system are designated "Projects II and III."

FIGURE 1: Comparison of electrode and fiber optic sensors.

AMPEROMETRIC
GAS SENSOR

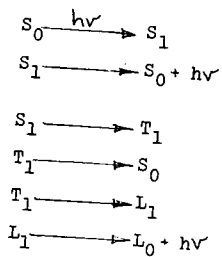
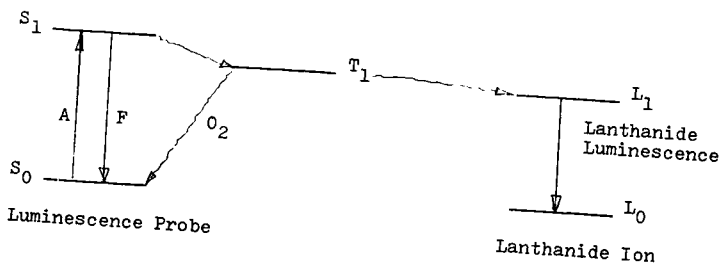


FIBER OPTIC
LUMINESCENCE SENSOR



← GAS-PERMEABLE MEMBRANE →

FIGURE 2: Triplet State Energy Transfer.



$A \equiv$ absorption

$F \equiv$ Fluorescence (reference band, oxygen insensitive)

intersystem crossing

oxygen quenching

energy transfer to lanthanide

lanthanide luminescence

EXPERIMENTAL

COMPOUNDS USED:

Structures of all compounds used are shown in Table 1. Compounds are listed under the Project in which they were first used. With the exception of the sodium dodecyl sulfate, all chemicals were used without further purification. Solutions were prepared using distilled, deionized water as solvent.

The following chemicals were purchased from Aldrich:

Dodecyl sulfate, sodium salt (SDS)
Terbium(III) chloride, hexahydrate.
Quin2.
Calcein Blue.
1-Bromonaphthalene.
3-Indoleglyoxylic acid.
1-Pyrenebutyric acid.

Chemicals provided by Instrumentation Labs.:

4-Morpholinepropanesulfonic acid (MOPS).
Europium(III) oxide.
L-6782.

Chemicals purchased from Molecular Probes, Inc.:

2-(trifluoroacetylacetyl)naphthalene (β -NFA).
5-dimethylaminonaphthalene-1-sulfonamido
ethyltrimethylammonium iodide.
1-Pyrenemethyltrimethylammonium iodide.
6-Dodecanoyl-2-methoxynaphthalene.

The following chemicals were purchased from Sigma:

1,2-bis(2-aminophenoxyethane-N,N,N',N'-tetra-
acetic acid, tetrapotassium salt.
Elastase, from porcine pancreas.

Purchased from Eastman Kodak, Co.:

Trioctylphosphine oxide (TOPO).

Synthesized by Dr. Diuakaran Masilamani of Allied Co.:

5-(4-bromo-1-naphthoyl)-1-pentyltrimethylammonium
bromide (BNK-5).

Synthesized by Dr. Eric Lee of the Allied Corp.

BNCOC17.

Obtained from 3M:

FC-95.

Purchased from the Fisher Scientific Company:

Magnesium(II) acetate.

Obtained from the Department of Chemistry of the University of
New Hampshire:

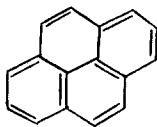
Pyrene.
Sodium dodecyl sulfate (SDS).

TABLE 1: Compounds used in this research project.

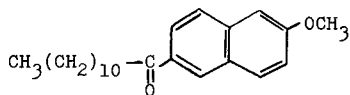
A) Project I

Compound	Structure
FC-95	$C_8F_{17}SO_3^- K^+$
L-6782	$C_8F_{17}SO_2NHCH_2CH_2CH_2N^+(CH_3)_3 Cl^-$
SDS	$CH_3(CH_2)_{11}OSO_3^- Na^+$

Pyrene



6-Dodecanoyl-2-methoxynaphthalene



5-Dimethylaminonaphthalene-1-sulfonamidoethyl-trimethylammonium iodide

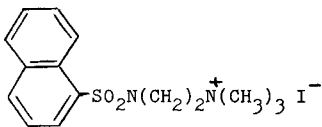
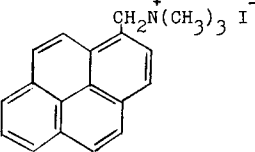
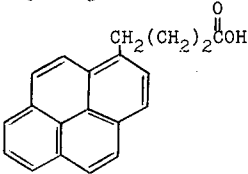
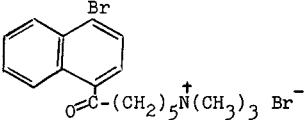
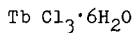


TABLE 1A, cont.

Compound	Structure
1-Pyrenemethyltrimethylammonium iodide	 $\text{CH}_2\text{N}^+(\text{CH}_3)_3 \text{I}^-$
1-Pyrenebutyric acid (PBA)	 $\text{CH}_2(\text{CH}_2)_2\overset{\text{O}}{\parallel}\text{COH}$
BNK-5	 Br^-

B) Project II

Terbium(III) chloride hexahydrate



MOPS

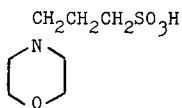
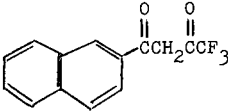
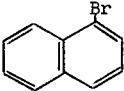
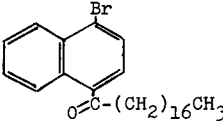


TABLE 1, cont.

C) Project III

Compound	Structure
Europium(III) oxide	Eu_2O_3
Quin2	
Calcein Blue	
BAPTA	
3-Indoleglyoxylic acid	

TABLE 1C, cont.

Compound	Structure
β -NTA	
1-bromonaphthalene	
ENCOC17	
TOPO	$(\text{CH}_3(\text{CH}_2)_7)_3\text{PO}$
Magnesium acetate	$(\text{CH}_3\text{COO})_2\text{Mg}$

INSTRUMENTATION:

Instrumentation used to record excitation, emission, and absorption spectra include a SLM Spectrofluorometer and a Perkin-Elmer 204 Fluorescence Spectrophotometer at the University of New Hampshire, and a Hitachi Perkin-Elmer MPF-2A Fluorescence Spectrophotometer and a Perkin-Elmer Lambda 3B UV/VIS Spectrophotometer at Union College. A Jenway 9070 Oxygen Meter was used to measure oxygen concentration in samples.

PROCEDURES:

The emission and excitation spectra of the compounds and systems tested were first recorded in air-saturated solution (containing approximately 9ppm oxygen). Solutions were then degassed with nitrogen gas for a period of ten to fifty minutes, and the emission and excitation spectra were again recorded. In some cases, buffers were used to maintain a pH near that in the literature for a certain compound. In others, a surfactant was added to inhibit oxygen quenching of the luminescence.

RESULTS

PROJECT I:

The initial idea was to try to form a complex between pyrene and a fluorinated surfactant. Hydrocarbon surfactants had been shown to protect fluorophores from oxygen quenching. Pyrene has a very distinctive emission spectrum. It was hoped that such a complex could also be formed with the fluorinated surfactants.

The first system tested of this type was pyrene and a fluorinated surfactant, FC-95. Pyrene was chosen because it has a very distinctive emission spectrum. The relative intensities at which the various emission bands of pyrene occur are based on the polarity of the solution (8). Pyrene is a highly nonpolar molecule and has been shown to associate with the nonpolar part of hydrocarbon micelles (9). It was hoped that pyrene would substitute more strongly into the fluorinated micelles.

The fluorinated surfactant, FC-95, was dissolved in a saturated solution of pyrene in water. Final concentration of the surfactant is 3.4×10^{-3} M; concentration of pyrene is 5.6×10^{-6} M (100 ppb). Spectra of the pyrene solution alone

were also recorded. The pyrene spectra with and without the anionic surfactant FC-95 are the same. Pyrene is, therefore, not substituting into the micelle.

Various combinations of cationic fluorescing compounds (including derivatives of pyrene) and the anionic surfactant FC-95 were tested. In none of these cases is a difference observed between the spectra of solutions with and without the surfactant present (see Table 2). Thus, none of these systems are suitable for an optical oxygen sensor. In addition, no complex was formed through a combination of cationic surfactant L-6782 and anionic pyrene derivative 1-pyrenebutyric acid. Although the 1-pyrenebutyric acid is much less soluble in the micellar solution than in water alone, no difference is observed in the spectra.

It was concluded that the fluorinated surfactants did not complex with fluorophores of this sort. Although we would expect the nonpolar pyrene derivatives to be dissolved in such nonpolar micelles, it is apparent that the fluorinated surfactants' micelle interiors are so exclusive as to reject even as nonpolar a compound as pyrene. For this reason, Project I was abandoned.

TABLE 2: Systems tested in Project I.

