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REPORT DOCUMENTATION PAGE

1a. REPORT SECURITY CLASSIFICATION UNCLASSIFIED			1b. RESTRICTIVE MARKINGS	
2a. SECURITY CLASSIFICATION AUTHORITY			3. DISTRIBUTION/AVAILABILITY OF REPORT Approved for public release; distribution is unlimited.	
2b. DECLASSIFICATION/DOWNGRADING SCHEDULE			5. MONITORING ORGANIZATION REPORT NUMBER(S)	
4. PERFORMING ORGANIZATION REPORT NUMBER(S)			7a. NAME OF MONITORING ORGANIZATION	
6a. NAME OF PERFORMING ORGANIZATION Naval Ocean Systems Center	6b. OFFICE SYMBOL (if applicable) NOSC	7b. ADDRESS (City, State and ZIP Code)		
6c. ADDRESS (City, State and ZIP Code) San Diego, California 92152-5000			9. PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER	
8a. NAME OF FUNDING/SPONSORING ORGANIZATION Office of Chief of Naval Research	8b. OFFICE SYMBOL (if applicable)	10. SOURCE OF FUNDING NUMBERS		
8c. ADDRESS (City, State and ZIP Code) Independent Exploratory Development Programs (IR) OCNR-10P Arlington, VA 22217-5000		PROGRAM ELEMENT NO. 0601152N	PROJECT NO. ZT59	AGENCY ACCESSION NO. RR00001 DN306 250
11. TITLE (include Security Classification) FIBER OPTIC-FLUORESCENCE SENSORS FOR REMOTE DETECTION OF CHEMICAL SPECIES IN SEAWATER				
12. PERSONAL AUTHOR(S) S. H. Lieberman, S. M. Inman, and E. J. Stromvall				
13a. TYPE OF REPORT presentation/paper	13b. TIME COVERED FROM TO	14. DATE OF REPORT (Year, Month, Day) September 1989	15. PAGE COUNT	
16. SUPPLEMENTARY NOTATION				
17. COSATI CODES			18. SUBJECT TERMS (Continue on reverse if necessary and identify by block number)	
FIELD	GROUP	SUB-GROUP	fluorescence spectrometry fiber optics seawater analyses	
19. ABSTRACT (Continue on reverse if necessary and identify by block number) Development of fiber optic based chemical sensors that use fluorogenic indicators for measurements in seawater is discussed. A sensor configuration for avoiding reversibility problems in which the indicator is constantly renewed at the probe tip by forcing it through an ultrafiltration membrane is described. The sensor is shown to respond reversibly on time scales of 1-sec to changes in analyte concentrations. Methods for increasing the specificity in complex matrices are discussed.				
Published in <i>Proceedings of the Symposium on Chemical Sensors</i> , Vol. 87-9, 1987.				
20. DISTRIBUTION/AVAILABILITY OF ABSTRACT ☒ UNCLASSIFIED/UNLIMITED ☐ SAME AS RPT ☐ DTIC USERS			21. ABSTRACT SECURITY CLASSIFICATION UNCLASSIFIED	
22a. NAME OF RESPONSIBLE INDIVIDUAL S. H. Lieberman			22b. TELEPHONE (include Area Code) (619) 553-2778	22c. OFFICE SYMBOL Code 522

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FIBER OPTIC-FLUORESCENCE SENSORS FOR REMOTE DETECTION OF CHEMICAL SPECIES IN SEAWATER

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ABSTRACT

Development of fiber optic based chemical sensors that use fluorogenic indicators for measurements in seawater is discussed. A sensor configuration for avoiding reversibility problems in which the indicator is constantly renewed at the probe tip by forcing it through an ultrafiltration membrane is described. The sensor is shown to respond reversibly on time scales of 1-sec to changes in analyte concentrations. Methods for increasing the specificity in complex matrices are discussed.

INTRODUCTION

Increased use of microprocessors and robotics has created a need for chemical sensors that link the computer to the outside world. For oceanographic applications there is a need for "passive" sensors that make continuous or nearly continuous measurements in seawater. The availability of such sensors would facilitate the understanding of small scale chemical variability in the ocean.

