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## Development of Membrane Film Fiber Optic Based Sensors for the Remote Monitoring of the Quality of Surface and Groundwater

Leonidas G. Bachas  
*University of Kentucky*

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RESEARCH REPORT NO. 184

DEVELOPMENT OF MEMBRANE FILM FIBER  
OPTIC BASED SENSORS FOR  
THE REMOTE MONITORING OF THE QUALITY  
OF  
SURFACE AND GROUNDWATER

By

Leonidas G. Bachas  
Principal Investigator

Project Number: G2021-03

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Water Resources Research Institute  
University of Kentucky  
Lexington, KY

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August 1992

## **DISCLAIMER**

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## ABSTRACT

The objective of this study was the development of chromo- and fluoroionophores and their subsequent use in sensors capable of surface and groundwater monitoring. Several reagents (modified crown ethers and porphyrins) were synthesized based on principles of chemical recognition and used for metal and pH sensing. The modified crown ether reagents include a chromogenic or fluorogenic group. The selectivity of these reagents is controlled by the size of the crown ether ring and the type of the chromogenic or fluorogenic side-arm. In addition, a fluorogenic crown ether was synthesized that incorporates a fluorogenic side-arm and a perfluorinated carbon chain. The later was used to immobilize the fluoroionophore on PTFE membranes, which were placed at the tip of an optical fiber bundle. This configuration led to the development of a divalent ion-selective fiber optic sensor. Finally, cobalt(II)tetra(*p*-hydroxyphenyl)porphyrin, [Co(*p*-OH)TPP] was electropolymerized on the surface of optically transparent electrodes to form membranes composed of the polymerized [Co(*p*-OH)TPP] chromoionophore. These membrane phases, capable of molecular recognition, were used for the construction of fiber optic sensors.

## DESCRIPTORS

Monitoring, Membrane Processes, Surface Water, Groundwater

## ACKNOWLEDGMENTS

The work described in this report was carried out by graduate students Timothy L. Blair and John Allen, and by Truis Smith-Palmer, who was on sabbatical leave from St. Francis Xavier University, Antigonish, Nova Scotia, Canada.

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(d) 200  $\mu\text{L}$  of 0.7% benzyltrimethylammonium hydroxide and 100  $\mu\text{L}$  of 0.003 M potassium chloride; and (d) 200  $\mu\text{L}$  of 0.7% benzyltrimethylammonium hydroxide and 100  $\mu\text{L}$  of 0.006 M potassium chloride. p. 14

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## CHAPTER I - INTRODUCTION

The objective of this study was the development of chromo- and fluoroionophores and their subsequent use in sensors capable of surface and groundwater monitoring. There is an overall need for developing sensitive and reliable techniques for measuring chemical parameters (in the ppm to sub-ppm range), which is needed to characterize present conditions and predict future changes in water quality. This research project combines fiber optic and membrane technologies in the development of sensing systems for metals and pH.

It is well established that crown ethers are capable of forming stable complexes with a variety of species, especially alkali and alkaline earth metal cations. This attribute has led to analytical applications in ion-selective electrodes and ion chromatography. In addition, spectrophotometric determinations of cationic species have been accomplished by ion-pair extractions involving crown ethers and anionic-dye counter ions. Alternatively, chromophores can be attached to the crown ether skeleton to yield cation-selective dyes (chromoionophores).

Modified crown ethers that include a chromogenic group were first reported by Dix and Vögtle,<sup>1</sup> Takagi and co-workers,<sup>2</sup> and Pacey and co-workers<sup>3</sup> and were more recently reviewed in an article by Forrest and Pacey.<sup>4</sup> Among the most successful of these reagents for the alkaline earth cations were diaza-crown ethers possessing two pendant side-arms, which included a dissociable proton as illustrated in Figure 1. The structure of these chromoionophores can be modified easily by varying the size of the crown ether ring or altering the chromophoric side-arm. Such changes

produce reagents with different sensitivities and selectivities. Reagents that allow the formation of two six-membered chelate rings (each comprised of one crown nitrogen, the complexed ion and the chromophore) have been found to be particularly useful for extraction of divalent cations due to a strong anion-cation electrostatic interaction.<sup>5</sup>

Attempts to develop crown ether ionophores with an attached fluorogenic group (i.e., fluoroionophores) have been less successful. Early fluoroionophores showed very small changes in fluorescence upon complexation with cations or were extremely unstable.<sup>6</sup> Nishida reported that a diaza-18-crown-6 with a coumarin-type fluorescent tag forms  $\text{Ca}^{2+}$ -chelates similar to those described above, but gave no details about the change in fluorescence as a function of metal ion concentration.<sup>7</sup>

Because of the current interest in the development of ion-selective fiber optic sensors, an evaluation of the selectivity and response characteristics of crown ether-based chromo- and fluoroionophores is desirable. In that respect, we have synthesized several crown ethers for use in determinations of metal cations.

Fiber optic chemical sensors (FOCS) are based on interactions of an immobilized reagent phase with an analyte in a sample solution. Typically, optical fibers are used as optical waveguides that carry light to and from a species-selective reagent phase. Interactions with the analyte induce changes in the optical properties of the reagent phase. Monitoring of this change in the optical signal allows for the determination of the analyte.

Immobilization of reagents for optical sensors can be achieved in a number of ways. Reagents have been entrapped within polymer matrices<sup>8,9</sup> or behind semi-permeable membranes.<sup>10,11</sup> Solid supports (e.g., glass beads or polymer films) with covalently-attached reagents can

be affixed at the sensing tip,<sup>12,13</sup> or the reagents can be covalently attached to the fiber itself.<sup>14</sup> Recently, Ogasawara *et al.* reported a FOCS based on a dynamic immobilization scheme.<sup>15</sup> In this approach, C<sub>18</sub> chains were covalently attached to an optical fiber, resulting in a hydrophobic region at the fiber surface. Hydrophobic reagents were then associated with this surface. The technique was used to develop a sensor for riboflavin binding protein based on its ability to quench the fluorescence of 3-octylriboflavin which was associated with the fiber optic surface. Immobilization has also been accomplished by adsorption of reagents on polymeric supports,<sup>16-18</sup> such as poly(tetrafluoroethylene) (PTFE).<sup>19</sup>

Two different approaches to reagent immobilization have been explored in the present study. In one of them, a perfluorinated alkyl chain was used to anchor the reagent on PTFE membranes. The second approach involved incorporation of a functional group that can be used to directly polymerize the reagent on surfaces.

## CHAPTER II - RESEARCH PROCEDURES

### 1. Evaluation of 10-[(2,4-dinitrophenyl)azol-9-hydroxy-1,8-anthracene-18-crown-6 as a chromoionophore.

The chromogenic crown ether **1** was prepared as described previously.<sup>20</sup> This crown ether dissolves extremely slowly in ethanol, and therefore, solutions in this solvent were prepared by first dissolving **1** in a minimum amount of chloroform (ca 0.5 mL per mg) and then diluting to volume with absolute ethanol.