<u>Surfactant</u>	<u>Fluorescing Compound</u>
1) FC-95 (3.4×10^{-3} M)	Pyrene (5.6×10^{-6} M).
2) FC-95 (2.3×10^{-3} M)	6-Dodecanoyl-2-methoxynaphthalene (1.1×10^{-5} M).
3) FC-95 (2.1×10^{-3} M)	5-Dimethylaminonaphthalene-1- sulfonamidoethyltrimethylammonium iodide (2.0×10^{-4} M).
4) FC-95 (2.3×10^{-4} M)	BNK5 (concentration unknown).
5) FC-95 (4.0×10^{-4} M)	1-Pyrenemethyltrimethylammonium iodide (4.5×10^{-6} M).
6) SDS (1.7×10^{-3} M)	5-Dimethylaminonaphthalene-1- sulfonamidoethyltrimethylammonium iodide (4.4×10^{-4} M).
7) L-6782 (2.0×10^{-3} M)	1-Pyrenebutyric acid (5.5×10^{-5} M).

PROJECT II:

Another possibility for a suitable chemical system for an optical oxygen sensor is to find a system whereby a fluorescing compound, through triplet state energy transfer, would cause a lanthanide to luminesce. In this ideal system there would be two emission bands: one band would be oxygen-sensitive, and the other would be insensitive to oxygen quenching.

The method picked for testing this possibility was to reproduce the successful results of Prendergast et.al. (7), and then expand upon that research.

The system in question is an elastase-terbium complex. Prendergast et.al. found that this system exhibited the desired characteristics. They performed all runs in a pH 6.6 MOPS (4-morpholinepropanesulfonic acid)/KCl buffer. As MOPS was not immediately available, we substituted other buffers.

A pH 6.71 potassium dihydrogen phosphate/NaOH buffer was made. Upon the addition of $TbCl_3$, a large quantity of white precipitate formed. No information on the solubility of terbium hydroxide or terbium phosphate was available. A simple test proved them both to be insoluble in aqueous

solution.

In a pH 6.17 acetic acid/sodium acetate buffer, and in a pH 6.76 tris(hydroxymethyl)aminomethane buffer, the emission spectrum of elastase in the presence of terbium(III) ions and that of elastase alone are the same.

Finally, some MOPS was obtained. Following the directions in Prendergast et.al., a pH 6.60 MOPS/KCl buffer was made. On first glance the emission spectra for the solutions with and without terbium present appeared the same. Upon closer scrutiny a tiny peak at about 545nm is observed (see Figures 3 and 4). This is one of the wavelengths at which terbium luminesces. Unfortunately, this peak was too small to be measured with any degree of accuracy.

FIGURE 3:

Emission spectrum (320-600nm)
Elastase in pH 6.6 MOPS/KCl buffer
excitation wavelength 295nm
8/2/85

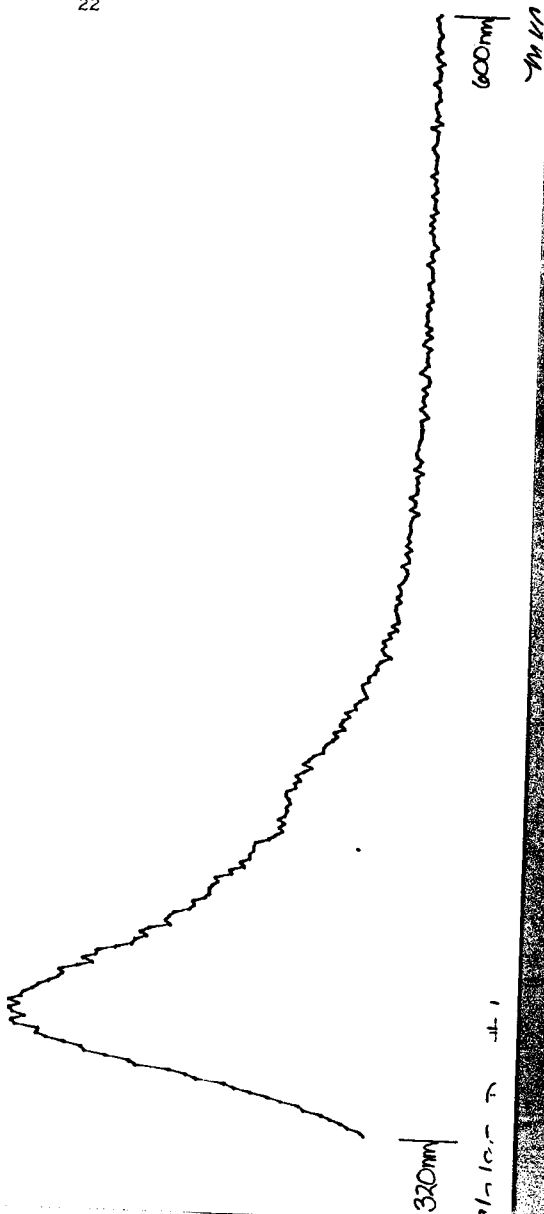
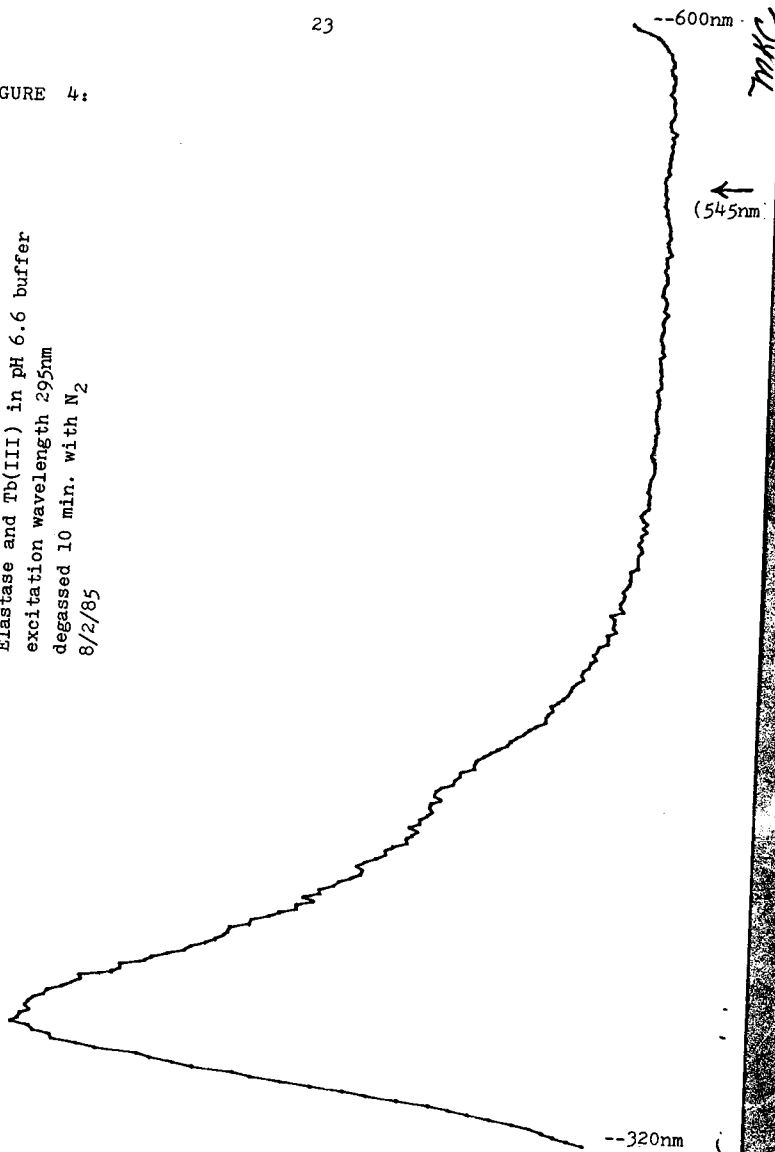


FIGURE 4:

Emission spectrum (320-600nm)
Elastase and Tb(III) in pH 6.6 buffer
excitation wavelength 295nm
degassed 10 min. with N₂
8/2/85



PROJECT III:

Since the elastase/terbium(III) solutions did not exhibit triplet state transfer, other combinations of fluorophore and lanthanide ion were tried. Table 3 lists those systems tested in Project III.

In the majority of the systems tested there is no difference observed between the emission spectra with and without lanthanide ion present. No energy transfer is observed for in the systems listed in Table 4. These systems are not suitable for a two-band optical oxygen sensor.

The system Quin2 and europium(III) was also tried. Two solutions were prepared: 1×10^{-4} M Quin2 in pH 6.60 MOPS/KCl buffer, and 1×10^{-4} M Quin2, 1×10^{-4} M Eu(III) in pH 6.60 MOPS/KCl buffer. Excitation and emission spectra were run for these solutions.