Optical sensors that couple fluorescence measurements with fiber optic cables offer several advantages. Fluorescence measurements are inherently sensitive. Concentrations as low as sub-parts-per-trillion can be determined (1). Fluorescence measurements are fast. Individual determinations can be made in less than a micro-second. Use of UV-visible transmitting fiber optic cable to couple the sensing element to the measurement instrument permits

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measurements over large distances and multiplexing of sensors from numerous remote locations.

The disadvantage of sensors that depend on fluorescence measurements is that only some organic compounds and almost no inorganic constituents fluoresce naturally. Therefore, development of viable fluorescence based sensors depend on the availability of indicator molecules that form fluorescent complexes with the chemical species of interest. The principle behind the sensor is that the fluorescence of the indicator molecule is either enhanced or quenched by the ion or molecule of interest.

Several groups have reported studies in which fluorescence spectroscopy was coupled to fiber optics to provide a remote detection capability for a variety of chemical constituents. Applications include: biomedical sensors (2-4); process control and monitoring (5-6); and general analytical chemistry (7-10). Most of these studies have used the same approach. A fluorogenic ligand is immobilized on the end of a single or bifurcated cable, excitation energy is transmitted down the fiber optic light guide, fluorescence emission from the sample is transmitted back up the cable, and the wavelength(s) of interest are selected and measured at the detector.

It is important to note, that with the exception of sensors employing pH sensitive indicator molecules, sensors for dissolved ionic species have not, to date, been demonstrated to respond reversibly. Seitz (11) has, however, presented a theoretical discussion of requirements for reversibility based on stability of the complex formed between the indicator molecule and the analyte species.

In this report we discuss results of our efforts to develop and characterize fluorogenic indicator systems for use as optical chemical sensors for chemical species in seawater. Specific issues that are addressed include: (1) Interference from competing species (non-specificity of the organic indicator molecule for the species of interest) (2) Non-reversibility of complex formation between the indicator molecule and the species of interest. Specificity is critical because almost every element in the periodic table is present in seawater. In



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addition concentrations of different ions with affinities for the same indicator ligand commonly vary by six orders of magnitude (or more). Reversibility is important because if the sensor is to be useful for quantifying small scale variations it must respond quickly to changes in concentrations.

METHODS AND MATERIALS

Instrumentation. Two different instruments were used for making fluorescence measurements. Early experiments were conducted using a Guided Wave (Rancho Cordova, CA) Model 200 Optical Waveguide Spectrum Analyzer. The Guided Wave instrument is an integrated spectrophotometer system designed for making measurements over fiber optic cables. The second instrument was an EG&G PARC Optical Multichannel Analyzer System consisting of the Model 1460 Optical Multichannel Analyzer with a Model 1463 Detector Controller coupled to a Model 1420 Intensified Diode Array Detector. Sample modified light was dispersed over the detector using a EG&G Model 1232 Compact Spectrograph with a 600g/nm holographic grating.

Excitation light was provided by a 75 watt Xenon arc lamp mounted in a PTI (Photon Technology International, Inc., Princeton, N.J.) Model 02-A1000 Arc Lamp Housing and powered by a Model 02-LPS200 Universal Regulated D.C. Arc Lamp Power Supply.

All measurements were made using a bifurcated fiber optic cable bundle (Six-to-One Probe, Guided Wave, Inc.). The bundle consists of seven 325 um core diameter uv-transmitting fiber optic fibers. A single fiber is used to transmit the excitation energy to the sample and the other six fibers are arranged concentrically around the excitation fiber at the probe tip and are stacked vertically at end of the cable that mates to the spectrograph.