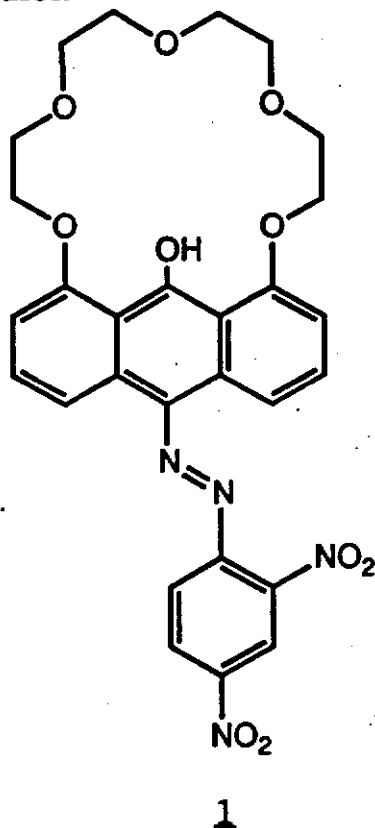


Figure 1. Structure of the chromogenic crown ether **1**.

For a typical analysis, 4 mL of a  $9.5 \times 10^{-5}$  M solution of **1** in absolute ethanol was transferred to a 5-mL volumetric flask, and 100  $\mu$ L of an

aqueous solution of the chloride salt of the cation of interest was added. Benzyltrimethylammonium hydroxide (200  $\mu$ L of 0.7% in ethanol) was added just before the absorbance was measured, and the final volume was adjusted to 5 mL with ethanol.

## 2. Chromo- and fluoroionophores based on diaza-crown ethers

*N,N'*-Bis[1,2-dihydroxyanthraquinon-3-ylmethyl]-diaza-18-crown-6

(2). Diaza-18-crown-6 (1 g, 3.8 mmol) and alizarin (2 g, 8.3 mmol) were dissolved in 40 mL dimethylformamide, and triethylamine (2.1 mL, 16 mmol) and formalin (1.2 mL, 16 mmol) were added. The mixture was stirred under nitrogen at room temperature for 2 h and then at 60 °C for 18 h. The reaction mixture was concentrated under reduced pressure and the residue was dissolved in chloroform and extracted with 10% aqueous tartaric acid. The tartaric acid layer was neutralized with NaOH and then the product was extracted with chloroform. The chloroform solution was concentrated under reduced pressure and washed with dilute aqueous NaOH to remove residual alizarin. After drying with magnesium sulfate, the solvent was removed resulting in rust-colored crystals. [<sup>1</sup>H-n.m.r. (CDCl<sub>3</sub>)  $\delta$ = 8.4 - 7.5 (10H, m, aromatic H), 4.1 (4H, s, ArCH<sub>2</sub>), 3.6 - 3.8 (16H, m, OCH<sub>2</sub>), 2.9 (8H, t, NCH<sub>2</sub>). Found: 60.96% C, 5.23% H, 3.47% N, 3.88% Na. Calcd. for C<sub>42</sub>H<sub>42</sub>N<sub>2</sub>O<sub>12</sub> • 1.5 Na • 1.5 H<sub>2</sub>O: 60.90% C, 5.48% H, 3.38% N, 4.17% Na.]

*N,N'*-Bis[4-methyl-7-hydroxycoumarin-8-ylmethyl]-diaza-15-crown-

5 (3). Diaza-15-crown-5 (655 mg, 2.5 mmol) and 4-methylumbelliferone (950 mg, 5.4 mmol) were dissolved in 20 mL dimethylformamide. Triethylamine (2.8 mL, 21 mmol) and formalin (2.8 mL, 37 mmol) were

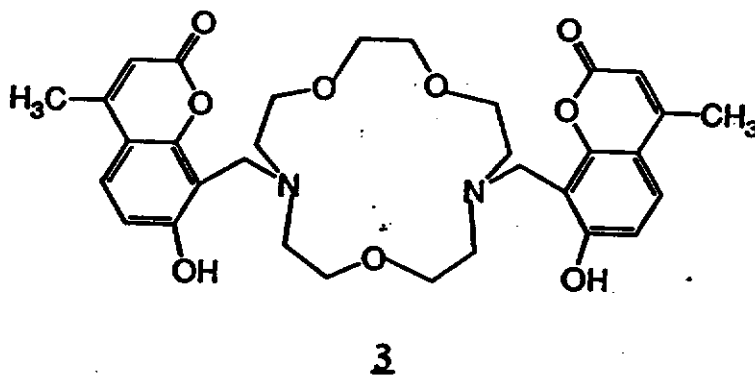
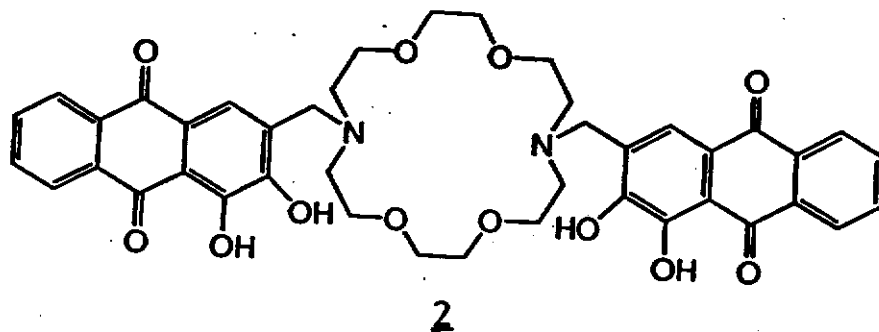


Figure 2. Structures of ionophores **2** and **3**.

added, and the mixture was stirred under nitrogen at 60 °C for 18 h. After filtering, the resulting white precipitate was washed with methanol and dried under vacuum. [<sup>1</sup>H-n.m.r. (CDCl<sub>3</sub>) δ = 7.4 - 6.0 (6H, aromatic H), 4.1 (4H, s, ArCH<sub>2</sub>), 3.8 - 3.6 (12H, m, OCH<sub>2</sub>), 2.8 - 3.0 (8H, m, NCH<sub>2</sub>), 2.4 (6H, s, CH<sub>3</sub>). Found: 64.39% C, 6.42% H, 4.69% N. Calcd. for C<sub>32</sub>H<sub>38</sub>N<sub>2</sub>O<sub>9</sub>: 64.63% C, 6.44% H, 4.71% N.]

*Extraction-Spectrophotometry with 2.* A 5.00 x 10<sup>-5</sup> M solution of **2** in HPLC-grade 1,2-dichloroethane (2.00 mL) and 2.00 mL of a buffered aqueous solution (0.0500 M Bis-Tris-Propane/HCl, pH 9.5) containing alkaline earth metal chlorides were mixed end-over-end for 30 min. The phases were then separated and the absorbance of the organic phase at

545 nm was measured on a Perkin Elmer (Norwalk, CT) Lambda-6 spectrophotometer.

*Fluorometric Determinations Using 3.* A buffered solution of alkaline earth metal salt (2.00 mL) was added to 2.00 mL of **3** in HPLC-grade methanol, resulting in a solution of  $1.25 \times 10^{-8}$  M fluoroionophore, 0.0250 M Tris/HCl (pH 8.5). The solution was allowed to equilibrate for 8 min, and the fluorescence spectrum was obtained on a SPEX (Edison, NJ) Fluorolog-2 F111 fluorometer in the ratio mode with the excitation monochromator at 360 nm.