At the University of New Hampshire, results were obtained which initially looked promising for the Quin2/europium system. The observed emission maxima are listed in Table 5. When samples were excited at 343nm, three emission bands were observed for Quin2 alone; with the addition of europium(III)

two smaller bands appeared at longer wavelengths and the longest wavelength band from Quin2 increased in intensity. The bands at 373nm and 392nm were sensitive to oxygen quenching; the others were not.

These results are misleading. There was some sample contamination, most probably due to pyrene or a pyrene derivative, which caused the appearance of the emission bands at 373nm and 392nm. These were the only oxygen-sensitive bands in the spectra. Samples subsequently run at Union College on fresh solutions with the same concentrations present yielded one Quin2 emission band, which has its maximum emission at about 496nm (see Figure 5). Literature results confirm that this is due to Quin2 fluorescence ($\lambda_{\text{ex}}=339\text{nm}$, $\lambda_{\text{em}}=492\text{nm}$) (10). When spectra of solutions containing europium(III) ions are run, the Quin2 fluorescence is nearly completely quenched and europium luminescence is observed (Figure 6). A solution of europium(III) oxide alone in the same buffer is not directly excited by 339nm radiation. The europium luminescence in the presence of Quin2 is, thus, not due to direct excitation, but due to energy transfer.

Although energy transfer did occur, it is not triplet state energy transfer, but, rather, singlet state transfer (Figure 7). This transfer is so fast that it quenches the

Quin2 emission itself; it is also much more rapid than the diffusion of oxygen through the solution. Thus, no oxygen quenching is observed for the lanthanide emission, and this system is not suitable for an optical oxygen sensor.

The next set of systems tested was beta-NTA (2-naphoyltrifluoroacetone) and europium or terbium. Hemmila et.al. indicated that energy transfer from β -NTA to chelated lanthanide metals occurs (11). They report optimal conditions when a solution of 1.5×10^{-5} M β -NTA, 5.0×10^{-5} M TOPO, and 0.1% Triton X-100 in pH 3.2 phthalate buffer is used. A similar solution was prepared at Union. As naphthalene and its derivatives are relatively insoluble in aqueous solutions, a saturated β -NTA stock solution was prepared in pH 3.3 phthalate buffer. The absorption spectra for various dilutions of the stock solution are extremely intense, and dilutions of 1:1000 are necessary before fluorescence measurements can be taken.

For a 1:100 dilution of the β -NTA stock solution, the maximum absorbance is found to be 0.608 at 279nm. As no literature value for the molar absorptivity of beta-NTA could be found, data for a similar compound, 2-acetylnaphthalene, was used. At 282nm the molar absorptivity (ϵ) of

2-acetylnaphthalene is 9100 L/cm/mol in methanol (12). This relates to a β -NTA concentration of 6.7×10^{-5} M using Beer's Law ($A=abc$). This corresponds to a β -NTA stock solution concentration of 6.7×10^{-3} M.

As the maximum concentration of β -NTA possible in the stock solution was only 5.6×10^{-6} M, these results are misleading. A phthalate buffer should not have been used for the measurements; it interfered with the absorption spectra as it is a fluorescing compound, and was present in high concentrations (0.1 M) in the buffer. The potassium hydrogen phthalate strongly absorbed and gives a huge background absorption for the solution. Another buffer must be used.

A pH 4.00 acetic acid/sodium acetate buffer was prepared, and another β -NTA stock solution made. If all the β -NTA was dissolved in the buffer, the concentration of β -NTA in solution would be 5.8×10^{-5} M. Absorption spectra were run for this stock solution and the value of a for 2-acetylnaphthalene again used to calculate the approximate β -NTA in the solution (see Table 6). It is apparent from this table that the concentration of β -NTA increased with time. The approximate concentrations calculated are reasonable when viewed in light of the maximum concentration possible.

The excitation and emission spectra of the stock solution were run. Excitation maxima occur at approximately 260nm, 300nm, and 350nm (as expected from the absorption spectrum). The emission is very broad, with a maximum at about 450nm. This is somewhat surprising since most naphthalene derivatives fluoresce at about 350nm. A possible explanation for the dramatic shift in emission wavelength is that the β -NTA is in an enol form in aqueous solution at pH 4.00. Two emission bands might be expected, one for the β -diketone form, the other for the enol form. As only one band was observed, there is only one excited-state form present in this solution. Addition of aliquots of concentrated HCl result in no shift in emission band wavelength over a range of pH 4.00 to pH 1.45. This indicates that no change of form occurs in this pH range, and that, therefore, the same form had been present for Hemmila et.al. at pH 3.2 (11). That group reported an absorption maximum for the enol form of β -NTA at 340nm; this agrees with our results.

When aliquots of a 1×10^{-3} M terbium(III) chloride solution are added to 3.00ml of the β -NTA stock solution, no lanthanide luminescence is observed. Although it is possible that some transfer occurs, terbium luminescence peaks expected at 490nm and 545nm would be obscured by the broad (380nm-560nm) fluorescence band of β -NTA.

A stock solution containing the same concentration of β -NTA, 5×10^{-5} M TOPO, and 0.1% Triton X-100 was prepared for the titration with europium. The emission spectrum of this solution has a maximum at about 450nm when excited with 350nm radiation (Figure 8). With the addition of ten microliters of a 1×10^{-3} M Eu(III) solution, strong luminescence bands are observed at about 580nm, 595nm, and 615nm and the intensity of the β -NTA fluorescence decreases (Figure 9). Subsequent addition of aliquots of europium solution results in a dramatic decrease in the band at 450nm, while the longer wavelength bands increase in intensity.

No direct excitation of europium(III) is observed when a solution without β -NTA is tested. No increase in the intensity of any of the emission bands is observed upon degassing with nitrogen. Therefore, neither the β -NTA fluorescence nor the europium luminescence is sensitive to oxygen quenching. The evidence overwhelmingly points to a singlet state energy transfer mechanism. The Eu(III)/ β -NTA system is not suitable for an optical oxygen sensor.

The systems in which singlet state energy transfer is observed are listed in Table 7. These systems cannot be used

for a two emission-band oxygen sensor.

Fendler et.al. report that micelle-solubilized naphthalene transferred from its triplet state to terbium(III) chloride (13). The micelle increases the solubility of naphthalene in water, as its nonpolar interior provides an environment in which the nonpolar molecule will dissolve. At the same time, the negatively charged sodium dodecyl sulfate (SDS, a surfactant) ions attract the positively charged terbium ions to the surface of the micelle. In the presence of SDS, triplet state transfer occurs. In aqueous solution containing no SDS, triplet-triplet annihilation prevents the triplet state transfer (13). The use of micelles with other nonpolar molecules (primarily naphthalene- and pyrene-derivatives) has been studied (9,14).

Almgren et.al. observed that 1-bromonaphthalene solubilized in SDS sensitizes terbium(III) on the micelle surface (14). The solutions used contained 0.1 M SDS, $10E-4$ M aromatic compound, and varying concentrations of terbium(III). Triplet state transfer was observed (14).

This experiment was repeated at Union College, with minor changes. 1-bromonaphthalene was dissolved in toluene, not

benzene, and then injected into a solution containing SDS and terbium ions. Concentrations used were 0.1 M SDS, 0.01 M Tb(III), and $10E-4$ M 1-bromonaphthalene. Toluene emission is observed for this solution.

To avoid this toluene interference, new solutions were prepared by micropipeting the 1-bromonaphthalene directly into the SDS/Tb(III) solution and stirring the solution overnight.

SDS, obtained from Aldrich, was 98% pure. As a result of the impurities, the SDS solution emitted at about 320nm. This emission interferes with the measurement of the 1-bromonaphthalene fluorescence intensity. The SDS was recrystallized twice with 95% ethanol; an approximately 80% yield was obtained. A new stock solution of the recrystallized SDS shows no emission at 320nm.

Terbium(III) chloride hexahydrate and 1-bromonaphthalene were dissolved into the recrystallized SDS stock solution. In air-saturated solution there is no apparent transfer from the 1-bromonaphthalene to terbium(III); the lanthanide luminescence observed is due to direct excitation of terbium (Figure 10). Upon degassing the solution in the cuvette for 45 minutes with nitrogen, a large increase in Tb(III) luminescence is observed (Figure 11), while the

1-bromonaphthalene fluorescence intensity remains relatively constant. This indicates that transfer is occurring by a triplet state energy path.

Since the system SDS/Tb(III)/1-bromonaphthalene has suitable properties for an optical oxygen sensor, quantitative data were obtained for the system. The oxygen electrode is used to measure the concentration of oxygen in each sample of the solution. Transfer of degassed sample from the main solution to a cuvette results in quenching by oxygen from the atmosphere. Since the system is so sensitive to oxygen quenching, samples are now degassed in a glove bag under nitrogen atmosphere. The glove bag is filled with nitrogen and then purged a total of three times, and then filled a fourth time with nitrogen.