Experimental Apparatus. A schematic of the sensor envisioned for use in the ocean is given in Fig. 1. For laboratory tests, a flow through manifold was machined from clear PVC for evaluating the sensor configuration shown in the schematic. With this system it is possible to control the flow rate

past the membrane to simulate different flow regimes that might be experienced with a bare-ended probe. The manifold contains provisions for mounting a 12mm diameter ultrafiltration membrane with a nominal molecular weight cut-off of 500 (Amicon, Inc., Danvers, MA). The fiber optic cable probe is threaded into the block so that it "looks" directly at the membrane. Distance from the end of the probe to the membrane can be adjusted from approximately 0.5 to 2 cm. The solution to be analyzed is pumped through the manifold using a peristaltic pump (flow rate variable from 2-100 ml min⁻¹). Pressure on the indicator reservoir was controlled using a two-stage regulator on a N² cylinder.

Chemicals and reagents. Quinolin-8-ol-sulfonate (Q-80S) was purchased from Aldrich and recrystallized in water. p-Tosyl-amino quinolin (PTAQ) was synthesized by Chemica, Inc. (Gardena, CA) and recrystallized in EtOH. pH was adjusted using Baker NH⁴OH or GFS Chemicals (Columbus, OH) nitric acid. Seawater for most experiments was obtained from the seawater pumping system at the Scripps Institution of Oceanography (La Jolla, CA).

RESULTS AND DISCUSSION

Efforts have concentrated on the development of a sensor for zinc in seawater. However, because fiber optic sensors using fluorogenic indicators have potential for wide application we have attempted to take a generic approach to solving problems associated with this type of sensor. Solutions that require active manipulations at the point of measurement have been avoided.

Studies have focused on evaluating techniques for solving the problems of reversibility and specificity. These include:

- (1) Development of a novel ultrafiltration membrane system for constant renewal of the fluorogenic indicator.

- (2) Investigation of several means to increase specificity of the indicator system for species of interest.

Development of a Reversible Indicator Ligand System

One of the most critical issues for the development of a viable sensor is the question of reversibility. Because the fluorescence signal is the direct result of complex formation with the fluorogenic ligand, the complex must dissociate when analyte concentration decreases. This requires moderate stability constants so that the formation of the complex is thermodynamically favored, but not so strong that complex formation cannot be reversed (11). Reversibility also requires relatively fast kinetics for the dissociation reaction.

These thermodynamic and kinetic considerations can present a serious limitation to the approach of immobilizing the fluorogenic indicator molecule on the end of the fiber optic cable. A related problem is that fluorogenic ligands often require chemical modification before they can be immobilized on a surface. This can alter the fluorescence properties of the molecule relative to its behavior in solution.

Based on these considerations, we are evaluating what we believe to be a simple but novel approach to sensor design. Rather than physically immobilizing the fluorogenic ligand on the end of the fiber optic cable via chemical and/or electrostatic interactions we make the indicator available within the optical viewing volume of the fiber optic probe by pushing the ligand through an ultrafiltration membrane. Complexation occurs on the sample side of the membrane at or near the surface of the membrane. The fluorescence signal is transmitted back through the fiber optic cable to the detector. The complexed indicator is constantly removed from the viewing volume by diffusive/convective mass transport processes and replaced by uncomplexed indicator from the reservoir. Because the flow through the membrane is quite slow (approximately 1 $\mu\text{l}/\text{min}$) 1.5 ml reservoir can provide a sensor with a lifetime of 24 hrs.

Studies show that this approach appears feasible. Results from an experiment using the OMA system in which a series of samples that contained 0.0 to 2.0 ppm added Mg^{2+} (0.5 ppm increments) in distilled water was pumped through the sensor manifold is shown in Fig

2. Sensor reversibility is evident from the decline in signal observed when the samples were run in order of decreasing concentration.

In another experiment, distilled water that contained increasing concentrations of seawater produced a linear ($r^2=0.99$) fluorescence response on the Guided Wave instrument (Fig 3.) when pumped through the manifold. Concentrations are expressed in terms of added Mg^{2+} because Mg is the dominant ion in seawater that complexes with Q-80S. These results suggest that the indicator Q-80S could be used as a salinity sensor because Mg^{2+} varies directly as a function of salinity in seawater.