### 3. Fluorocarbon-based immobilization of a fluoroionophore for preparation of fiber optic sensors

The starting material for the synthesis of ionophore **8** (Figure 3) was commercially available diaza-18-crown-6, **4**. Attempted monoacylation of **4** with perfluorooctanoyl chloride failed to yield the desired monosubstituted derivative **7**. However, the diazacrown ether **4** could be readily converted to monocarbamate **5** by treatment with benzyl chloroformate.<sup>21</sup>

*N-Carbobenzoxy-N'-perfluorooctanoyl-1,4,10,13-tetraoxa-7,16-diazacyclooctadecane 6.* Triethylamine (0.17 mL) was added to a solution of **5** (115 mg, 0.29 mmol) in 4 mL of anhydrous benzene, followed by perfluorooctanoyl chloride (130 mg, 0.30 mmol) in 1 mL of benzene. The resulting mixture was then stirred at room temperature under argon for 18 h. A 10% aqueous NaOH solution (1 mL) was added and the layers were separated after 10 min. After adding 15 mL benzene, the benzene solution was washed successively with 10% NaOH, 10% HCl, water and brine and was dried over sodium sulfate. Chromatography on silica gel yielded 181



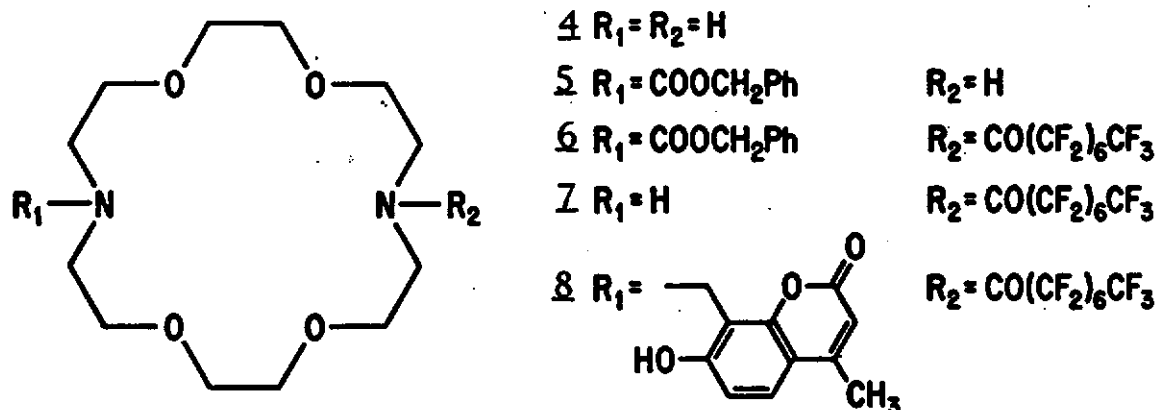


Figure 3. Structures of ionophore **8** and precursors **4-7**.

mg (0.23 mmol, 79% yield) of **6**. [ $^1H$ -NMR ( $CDCl_3$ )  $\delta = 3.5 - 3.9$  (24H, m), 5.15 (2H, s), 7.35 (5H, s)]

*N*-Perfluorooctanoyl-1,4,10,13-tetraoxa-7,16-diazacyclooctadecane, **7**. Product **6** (700 mg, 0.88 mmol) was hydrogenated in 10 mL methanol in the presence of 10% Pd/C (30 mg). The catalyst was filtered off and the filtrate evaporated. After recrystallization from benzene-heptane, 394 mg (0.60 mmol, 68% yield) of product **7** was obtained. [ $^1H$ -NMR ( $CDCl_3$ )  $\delta = 3.25$  (4H, t), 3.6 - 4.0 (20H, m)]

*Condensation of 7 with 4-methylumbelliferone*. Product **7** (131.7 mg, 0.20 mmol), 4-methylumbelliferone (37 mg, 0.21 mmol), triethylamine (0.2 mL) and formalin (0.15 mL) were dissolved in 6 mL of anhydrous DMF. The resulting solution was stirred at 55°C under argon for 22 h. The solvent was evaporated and the residue was dissolved in 20 mL of chloroform. The solution was washed with 10% tartaric acid and brine, and

was then dried over sodium sulfate. Chromatography on silica gel yielded 90 mg (0.11 mmol, 53% yield) of **8** as a colorless oil. [ $^1\text{H-NMR}$  ( $\text{CDCl}_3$ )  $\delta$  = 2.4 (3H, s), 2.9 (4H, s), 3.5 - 4.0 (20H, m), 4.15 (2H, s), 6.1 (1H, s), 6.8 (1H, d), 7.4 (1H, d), 8.0 (1H, s). FAB-MS 846 ( $\text{M}^+$ ), 601, 440, 231, 173]

*Apparatus.* All spectroscopic measurements were made using an Oriel (Stratford, CT) modular spectrophotometer, configured as described earlier.<sup>10</sup> The major components of the instrument include a tungsten-filament lamp, a grating monochromator (set at 358 nm), a glass bifurcated fiber optic bundle, a high-pass filter with a 420-nm cut off, a photomultiplier tube (PMT), and a photomultiplier readout device interfaced with a strip-chart recorder. The high-pass filter was placed in front of the PMT to discriminate against the exciting radiation. The probe construction is shown in Figure 4. A previously-swelled piece of PTFE

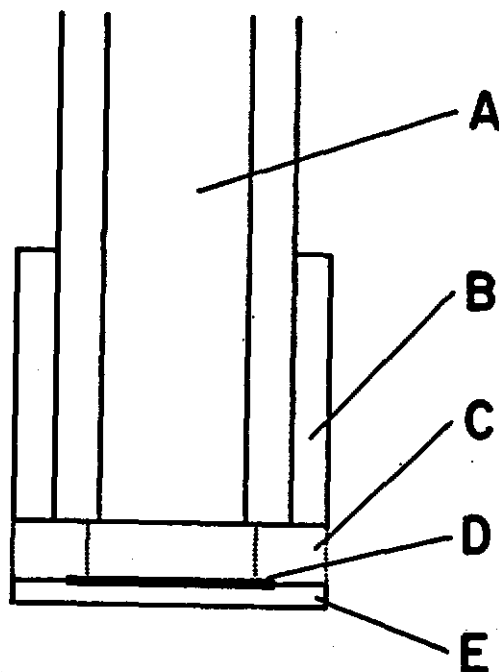


Figure 4. Construction of FOCS: (A) bifurcated fiber optic bundle, (B) PTFE sleeve, (C) 2-mm long spacer, (D) PTFE membrane with immobilized ionophore, and (E) PTFE support for membrane.

membrane (FHUP 047 00, Millipore) was placed in the PTFE housing and was immersed in a 0.50 mM solution of **8** (in HPLC-grade methanol) for several hours. The probe was washed with deionized water and placed in a 0.010 M Tris-HCl (pH 7.5)/0.50 mM EDTA for approximately 1 h. The probe was then immersed in fresh 0.010 M Tris-HCl (pH 7.5) and allowed to equilibrate. The change in signal was monitored as the concentration of analyte was varied by additions of standard solutions to the buffer. The probe was placed in the EDTA buffer before each experiment.

#### 4. Electropolymerized porphyrins for sensor development

The synthesis of tetra(*p*-hydroxyphenyl)porphine, (*p*-OH)TPP, was accomplished by following a two-step procedure reported by Little et al.<sup>22</sup> The metallation of tetra(*p*-hydroxyphenyl)porphine was carried out in a mixture of chloroform and methanol to yield cobalt(II)tetra(*p*-hydroxyphenyl)porphyrin, [Co(*p*-OH)TPP] (Figure 5).