Samples have been measured with reasonable accuracy in the range of 0.1 to 1.0 ppm oxygen. The ratio of the fluorescence intensity at 350nm to the luminescence band at 490nm is calculated for each concentration of oxygen measured. For the concentrations 0.6, 0.3, and 0.1 ppm oxygen, two samples were taken and the intensity ratios show good precision (see Table 8). Although the points seem to form a straight line (correlation coefficient 0.98), there is little reason to believe that it should be a straight line, since the

intensity of terbium luminescence is related to the rate of quenching of the energy transfer by oxygen. A plot of the ratio of intensities vs. concentration is shown in Figure 12.

The SDS/1-bromonaphthalene/Tb(III) system appears to be well-suited for an optical oxygen sensor. It is, however, extremely sensitive to oxygen quenching from the atmosphere. An increased upper range of oxygen determination is desired in order to render this system suitable for a variety of uses. Solutions with increased viscosity were studied. In a more viscous solution the rate of diffusion of oxygen to the 1-bromonaphthalene is slower, and, thus, the rate of quenching of the triplet state is also slower. This should increase the upper range of oxygen determination.

An SDS/Tb(III)/1-bromonaphthalene solution in a 10:90 mixture of glycerol and water has an increased range of oxygen determination. Measurements in the range 0.3 to 2.0 ppm have been made (see Figure 13). The two-fold increase in range does not eliminate the need to work in a glove bag; the solution is still very sensitive to oxygen quenching during transfer of solution under normal atmospheric conditions. Another problem inherent in this viscosity study is that increased viscosity most likely has some effect on the behavior of the oxygen electrode. The rate of mass transfer

across the membrane will be different for different solution viscosities.

The addition of magnesium(II) salts is reported to increase the viscosity of a solution of SDS (15). Magnesium(II) acetate was added to the stock solution, so that the concentration of Mg(II) ions was 0.04 M. A precipitate formed immediately. Upon titration in a cuvette containing 3.00ml of the SDS/Tb(III)/1-bromonaphthalene solution with 20 microliter aliquots of 1.0 M Mg(II), it was determined that the solubility of the magnesium salt of SDS is less than 0.02 M. The increased viscosity reported for solutions containing SDS and Mg (15) is most likely due to a lower critical micelle concentration for the magnesium dodecyl sulfate. Since no increase in triplet state transfer was observed during the titration, and since the solubility of this magnesium salt is lower than the concentration we desired to use, this method was abandoned.

In short, an SDS-solubilized solution of 1-bromonaphthalene and terbium(III) chloride is a suitable chemical system for a two-band optical oxygen sensor over a range of oxygen concentrations from 0.1 to 1.0 or 2.0 ppm.

TABLE 3: Systems tested in Project III.

-
- 1) Quin2 ($10E-4$ M); Eu(III) ($10E-4$ M) in pH 7 THAM/HCl buffer.
 - 2) Quin2 ($10E-4$ M); Eu(III) ($5 \times 10E-5$ M) in pH 6.6 MOPS/KCl buffer.
 - 3) Calcein Blue ($10E-4$ M); Eu(III) ($10E-4$ M) in pH 6.6 MOPS/KCl buffer.
 - 4) BAPTA ($2 \times 10E-4$ M); Eu(III) ($10E-4$ M) in pH 6.6 MOPS/KCl buffer.
 - 5) 3-Indoleglyoxylic acid ($1.7 \times 10E-5$ M); Eu(III) ($6 \times 10E-5$ M) in pH 6.6 MOPS/KCl buffer.
 - 6) Quin2 ($10E-4$ M); Tb(III) ($10E-4$ M) in pH 6.6 MOPS/KCl buffer.
 - 7) β -NTA ($2 \times 10E-5$ M); Eu(III) ($2 \times 10E-5$ M); TOPO ($5 \times 10E-5$ M); 0.1% Triton X-100 in pH 4.0 acetic acid/sodium acetate buffer.
 - 8) β -NTA ($2 \times 10E-5$ M); Tb(III) ($2 \times 10E-5$ M); TOPO ($5 \times 10E-5$ M); 0.1% Triton X-100 in pH 4.0 acetic acid/sodium acetate buffer.
 - 9) 1-bromonaphthalene ($10E-4$ M); Tb(III) (0.01 M); SDS (0.1 M).
 - 10) 1-bromonaphthalene ($10E-4$ M); Tb(III) (0.01 M); SDS (0.1 M) in 10:90 glycerol:water.
 - 11) 1-bromonaphthalene ($10E-4$ M); Tb(III) (0.01 M); SDS (0.1 M); Mg(II) (0.02 M).
 - 12) BNCOC17 ($4 \times 10E-6$ M); Tb(III) (0.01 M); SDS (0.1 M).

TABLE 4: Systems tested in Project III in which no transfer is observed.

<u>Lanthanide</u>	<u>Fluorescing Compound</u>
1. Europium	Calcein Blue.
2. Europium	1,2-bis(2-aminophenoxy)ethane-N,N,N',N'-tetraacetic acid, tetrapotassium salt.
3. Europium	3-Indoleglyoxylic acid.
4. Terbium	Quin2.
5. Terbium	β -NTA.
6. Terbium	ENCOC17.

TABLE 5: Quin2/Europium data from U.N.H.

Sample	Emission maximum	Characteristics
Quin2	373nm	Sharp bands, O ₂ sensitive Broad, intense band insensitive to O ₂ quenching
	392nm	
	496nm	
Quin2 and Europium	373nm	Sharp bands, O ₂ sensitive Broad, less intense insensitive to O ₂ quenching weak intensity bands insensitive to O ₂ quenching europium luminescence
	392nm	
	496nm	
	588nm	
	615nm	

TABLE 6: Calculation of approximate β -NTA concentration.

Date (1985)	Absorption maximum	Absorbance	a (approx.) in $L\text{cm}^{-1}\text{mol}^{-1}$	conc. (approx.) in M
10/30	250nm	0.895	48000	1.9×10^{-5}
	290nm	0.23	9100	2.5×10^{-5}

11/2	251nm	2.7	48000	5.6×10^{-5}
	293nm	0.31	9100	3.4×10^{-5}

TABLE 7: Systems tested in Project III in which singlet state transfer is observed.

<u>Lanthanide</u>	<u>Fluorescing Compound</u>
1. Europium	Quin2.
2. Europium	β -NTA.

TABLE 8: Ratio of intensities vs. concentration of O_2 for the 1-bromonaphthalene/Tb(III)/SDS system.

Sample	I(490)/I(350)	conc. O_2 in ppm	ave. I(490)/I(350)
A	0.3088	0.6	} 0.3094 ± 0.0006
B	0.3099	0.6	
C	0.5309	0.3	} 0.5226 ± 0.008
D	0.5143	0.3	
E	0.5824	0.1	} 0.6231 ± 0.04
F	0.6638	0.1	

FIGURE 5:

Emission spectrum (380-740nm)
Quin2 in pH 6.6 MOPS/KCl buffer
excitation λ 339nm
9/24/85
sample sensitivity 4

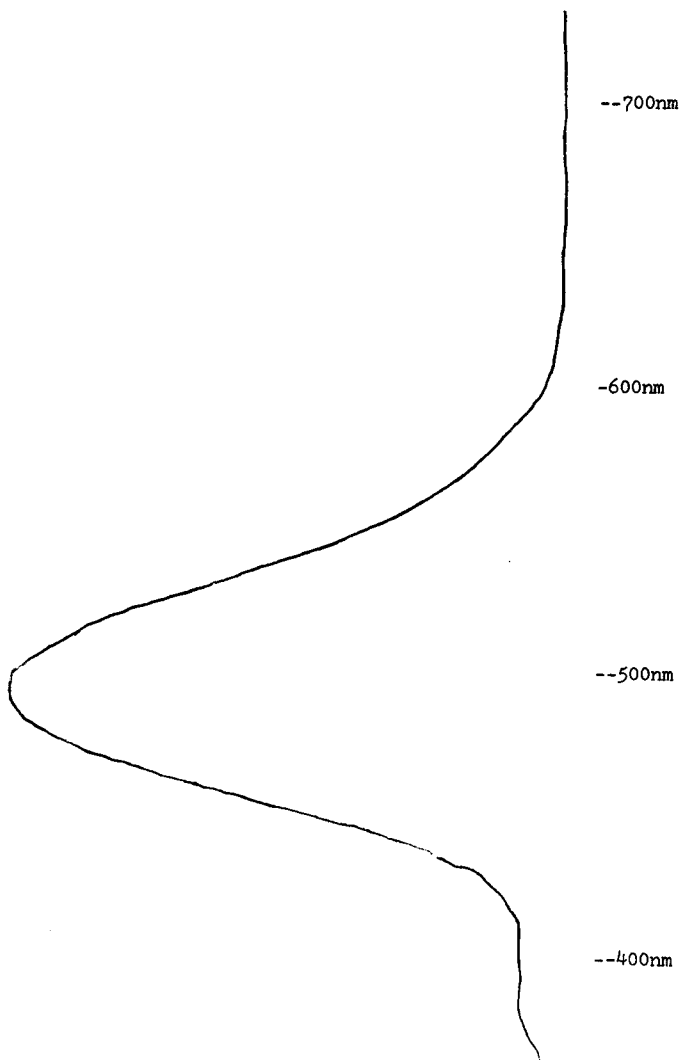


FIGURE 6:

Emission spectrum (360-720nm)
Quin2 and Eu(III) in pH 6.6 buffer
excitation λ 339nm
9/24/85
sample sensitivity 6

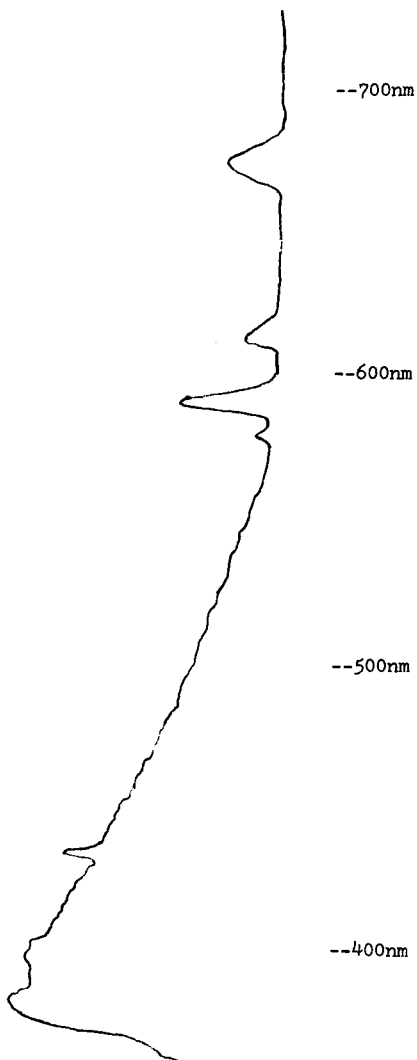
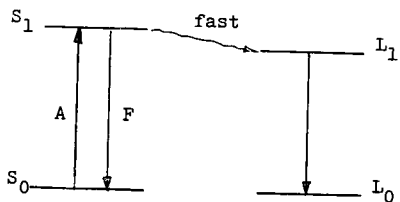


FIGURE 7: Singlet State Energy Transfer.



Luminescence Probe, Lanthanide Ion

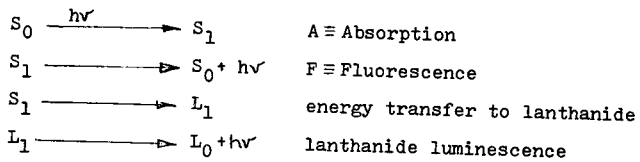


FIGURE 8:

Emission spectrum (360-680nm)
 β -NTA/TOPO/Triton X-100
excitation λ 350nm
11/12/85

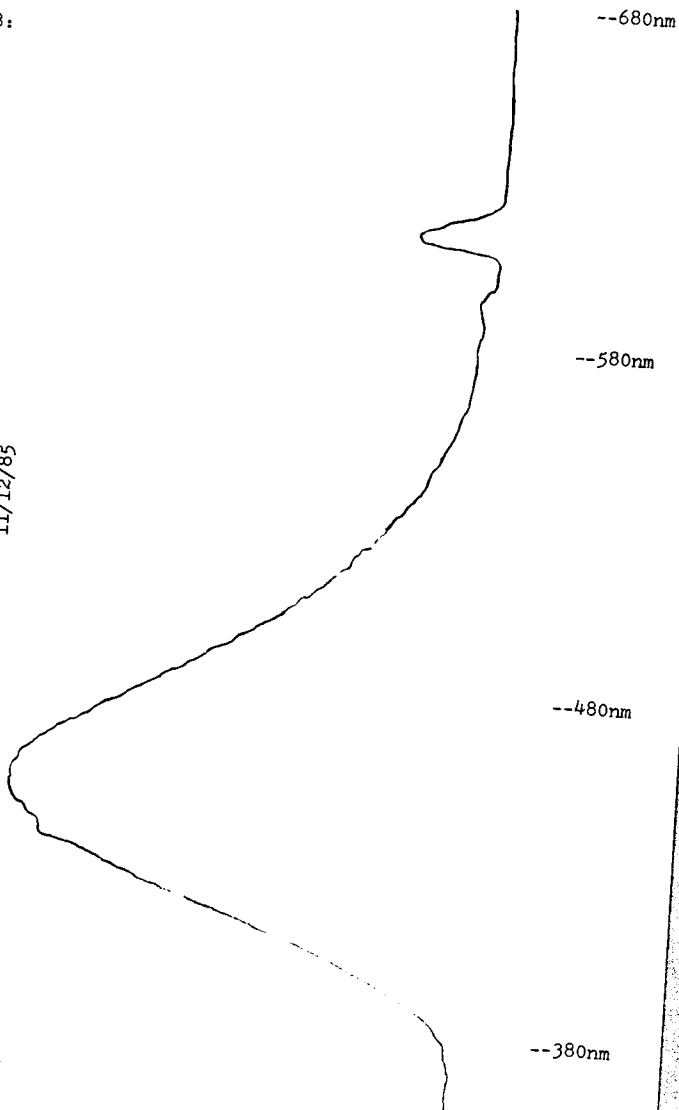


FIGURE 9 :

Emission spectrum (360-680nm)
 β -NTA/TOPO/Triton X-100
and Eu(III)
excitation wavelength 350nm
11/12/85

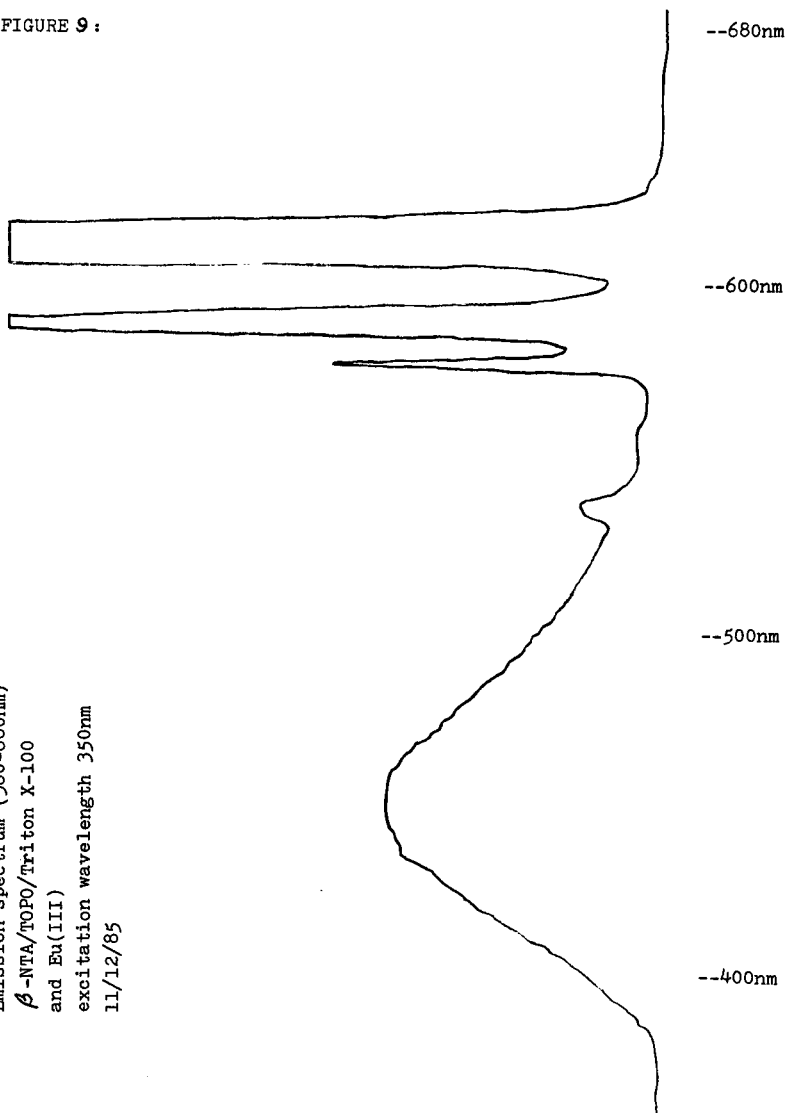


FIGURE 10:

Emission spectrum (300-600nm)
SDS/Tb(III)/1-bromonaphthalene
excitation λ 290nm
2/19/86