Experiments conducted using the OMA system permit detailed investigation of response time of the sensor to changes in concentration. Fig. 4 is a time history of the fluorescence emission at 517 nm measured at 1 second intervals for a sample solution pumped through the manifold system. The increase in signal at approximately 250 sec was the result of switching from distilled water to a solution of 20% seawater. The response time is estimated to be approximately 2-seconds. Fig. 4 shows that the fluorescence signal remained constant for the next 40-min as the seawater sample was pumped through the manifold. When the carboy containing the seawater solution ran dry at approximately 2700 sec after the start of the experiment a small volume (approximately 500 ul) of sample was trapped over the membrane. The linear increase in the fluorescence signal after 2700 sec shows that the flow of indicator ligand through the membrane into the sample is quite constant.

Response of the ultrafiltration membrane sensor to decreases in concentration is shown in Fig. 5. Successive scans of the fluorescence emission spectrum taken 1-second apart with the OMA system show that the signal returns to base line approximately 2-seconds after the sample is switched from a solution of 50% seawater to distilled water.

Studies using the ultrafiltration membrane with the indicator molecule PTAQ show increasing fluorescence with additions of Zn to seawater (Fig. 6). However, there is currently a problem with a memory effect on the membrane. When the concentration

of added Zn in the seawater is decreased the fluorescence signal does not change. Microscopic examination of the membrane under a UV light source suggests that the indicator ligand (which is insoluble in water) is precipitated and retained in the fibrous structure of the membrane. It may be possible to eliminate this problem by using a membrane with a smoother surface. Another possibility is to attach functional groups to the indicator molecule to increase its solubility in aqueous media.

The approach described above offers several advantages over sensors employing immobilized indicators. Because the indicator is constantly renewed by flow through the membrane, there is no requirement for the indicator to form a reversible complex with the species of interest. The sensor only depends on a constant supply of indicator through the membrane and on sufficiently fast kinetics for complex formation to occur while the indicator is in the optically active area of the sensor. Photodegradation of the immobilized indicator molecule by the excitation source should not be a problem with this system because the ligand is constantly renewed. No laborious immobilization chemistry is required in order to fix the indicator on the end of the fiber probe. This eliminates any undesirable modification of the fluorescent behavior of the ligand via the immobilization procedure. Finally, it is possible to modify the chemical characteristics of the fluorogenic indicator solution in order to optimize the sensor for determination of a particular species. For example, it may be possible to eliminate some interferences by adjusting the pH of the indicator solution because complex formation for different species often exhibits a variable pH dependence.

Specificity of the indicator ligand system for the chemical species of interest

There are several possible techniques for improving the specificity of the fluorogenic indicator system. These include: (1) employing indicator ligands that selectively complex the species of interest. (2) spectrally resolving fluorescence emission from complexes formed between different ions and the same indicator molecule. (3) using differences (nano-second time scales) in the fluorescent lifetimes of various

indicator ligand-metal complexes to resolve and eliminate interferences from competing ions. We are presently evaluating all of the above approaches.

Experiments to test for interferences from other ions showed that although the ligand Q-80S showed good response to zinc in low ionic strength solutions, in seawater other ions (notably Mg^{2+}) formed fluorescent complexes with the ligand and thereby interfered with the determination of low concentrations of zinc. Based on these results it was decided to investigate other fluorogenic indicators that would complex zinc but not the other ions in seawater. One ligand that appeared promising was PTAQ. Reportedly this ligand only forms complexes with Zn and Cd (12).

Initial experiments to evaluate PTAQ for measuring Zn in seawater were conducted in solution using a bare-ended fiber optic probe to provide excitation and collect the emitted fluorescence. Results (Fig. 7) show a linear ($r^2=0.96$) increase in the fluorescence signal for concentrations of added Zn ranging from approximately 20 to 200 ppb. In another experiment eight replicate determinations of separate seawater aliquots containing 50 ppb added Zn yielded a precision for the analysis of $\pm 6\%$. The above results were obtained even though PTAQ is insoluble in seawater and a finely dispersed precipitant was observed upon addition of PTAQ to seawater. It appears that PTAQ may be useful as a indicator for Zn in seawater if the ligand can be made more soluble in aqueous media or if a membrane can be found that does not retain the precipitant.