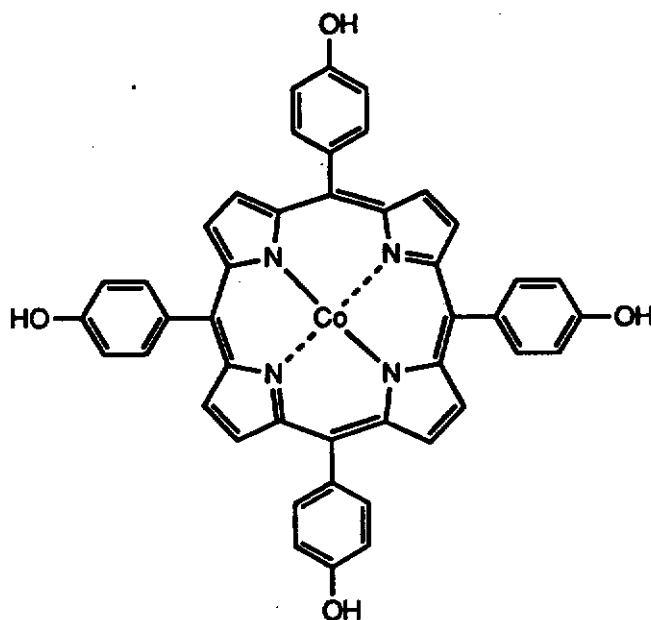


Figure 5. Structure of cobalt(II)tetra(*p*-hydroxyphenyl)porphyrin.

A conventional three-electrode set-up was employed for the electropolymerization of [Co(*p*-OH)TPP]. An indium tin oxide-coated glass slide (Delta Technologies, Stillwater, MN) was used as the working electrode, while a silver wire and a platinum wire were the "pseudoreference" and counter electrode, respectively. An acetonitrile solution containing 1.0 mM [Co(*p*-OH)TPP] and 0.10 M TEAP was used for the electropolymerization. The potential of the cell was cycled at a scan rate of 200 mV/s between 0 and 1.3 V. The poly[Co(*p*-OH)TPP] films were prepared using 200 scans. Once the polymerization was completed, the potential was set at a constant final potential of 0.0 V for two minutes. The poly[Co(*p*-OH)TPP] films were washed with water and stored in a 0.010 M thiocyanate solution.

Spectroscopic measurements were made using an Oriel (Stratford, CT) modular spectrophotometer. A 100-W tungsten filament lamp (Model 6333) was powered by an Oriel power supply (Model 68735). The light from the source passed through the monochromator (Model 77296) and was focused on one leg of a bifurcated bundle of randomized glass fibers (Oriel Model 77533). Changes in signal were monitored at 470 nm. Variable slits (77269) on each side of the monochromator were set at 0.12 mm. The glass slide coated with the electropolymerized porphyrin film was placed at the common end of the bundle and the other leg carried light to the photomultiplier tube (Model 77761) (Figure 6). The PMT readout device (Model 7070) was interfaced with a strip-chart recorder (Linear Model 1200).

The sensing tip was placed in a 0.10 M phosphoric acid solution and the pH was adjusted with a NaOH solution. The pH was also monitored using a Fisher glass pH electrode (Model 13-620-287) and an Accumet pH

meter (Model 925). The sensor was stored in a solution of pH ~ 10 between experiments.

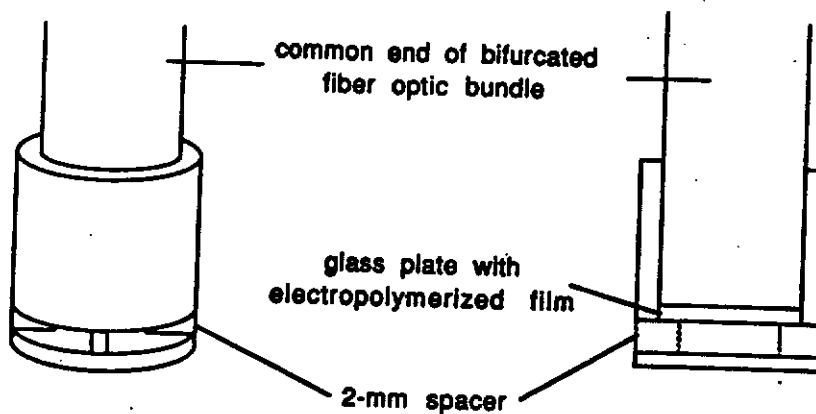


Figure 6. Construction of the fiber optic sensor based on electropolymerized porphyrin. Both a three dimensional and a crossectional view are shown.

## CHAPTER III - DATA AND RESULTS

Several reagents (modified crown ethers and porphyrins) were synthesized based on principles of chemical recognition and used for metal and pH sensing as described in the subsequent four sections.

### 1. Evaluation of 10-[(2,4-dinitrophenyl)azo]-9-hydroxy-1,8-anthracene-18-crown-6 as a chromoionophore.

10-[(2,4-dinitrophenyl)azo]-9-hydroxy-1,8-anthracene-18-crown-6 (**1**), was prepared and used as a chromogenic crown ether for the determination of  $K^+$  in water and organic solvents. This chromogenic crown ether dissolves in several organic solvents to form a yellow solution with absorption maxima at 362 nm and 427 nm. Triethylamine and pyridine cause no dissociation of the hydroxyl group at position 9 as evidenced by the lack of an absorption shift in the presence of these reagents. In the presence of a strong base (e.g., sodium hydroxide), a solution of **1** turned blue, with an absorption maximum at 576 nm indicating the dissociation of the hydroxyl moiety. With ethanol as the solvent and a small concentration of base, the absorption at 576 nm remained relatively low. When potassium was added, a complex was formed with **1** and the wavelength of maximum absorption shifted to 581 nm (Figure 7).

Linear calibration curves were obtained for potassium using a constant amount of benzyltrimethylammonium hydroxide as the base (200  $\mu$ L of a 0.7% solution in ethanol) (Inset, Figure 8). The slope of the calibration curve was dependent on the concentration of reagent **1** used. Therefore, the concentration of chromogenic crown ether was optimized by

taking into account that higher concentrations of **1** (with the same amount of base) also result in higher blank values, which can decrease the reproducibility of the technique. With a concentration of **1** equal to  $9.3 \times 10^{-5}$  M, the standard deviation was 8% and a detection limit of  $1 \mu\text{g}$  potassium (equivalent to 5 ppm) was achieved.