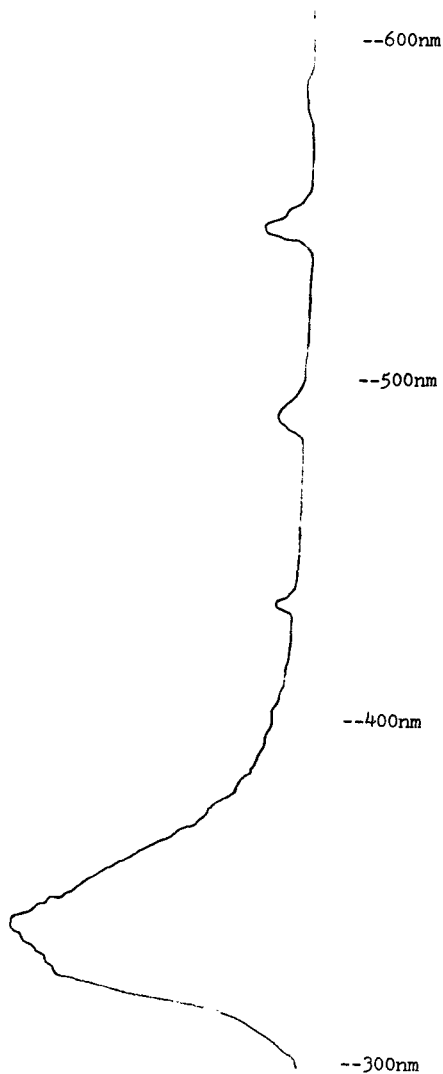


FIGURE 11:

Emission spectrum (300-600nm)
SDS/Tb(III)/1-bromonaphthalene
degassed 45 min. with N₂
excitation λ 290nm
2/19/86

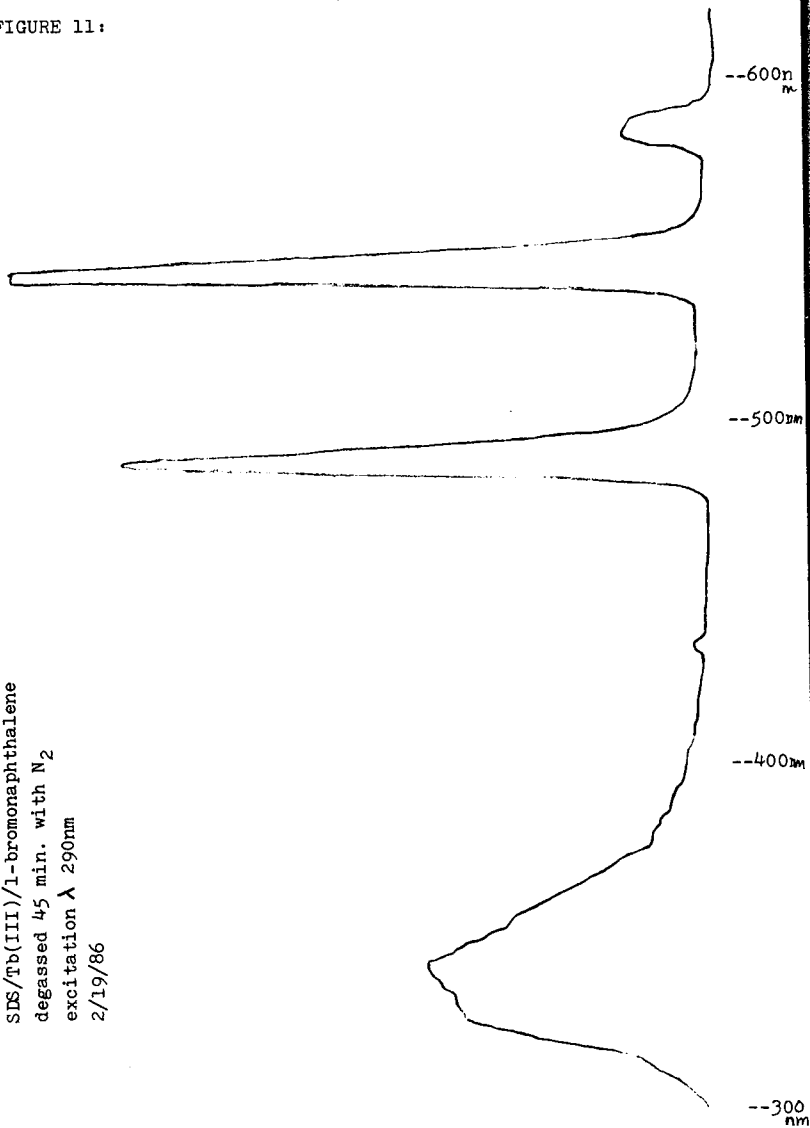


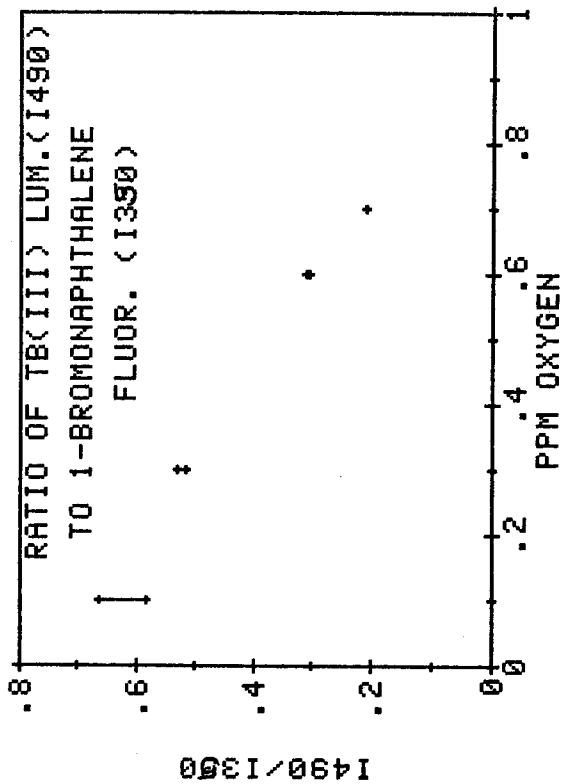
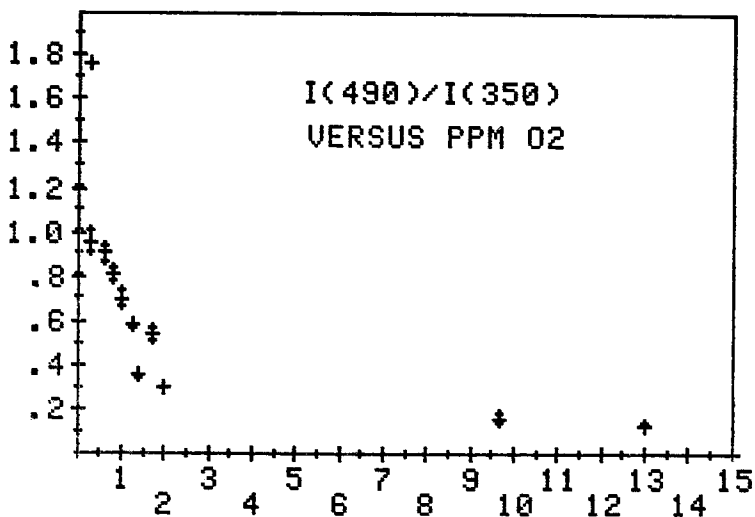
FIGURE 12: I(490)/I(350) vs. conc. O₂.

FIGURE 13: $I(490)/I(350)$ versus concentration of O_2
for a more viscous solution.

SDS/Tb(III)/1-bromonaphthalene in 10:90 glycerol:water.



DISCUSSION

Project I:

The anionic and cationic fluorinated surfactants studied form micelles which have more nonpolar interiors than their hydrocarbon counterparts. This is expected, since the carbon-fluorine bonds are more nonpolar than carbon-hydrogen bonds. We had hoped that this increase in nonpolarity of the micelle interior would result in an increased solubility of pyrene (or a similar nonpolar compound) in an aqueous solution, as a result of the nonpolar fluorophore migrating into the fluorinated micelle. Since the micelle environment would be so nonpolar, we would expect the fluorophore to remain in the micelle longer than in a hydrocarbon micelle. This was not the case.

Although the fluorinated micelles are, indeed, more nonpolar than the hydrocarbon micelles, they are so nonpolar that they will not allow pyrene to occupy their interior. Pyrene is normally thought of as an extremely nonpolar molecule. Its aromatic ring structure indicates its nonpolarity (see Table 1). However, the fluorinated

surfactants are apparently even more nonpolar than pyrene; they therefore exclude pyrene from dissolving in their interior. Pyrene, very insoluble in water, is actually more soluble in water than in these surfactants.

Since spectra of pyrene and the other fluorophores studied in Project I appear the same in the presence and absence of a fluorinated surfactant, the idea of a two-band sensor based on the fluorescence of a molecule inside and outside the fluorinated micelle cannot be realized. It is interesting to note that a great deal of information on these fluorinated surfactants was gained by this experiment, and, although it was a failure as far as oxygen sensors are concerned, it provided data on the behavior of the surfactants in aqueous solution.