The feasibility of using differences in the fluorescent lifetimes of various indicator ligand-metal complexes for resolving and eliminating interferences is being investigated. Experimentally this consists of exciting the fluorescence of the indicator molecule-metal complex with a pulse from a tunable dye laser and then monitoring the decay of the resulting fluorescence signal. Hiraki, et al. (13) have shown that it is possible to resolve fluorescence signals of Zn and Cd complexes from Mg and Al complexes with the ligand quinolin-8-ol-sulfonate.

Another possibility under investigation is to use

subtle spectral differences in fluorescence emission that can be measured with the OMA system to discriminate between different complexes with the same indicator.

If time resolution of fluorescence signals and/or differences in emission spectra can be used for resolving interferences in seawater then it may be possible to use more generic complexing agents, thereby eliminating the need and costly effort required to synthesize a specific indicator for each species of interest.

ACKNOWLEDGMENT

This work was supported by the NOSC IR program.

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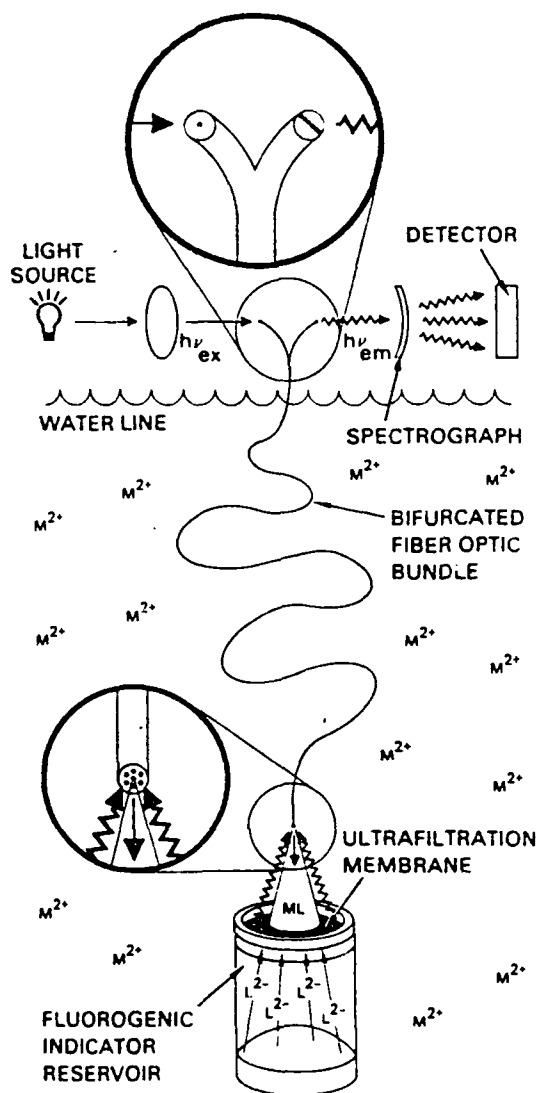


Fig. 1. Schematic of ultrafiltration membrane sensor.

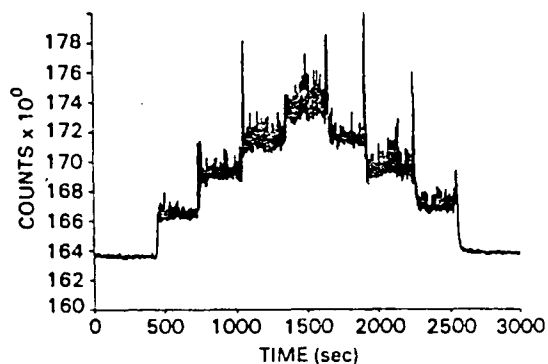


Fig. 2. Response of sensor to 0.5 ppm changes in Mg^{2+} concentration.