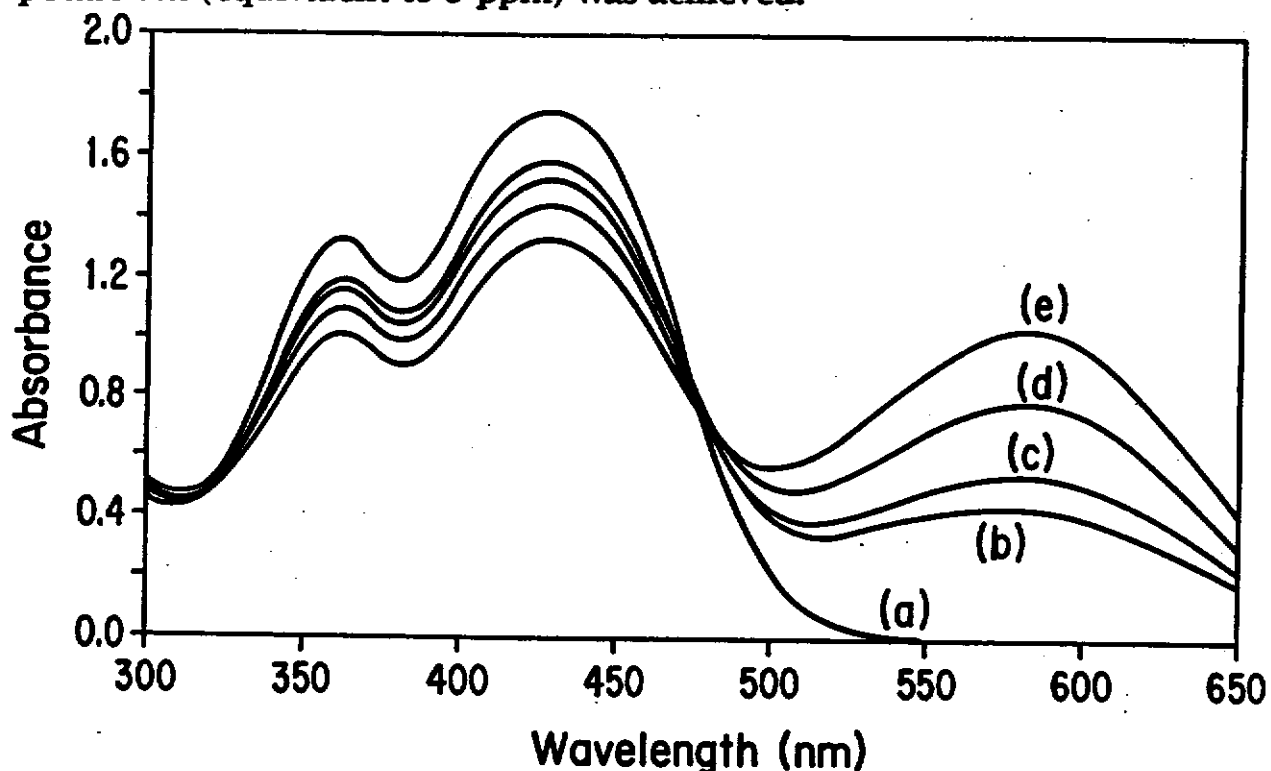


Figure 7. Absorbance spectra for 4 mL of  $9.3 \times 10^{-5}$  M of **1** in ethanol made up to 5 mL with ethanol after the addition of the following: (a) ethanol; (b) 200  $\mu\text{L}$  of 0.7% benzyltrimethylammonium hydroxide and 100  $\mu\text{L}$  of water; (c) 200  $\mu\text{L}$  of 0.7% benzyltrimethylammonium hydroxide and 100  $\mu\text{L}$  of 0.001 M potassium chloride; (d) 200  $\mu\text{L}$  of 0.7% benzyltrimethylammonium hydroxide and 100  $\mu\text{L}$  of 0.003 M potassium chloride; and (e) 200  $\mu\text{L}$  of 0.7% benzyltrimethylammonium hydroxide and 100  $\mu\text{L}$  of 0.006 M potassium chloride.

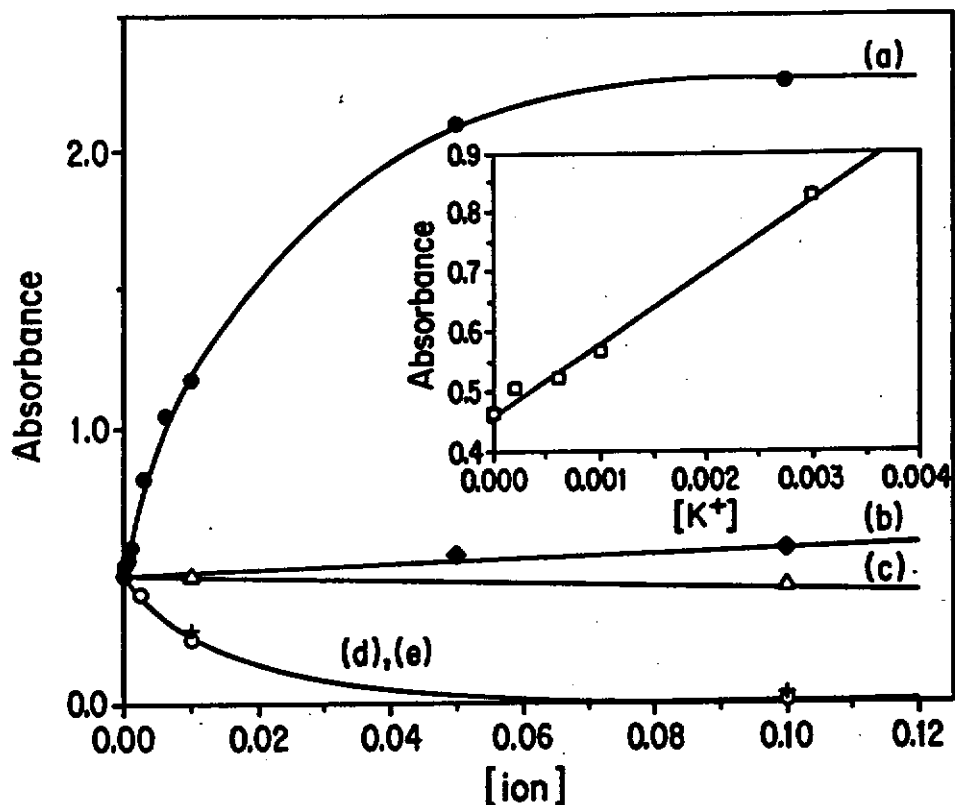


Figure 8 . Graphs of absorbance at 581 nm versus the concentration of ions in the 100- $\mu$ L aqueous sample aliquots (final volume of 5 mL): K<sup>+</sup> (a), Na<sup>+</sup> (b), Li<sup>+</sup> (c), Mg<sup>2+</sup> (d), Ca<sup>2+</sup> (e). The inset is an expansion of the low concentration region of the graph for potassium.

Sodium also reacted with **1** but to a much lesser extent. For the above concentration of **1** the slope of the calibration curve given by sodium was 200-fold lower than that for potassium (Figure 8). Sodium ion, therefore, did not interfere significantly. Lithium interfered to an even lesser extent, although in a negative direction. Magnesium and calcium ions produced a negative interference at concentrations greater than 10<sup>-3</sup> M. These ions prevent the formation of the phenolate ion, presumably by tying up the base.



## 2. Chromo- and fluoroionophores based on diaza-crown ethers

Katayama *et al.*<sup>5</sup> reported a monoaza-crown ether with an alizarin side-arm that allows for the efficient extraction of alkali metals. These extraction properties were attributed to the lipophilicity of the alizarin group and its ability to form a charge-delocalized anion. Substituting a diaza-crown and attaching two alizarin groups (or other chromophores) may further enhance these characteristics while producing selectivity for alkaline earth metal ions. Synthesis of such compounds and evaluation of their selectivity properties were one of the goals of our study.