Project II:

The results of Prendergast et.al. (7) were not reproducible. As all reaction conditions (concentration of reagents, buffer, temperature of runs, etc.) were identical, we conclude that there must be a difference between the elastase used by their group and the one used at the University of New Hampshire. As the system could not be used as the chemical basis for an optical oxygen sensor, Project II was abandoned.

Project III:

Most systems tested in Project III are not suitable for the optical sensing of oxygen. Table 4 lists those systems in which no energy transfer is observed; Table 7 lists the systems in which singlet state energy transfer occurs. Since singlet state energy transfer to the lanthanide ion is so rapid, the lanthanide luminescence bands are not sensitive to the oxygen concentration in the solution, and these systems cannot be used for an optical oxygen sensor.

The chemical system SDS/Tb(III)/1-bromonaphthalene is suitable for use as an optical oxygen sensor over a range of oxygen concentrations from 0.1 to 1.0 or 2.0, depending on the viscosity of the solution. A ratio of the terbium luminescence band at 490nm to the 1-bromonaphthalene fluorescence band at 350nm is used to determine oxygen concentration. Since the energy transfer from the triplet state of 1-bromonaphthalene to the singlet excited state of the terbium ion competes with the collisional quenching of the triplet state by oxygen, the lanthanide luminescence bands are sensitive to oxygen concentration. The fluorescence of the 1-bromonaphthalene is not oxygen-sensitive; the fluorophore in the micelle is protected from oxygen quenching.

FUTURE WORK

Goals of future work include a more in-depth study of the excitation and emission spectra of BNK-5 and similar compounds, in order to ascertain their possible uses in an optical oxygen sensor. It is also desirable to find a way to immobilize the probe molecule in the micelle more fully. By using more rigid surfactants, it may be possible to keep the fluorescing compound inside the micelle, thus protecting it from oxygen quenching more fully, and increasing the upper range of the system for oxygen detection. We would like to develop a system which has its excitation and emission bands in the visible region. A system of this sort will be able to be used with glass fiber optics. This would greatly decrease the cost of the sensor, as glass optics are much less expensive than quartz optics. In short, we are still striving for the ideal system for an optical oxygen sensor, and research into the possibilities will continue in the future.

APPENDIX

A spectral study of BNK-5 was performed. Compounds like BNK-5 have been studied by W.Rudolf Seitz at the University of New Hampshire. BNK-10, which has a longer hydrocarbon chain than BNK-5, has unusual spectral properties. Its absorption and excitation spectra do not resemble each other. There are more excitation bands than would be indicated by the absorption spectrum. It is possible that the BNK-10 forms an excited state dimer. We were interested in studying BNK-5 to see if its spectral properties were similar to those for BNK-10.

A solution of 21mg of BNK-5 in 1L distilled, deionized water was prepared. This solution has a BNK-5 concentration of 4.67×10^{-5} M. The absorption spectrum shows maxima at 211nm, 223nm, and 305nm. Absorbance values are 1.1012, 1.1537, and 0.3223A, respectively.

Since a 1:5 dilution of the stock solution exhibited only weak intensity emission, the stock solution was used for fluorescence excitation and emission studies.

When the BNK-5 solution is excited with 305nm radiation, a broad, intense emission band is observed at about 430nm

(Figure 14). Raman bands are sometimes observed at wavelengths approximately 40nm longer than the excitation wavelength; these can be eliminated by the use of a filter. Several additional excitation wavelengths were used; all yield the same emission spectrum.

The excitation spectrum of BNK-5, at a (λ_{em}) of 430nm, has four bands (Figure 15). These appear at 260nm, 305nm, 350nm, and 380nm. The band at 380nm is a Raman band, which appears at a wavelength approximately 50nm shorter than the emission wavelength. The other three bands do not vary with a change in wavelength.

The absorption and excitation spectra of BNK-5 are dissimilar.

A 3ml sample of the BNK-5 stock solution was degassed with nitrogen gas for 15 min. in the cuvette. After degassing, the emission spectrum ($\lambda_{ex}=305nm$) has two broad, intense bands: one at 430nm, the other at 540nm (Figure 16). This second band is the room temperature phosphorescence of BNK-5. The phosphorescence of BNK-5 is quenched by oxygen; therefore, this band is not observed for the air-saturated solution.

The excitation spectrum of the degassed stock solution at (λ_{em})=430nm appears to be the same as for the air-saturated solution (see Figures 15 and 17). A comparison of the relative intensities of the excitation bands for the solution with and without oxygen is shown in Table 9. There is little change in relative intensities observed when the solution is deaerated.

When the excitation spectrum is recorded for a (λ_{em})=540nm, only two bands are observed (Figure 18). The band at 275nm appears to be too narrow to be an excitation band. It is probably present due to the fact that it occurs at half the emission wavelength. The other band is broad and centered at 320nm.

An emission spectrum recorded at (λ_{ex})=320nm has bands at 430nm and 540nm (Figure 19). Although these bands occur at the same wavelengths as for the spectrum recorded at (λ_{ex})=305nm, there is a substantial change in the relative intensities of the two bands (see Table 10).

BNK-5 might be suitable for use in an optical oxygen sensor. Its phosphorescence is oxygen-sensitive; its fluorescence is excited in the visible region; and it might transfer to a lanthanide (as it is a 1-bromonaphthalene

derivative). Additional information on the spectral properties of BNK-5 will be obtained. A study of the effect of concentration on the excitation and emission spectra would be especially interesting, as BNK-10 has shown unusual behavior as its concentration is varied.

TABLE 9: Relative intensities of the ENK-5 excitation bands.

	Relative intensities of bands at:		
	60nm	305nm	350nm
air-saturated solution	0.73	1.00	0.91
degassed solution	0.81	1.00	0.95

TABLE 10: Relative intensities of the ENK-5 emission bands for the degassed stock solution.

Excitation	Relative intensities of bands at:	
	440nm	540nm
305nm	1.00	0.51
320nm	1.00	0.86

FIGURE 14:

Emission spectrum (380-600nm)
BNK-5 stock solution
excitation wavelength 305nm
5/23/86

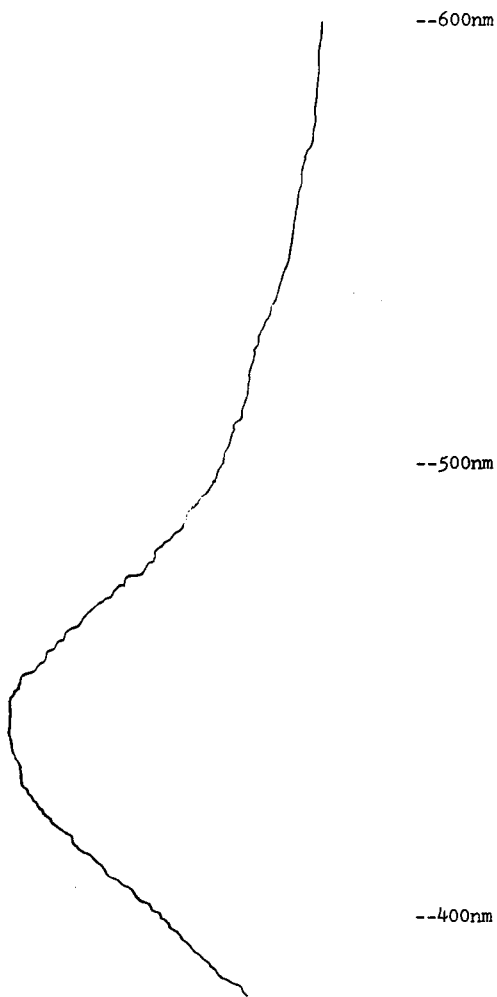
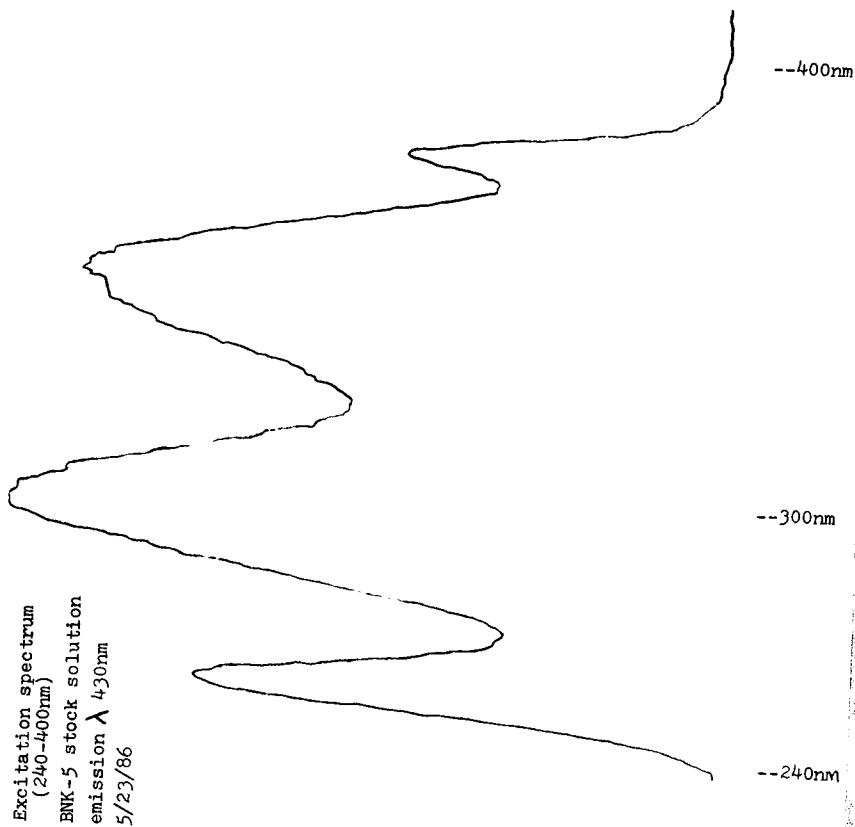