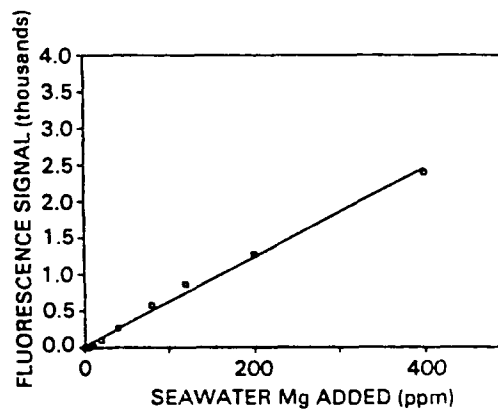


Fig. 3. Fluorescence signal vs. conc. of seawater added to distilled water (expressed in terms of added Mg^{2+}).

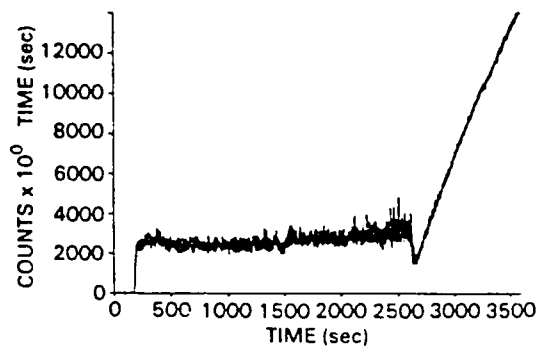


Fig. 4. Time history of fluorescence response for 50% seawater solution. Flow stopped at 2700 sec.

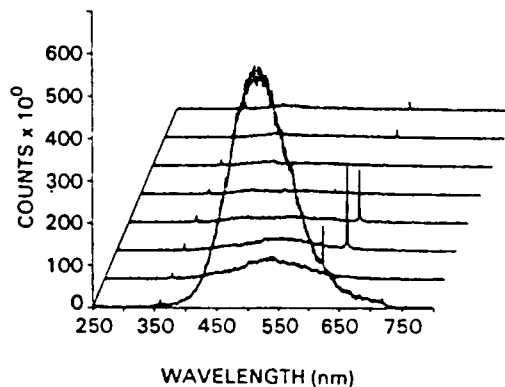


Fig. 5. Successive 1-sec scans of fluorescence emission measured while switching from 50% seawater to distilled water.

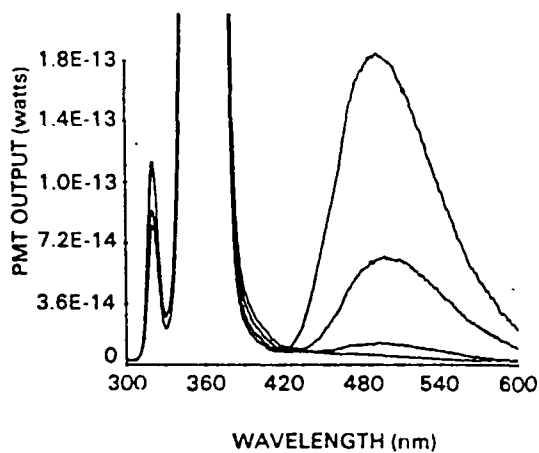


Fig. 6. Fluorescence response of the membrane sensor to 0.5, 5 and 50 ppm additions of Zn^{2+} to seawater.

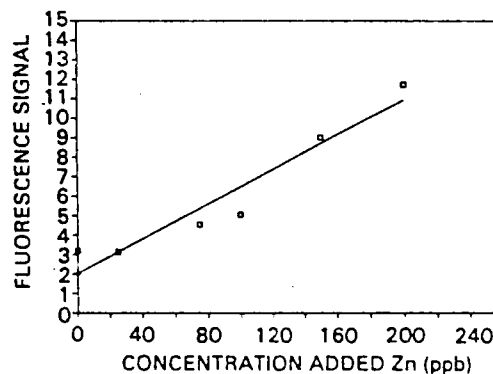


Fig. 7. Fluorescence vs. conc. Zn^{2+} added to seawater for PTAQ in solution.