The absorption spectra for the uncomplexed and the  $\text{Ca}^{2+}$ -complexed chromogenic diaza-18-crown-6 are shown in Figure 9. The spectrum of the complexed species exhibits a bathochromic shift and an increase in molar absorptivity as compared to the uncomplexed ligand. A spectrum of  $2 \times 10^{-5}$  M alizarin in 0.01 M NaOH showed that the spectra resulting from complexation of **2** have the same features as that of the alizarin anion. Further, no significant changes were observed in the spectrum of the alizarin anion after addition of  $\text{CaCl}_2$  to the solution. These observations indicate that, upon complexation, two of the protons of **2** (one from each alizarin -OH) are lost, and a neutral metal chelate is formed.

The absorbance signals observed for calcium and magnesium ion extractions are shown in Figure 10. The detection limit of the technique based on  $S/N = 3$  is  $1 \times 10^{-5}$  M  $\text{Ca}^{2+}$  and the calibration curve is linear to  $1 \times 10^{-4}$  M. The reagent shows excellent selectivity for calcium over magnesium ions with no significant absorbance signal to a magnesium concentration as high as  $5 \times 10^{-3}$  M. It should be noted that the signals due to strontium and barium are essentially equivalent to that of calcium,

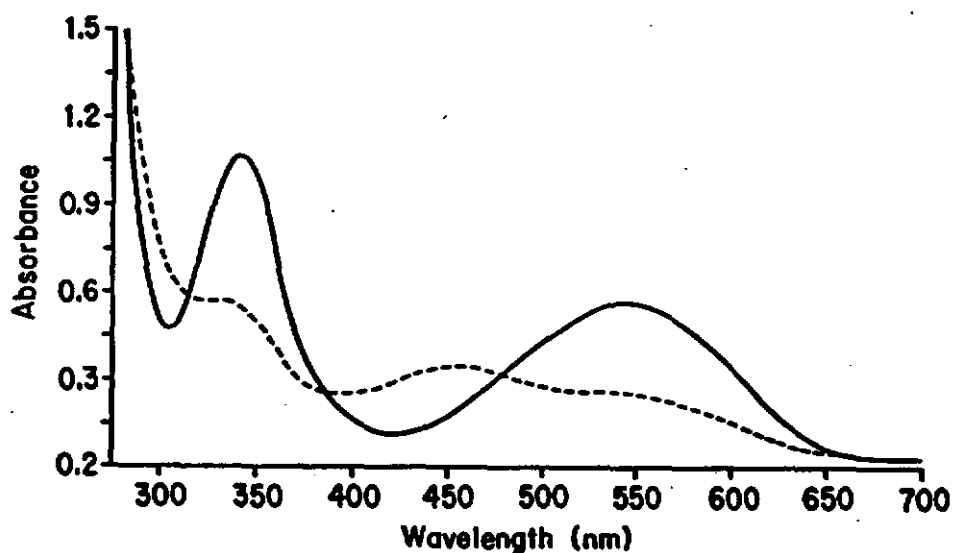


Figure 9. Spectra of calcium-complexed **2** (—) and the uncomplexed ligand (—). Conditions for complexation: buffered (pH 9.5) 0.010 M CaCl<sub>2</sub> extracted with 5.00 × 10<sup>-5</sup> M **2** in 1,2-dichloroethane.

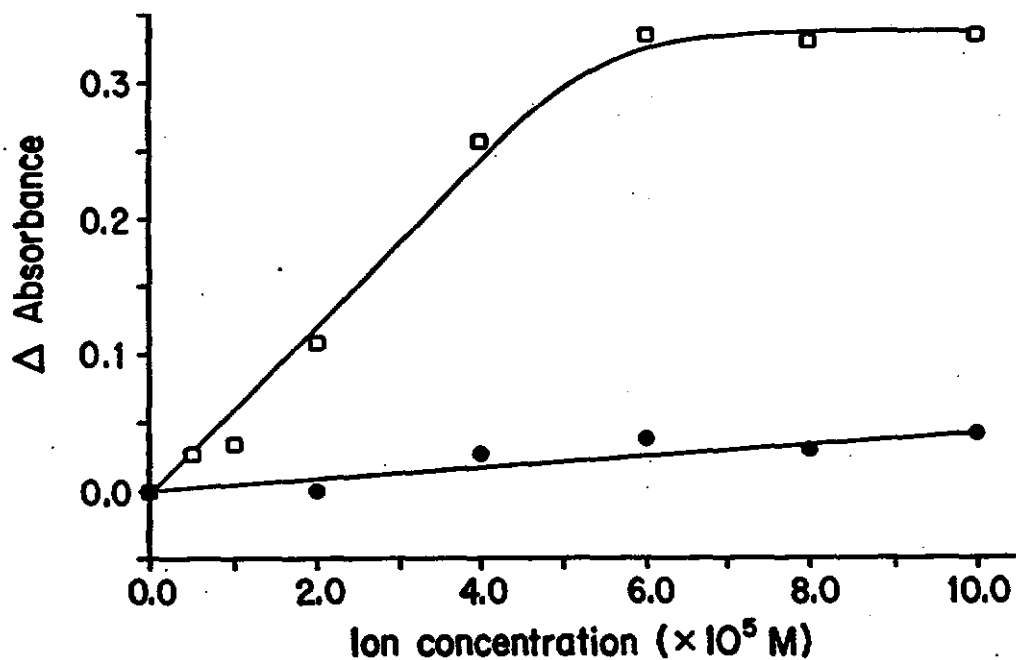


Figure 10. Calibration curves for 5 × 10<sup>-5</sup> M of **2** in 1,2-dichloroethane extracted with buffered chloride salts of calcium (□) and magnesium (O). Absorbance is measured at 545 nm.

however this should not pose a problem in most calcium determinations. There is also no interference from the alkali metal ions.

As mentioned earlier, the selectivity of crown ether ionophores like **2** can be modified by altering the side-arms and/or the ring size. In ionophore **3**, the ring size is reduced to a diaza-15-crown-5. This change should induce selectivity for ions smaller than  $\text{Ca}^{2+}$ . In addition, the alizarin chromophores were replaced by fluorogenic methylumbelliferone groups. Umbelliferone is a highly efficient fluorophore that is quite sensitive to changes in its environment. For these reasons, it has been employed in a number of analytical applications.<sup>23-26</sup> Unlike alizarin, methylumbelliferone is soluble in aqueous solutions over a wide pH range. Its diaza-15-crown-5 derivative **3** is soluble in a methanol/water mixture, allowing for one-phase determinations of  $\text{Mg}^{2+}$ .

To establish the optimum pH for the determinations, the fluorescence intensity of **3** was measured in solutions of different pH containing a fixed concentration of  $\text{Mg}^{2+}$ . A larger increase in intensity is observed at higher pH. However, umbelliferone decomposes at pH above 10.<sup>27</sup> Therefore, pH 8.5 was chosen in order to maximize the signal without serious problems with decomposition of the fluorophore.

Figure 11 shows the fluorescence spectra of **3** in solutions of various concentrations of  $\text{MgCl}_2$ . There is an increase in fluorescence intensity at 435 nm of about 40% upon complexation with  $\text{Mg}^{2+}$ . The calibration curve for  $\text{Mg}^{2+}$  is linear (Figure 12) from the detection limit of  $7 \times 10^{-6}$  to  $1 \times 10^{-4}$  M  $\text{Mg}^{2+}$ . Although the reagent shows the largest response to  $\text{Mg}^{2+}$ , the method suffers from interference from other alkaline earth metals, particularly  $\text{Ca}^{2+}$  and  $\text{Ba}^{2+}$ . Minimal response is observed for alkali metal ions.