FIGURE 15:



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FIGURE 16:

Emission spectrum (360-600nm)
BNK-5 stock solution
degassed 15 min with N₂
excitation wavelength 305nm
5/29/86



Figure 17:

Excitation spectrum (240-400nm)
BNK-5 stock solution
degassed 15 min. with N₂
emission λ 430nm
5/29/86

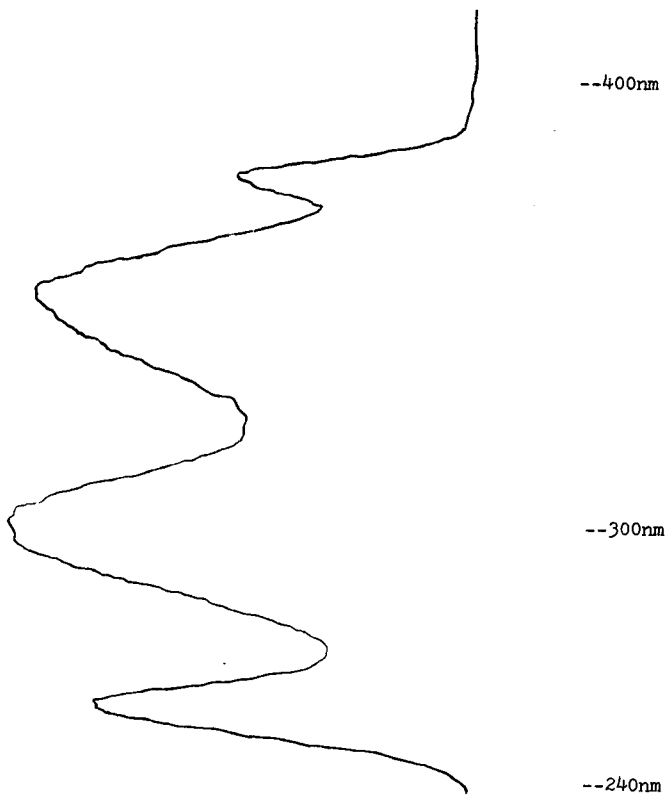


FIGURE 18:

Excitation spectrum (240-400nm)
EWK-5 stock solution
degassed 15 min with N₂
emission λ 540nm
5/29/86

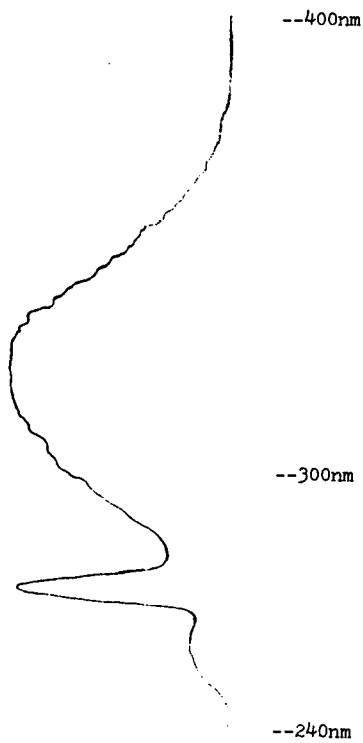
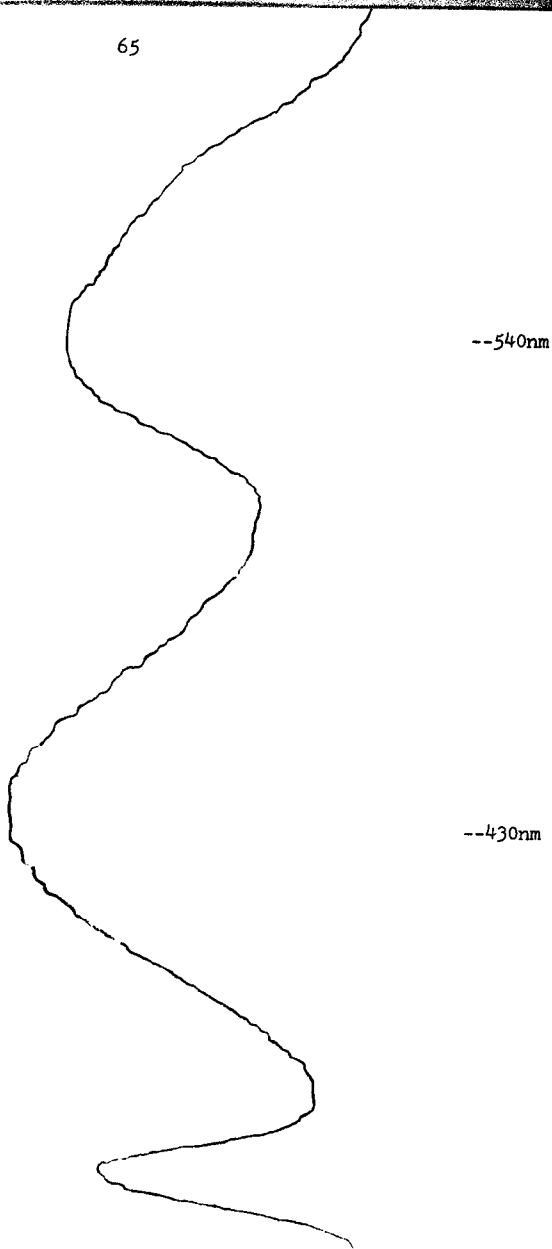


FIGURE 19:

Emission spectrum (340-640nm)
BNK-5 stock solution
degassed 15 min with N₂
excitation wavelength 320nm
5/29/86



REFERENCES

- (1) Turro, Nicholas J. "Modern Molecular Photochemistry"; Benjamin/Cummings Publishing Company, Inc.: Menlo Park, California, 1978.
- (2) Stevens, B. U.S.Pat., 3 612 866, 1971; Chem. Abstr. 1972, 76, 20945.
- (3) Lubbers, D.; Opitz, N. Ger.Pat., 2 508 637, 1976; Chem. Abstr. 1976, 85, 173867.
- (4) Fitzgerald, R. U.S.Pat., 363 425; Chem. Abstr. 1983, 98, 85777.
- (5) Wolfbeis, D.; Carlini, F. Anal. Chim. Acta. 1984, 160, 301.
- (6) Cline Love, L.J.; Habarta, J.G.; Dorsey, J.G. Anal. Chem. 1984, 56, 1132A.
- (7) Prendergast, F.G.; Lu, J.; Callahan, P.J. Journal of Biological Chemistry 1983, 258, 4075.
- (8) Dong, D.C.; Winnik, M.A. Photochemistry and Photobiology 1982, 35, 17.
- (9) Correll, G.D.; Cheser, III, R.N.; Nome, F.; Fendler, J.H. J. Amer. Chem. Soc. 1978, 100, 1254.
- (10) Tsien, R.Y.; Pozzan, T.; Rink, T.J. J. Cell. Bio. 1982, 94, 325.
- (11) Hemmila, I.; Dakubu, S.; Mukkala, V-M.; Siitari, H.; Lovgren, T. Analytical Biochemistry 1984, 137, 335.
- (12) DMS UV Atlas, IV, Table E1/T2.
- (13) Escabi-Perez, J.R.; Nome, F.; Fendler, J.H. J. Amer. Chem. Soc. 1977, 99, 7749.
- (14) Almgren, M.; Grieser, F.; Thomas, J.K. J. Amer. Chem. Soc. 1979, 101, 2021.
- (15) Gratzel, M.; Thomas, J.K. in "Modern Fluorescence Spectroscopy"; Wehry, E.L., Ed; Plenum Press: New York; 1976; Vol. 2, Ch. 4.