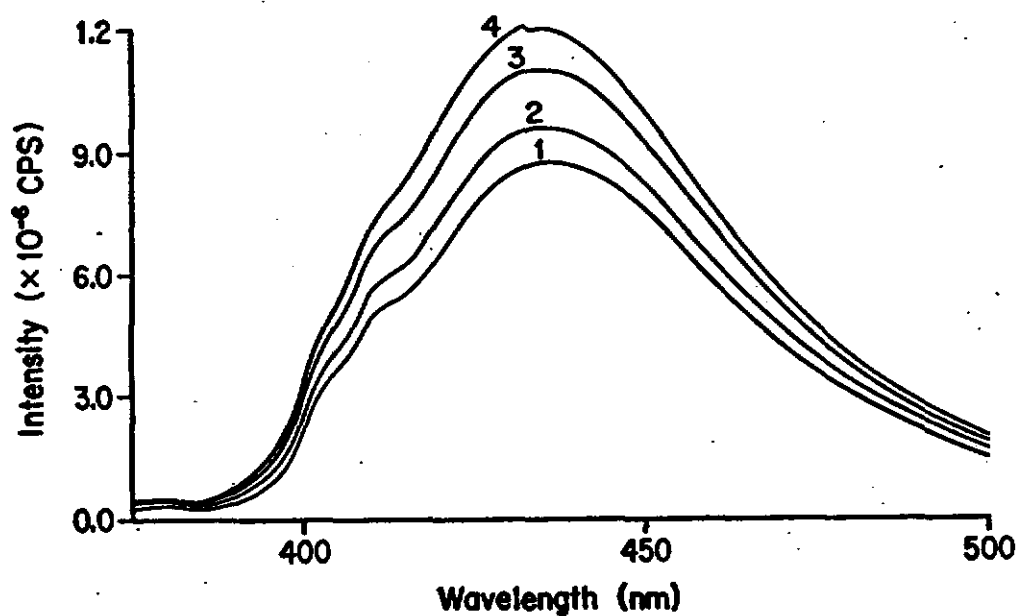


Figure 11. Fluorescence spectra of ionophore **3** in various concentrations of  $\text{MgCl}_2$ : (1) 0.0 M, (2)  $2.0 \times 10^{-5}$  M, (3)  $6.0 \times 10^{-5}$  M, (4)  $1.0 \times 10^{-4}$  M. Intensity is measured in counts per second (CPS).

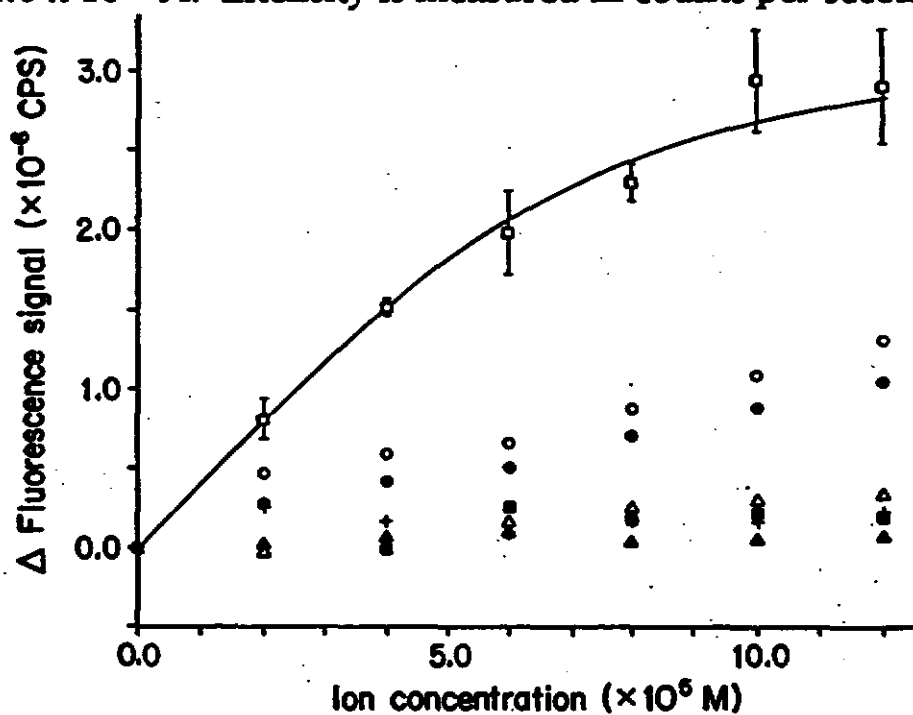


Figure 12. Response curves for ionophore **3**:  $\text{Mg}^{2+}$  ( $\square$ ),  $\text{Ca}^{2+}$  ( $\circ$ ),  $\text{Ba}^{2+}$  ( $\bullet$ ),  $\text{Sr}^{2+}$  ( $\Delta$ ),  $\text{Na}^+$  (+),  $\text{K}^+$  ( $\blacksquare$ ),  $\text{Li}^+$  ( $\blacktriangle$ ). The error bars denote  $\pm$  one standard deviation ( $n = 4$ ).

### 3. Fluorocarbon-based immobilization of a fluoroionophore for preparation of fiber optic sensors

PTFE provides an excellent surface for immobilization of reagents. The reagent phase adsorbed on the PTFE is more accessible to the analyte than when immobilized on resin beads.<sup>19</sup> The inert surface also eliminates problems associated with non-specific adsorption on the surface of the FOCS. However, this characteristic also makes immobilization difficult. In 1988, Kobos *et al.* reported an enzyme electrode in which the enzyme was immobilized on a PTFE membrane.<sup>28</sup> This was achieved by modifying the enzyme with perfluoroalkyl groups that could be embedded in the membrane.

The fluorogenic crown ether **8** was synthesized in order to evaluate the feasibility of using fluorocarbon-based immobilization of chromoionophores in fiber optic sensors. A perfluorinated alkyl chain was substituted on one of the nitrogens of the diaza-crown ether, and 4-methylumbelliferone (a fluorogenic tag) was attached to the other. Monoacylation of the diaza-crown ether skeleton with perfluorooctanoyl chloride proved to be impractical, producing only the disubstituted species. However, a monosubstituted carbamate could easily be prepared.<sup>21</sup> After acylation of the remaining nitrogen, the carbamate group could be cleaved to allow attachment of the fluorophore. The fluoroionophore was immobilized by the covalently-bound perfluoroalkyl group, which embedded itself in a PTFE membrane positioned at the tip of a bifurcated fiber optic bundle.

Diaza-crown ethers with two pendant chromogenic side arms have been shown to exhibit selectivity for divalent alkaline earth metal ions

over monovalent alkali metals.<sup>13,29</sup> Similar monoaza-crowns with a single chromogenic side arm are also selective for divalent cations.<sup>30,31</sup> This suggests that the diaza-crown **8** with a single pendant side arm would also be selective for alkaline earth metal ions. Calibration curves for  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  in the 0-5 mM range are shown in Figure 13. The probe exhibits similar responses to the two divalent cations. The curves are linear up to 1 mM and the detection limit for  $\text{Ca}^{2+}$  is 0.1 mM. The response can be reversed by immersing the probe in an EDTA solution.

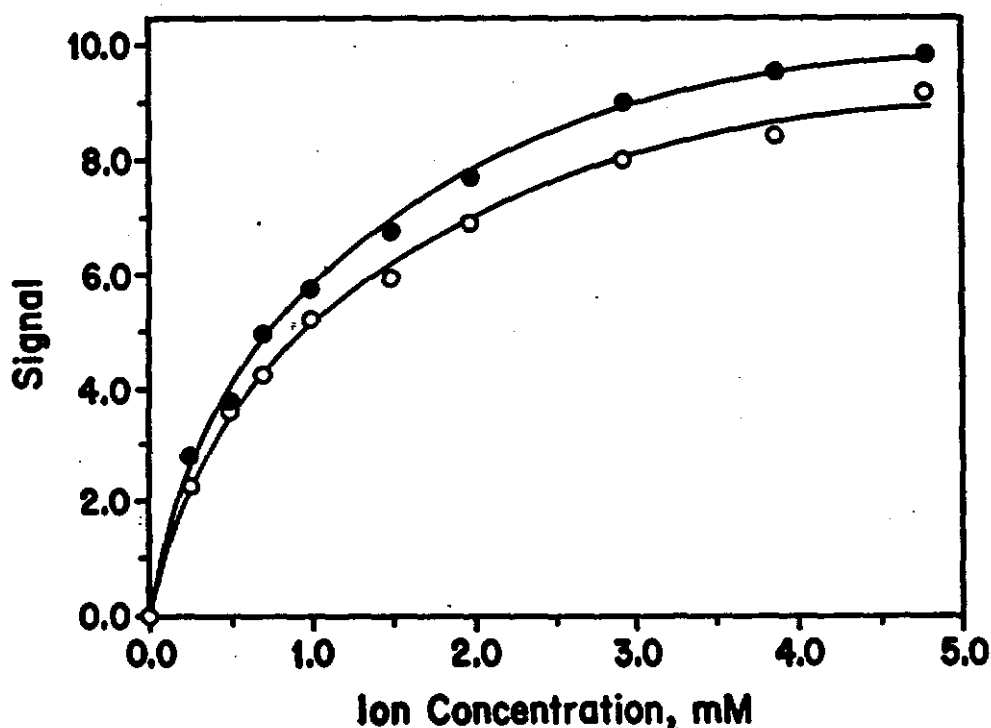


Figure 13. Calibration curves for  $\text{Ca}^{2+}$  (●) and  $\text{Mg}^{2+}$  (○). "Signal" refers to percent change in fluorescence intensity.

The probe gives a response time of less than 1 min in all cases. This is a significant improvement over the response times obtained with immobilization techniques that involve adsorption of the reagent on

particles that are entrapped behind a membrane at the tip of the sensor.<sup>32</sup> The response time of the latter type of probe is limited by the time required for diffusion of the analyte across the PTFE membrane and to the region of the probe where the optical interaction occurs. With immobilization on the surface of the PTFE membrane, the need for such diffusion is eliminated and the response time is limited only by mass transport to the FOCS surface and the kinetics of the immobilized reagent-analyte interaction.

#### 4. Electropolymerized porphyrins for sensor development

Fiber optic sensors were prepared by electrodeposition of polymeric films on the surface of indium-tin oxide glass slides that were immersed in solutions of the monomer [Co(*p*-OH)TPP]. The spectrochemical properties of porphyrins have been extensively studied, and their spectral characteristics are well known. Porphyrins absorb in the visible region, and present bands, like the Sorret band, that are characteristic and specific of each porphyrin. It has been demonstrated that the spectra of [Co(*p*-OH)TPP] in solution present significant differences depending on the pH of the solution. Given the ability to form polyporphyrin films, it was envisioned that fiber optic pH sensors based on polyporphyrin films could be developed.

The pH-sensitive poly[Co(*p*-OH)TPP] films were deposited on transparent indium-tin oxide slides and tested for use in a fiber optic sensor. The number of scans used in the electropolymerization was sufficient to produce films with an absorbance of about 0.5. Figure 14 shows the calibration curve for the optical sensor. The linear range extends from pH 8-12 with a slope of  $-0.1 \mu\text{A}/\text{decade}$ . The relative

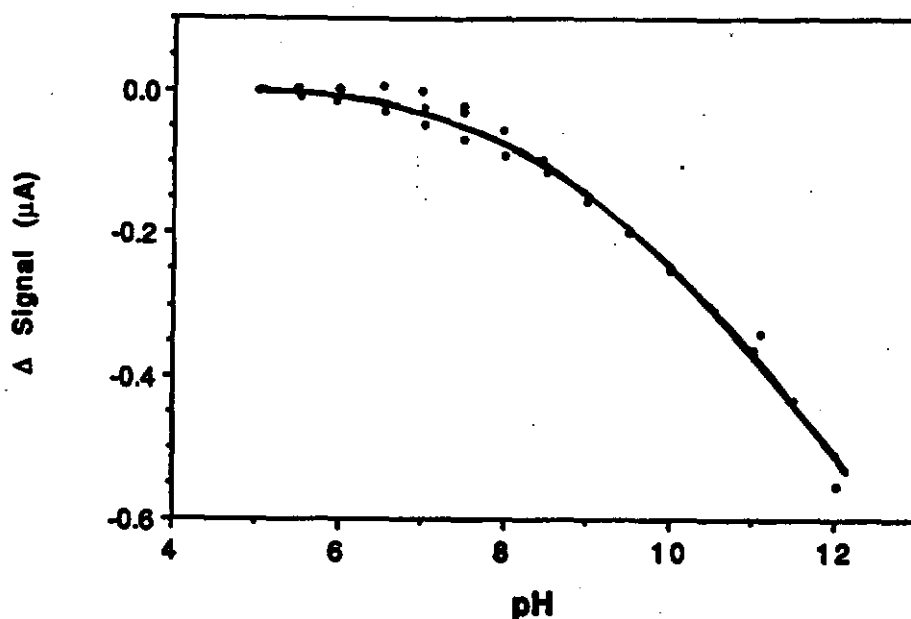


Figure 14. pH Response of an electropolymerized poly[Co(p-OH)TPP] film electrode.

standard deviation was typically less than 5% ( $n = 3$ ). Steady state response was reached in about 5 min.

This sensor does not respond to anions. No change in signal is observed for acetate, iodide, nitrate, nitrite, phosphate, salicylate, sulfate, or thiocyanate in concentrations as high as 0.1 M. This lack of anion response may be explained by a possible coordination between the cobalt center of the porphyrin and the hydroxyl group of the phenol from a nearby porphyrin unit in the polyporphyrin film.



## CHAPTER IV - CONCLUSIONS

New metal-selective chromo- and fluoroionophores were synthesized in our laboratory and used to develop methods for determining metal ions. In addition, we have demonstrated the feasibility of using a fluorocarbon-based immobilization method for the development of fiber optic sensors. A fiber optic probe using an immobilized fluorogenic crown ether ionophore was constructed that responds to  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  with response times of less than 1 min. Finally, fiber optic sensors based on electropolymerized [Co(*p*-OH)TPP] were used to monitor the pH of aqueous solutions in the pH 8 to 12 range. The developed sensors could find potential applications in the direct and *in situ* monitoring of metals and pH in both surface and groundwater.

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