TECHNICAL NOTES

Fluorocarbon-Based Immobilization of a Fluoroionophore for Preparation of Fiber Optic Sensors

Timothy L. Blair, Tadeusz Cynkowski, and Leonidas G. Bachas*

Department of Chemistry and Center of Membrane Sciences, University of Kentucky, Lexington, Kentucky 40506

INTRODUCTION

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[1,2] or behind semipermeable membranes.[3,4] Solid supports (e.g., glass beads or polymer films) with covalently-attached reagents can be affixed at the sensing tip[5,6] or the reagents can be covalently attached to the fiber itself.[7] Recently, Ogasawara et al. reported a FOCS based on a dynamic immobilization scheme.[8] In this approach, C_{18}-alkyl 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,[9-11] such as poly(tetrafluoroethylene) (PTFE).[12]

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.[12] The inert surface may also eliminate problems associated with nonspecific adsorption on the surface of the FOCS. Indeed, Bright et al. have reported that the stability of immunosurfaces prepared using functionalized PTFE membranes is improved over conventional quartz surfaces,[6] a fact that can be attributed to reduced denaturation due to nonspecifically adsorbed portions of the antibody.[13] However, the inertness of PTFE also makes immobilization difficult. In 1988, Kobos et al. reported an enzyme electrode in which the enzyme was immobilized on a PTFE membrane.[14] This was achieved by modifying the enzyme with perfluoroalkyl groups that could be embedded in the membrane.

We have synthesized the fluorogenic crown ether **5** (Figure 1) for the development of an optical sensor for alkaline earth metal ions. This fluoroionophore is immobilized at the tip of the FOCS through the covalently-attached perfluorinated alkyl chain, which can be embedded in a PTFE membrane.

EXPERIMENTAL SECTION

Reagents. The following reagents were used as received from Aldrich (Milwaukee, WI): diaza-18-crown-6 (1,4,10,13-tetraoxa-7,16-diazacyclooctadecane), formalin (37% formaldehyde solution in water with 10–15% methanol), benzyl chloroformate, Sure/Seal dimethylformamide (DMF), and triethylamine. Perfluorooctanoyl chloride and 4-methylumbelliferone were obtained from Strem Chemicals (Newburyport, MA) and Eastman Kodak (Rochester, NY), respectively. Tris(hydroxymethyl)aminomethane (Tris) was from Research Organics (Cleveland, OH) and (ethylenedinitrilo)tetraacetic acid (EDTA) from Mallinkrodt (Paris, KY). All salt solutions were prepared using analytical reagent-grade chemicals and deionized (Milli-Q water purification system, Millipore, Bedford, MA), distilled water.

Synthesis. The starting material for the synthesis of ionophore **5** (Figure 1) was commercially available diaza-18-crown-6, **1**. Attempted monoacylation of **1** with perfluorooctanoyl chloride failed to yield the desired monosubstituted derivative **4**. However, the diazacrown ether **1** could be readily converted to monocarbamate **2** by treatment with benzyl chloroformate.[15]

N-Carbobenzoxy-N'-(perfluorooctanoyl)-1,4,10,13-tetraoxa-7,16-diazacyclooctadecane (**3**). Triethylamine (0.17 mL) was added to a solution of **2** (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 of 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 mg (0.23 mmol, 79% yield) of **3** [^{1}H NMR ($CDCl_3$) δ 3.5–3.9 (24 H, m), 5.15 (2 H, s), 7.35 (5 H, s)].

N-(Perfluorooctanoyl)-1,4,10,13-tetraoxa-7,16-diazacyclooctadecane (**4**). Product **3** (700 mg, 0.88 mmol) was hydrogenated in 10 mL of 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 **4** was obtained [^{1}H NMR ($CDCl_3$) δ 3.25 (4 H, t), 3.6–4.0 (20 H, m)].

(1) Seiler, K.; Wang, K.; Kuratli, M.; Simon, W. *Anal. Chim. Acta* **1991**, *224*, 151–160.
(2) Kawabata, Y.; Kamichika, T.; Imasaka, T.; Ishibashi, N. *Anal. Chem.* **1990**, *62*, 2054–2055.
(3) Freeman, M. K.; Bachas, L. G. *Anal. Chim. Acta* **1990**, *241*, 191–125.
(4) Arnold, M. A.; Ostler, T. J. *Anal. Chem.* **1986**, *58*, 1137–1140.
(5) Posch, H. E.; Leiner, M. J. P.; Wolfbeis, O. S. *Fresenius' Z. Anal. Chem.* **1989**, *334*, 162–165.
(6) Bright, F. V.; Litwiler, K. S.; Vargo, T. G.; Gardella, J. A. *Anal. Chim. Acta* **1992**, *262*, 323–330.
(7) Tromberg, B. J.; Sepaniak, M. J.; Vo-Dinh, T.; Griffin, G. D. *Anal. Chem.* **1987**, *59*, 1226–1230.
(8) Ogasawara, F. K.; Wang, Y.; Bobbitt, D. R. *Anal. Chem.* **1992**, *64*, 1637–1642.
(9) Zhujun, Z.; Seitz, W. R. *Anal. Chem.* **1986**, *58*, 220–222.
(10) Narayanaswamy, R.; Russell, D. A.; Sevilla, F. *Talanta* **1988**, *35*, 83–88.
(11) Chau, L. K.; Porter, M. D. *Anal. Chem.* **1990**, *62*, 1964–1971.
(12) Wyatt, W. A.; Bright, F. V.; Hieftje, G. M. *Anal. Chem.* **1987**, *59*, 2272–2276.
(13) Betts, T. A.; Catena, G. C.; Haung, J.; Litwiler, K. S.; Zhang, J.; Zagrobelny, J.; Bright, F. V. *Anal. Chim. Acta* **1991**, *246*, 55–63.
(14) Kobos, R. K.; Eveleigh, J. W.; Stepler, M. L.; Haley, B. J.; Papa, S. L. *Anal. Chem.* **1988**, *60*, 1996–1998.
(15) Lehn, J. M.; Simon, J.; Wagner, J. *Nouv. J. Chem.* **1977**, *1*, 77–84.

0003-2700/93/0365-0945$04.00/0

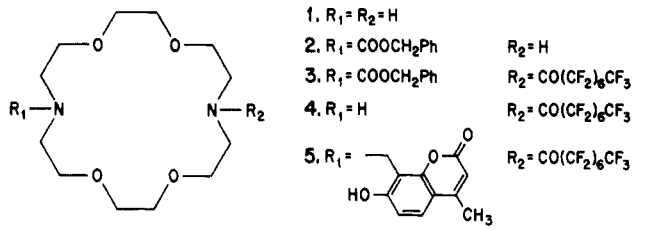

Figure 1. Structures of ionophore **5** and precursors **1–4**.

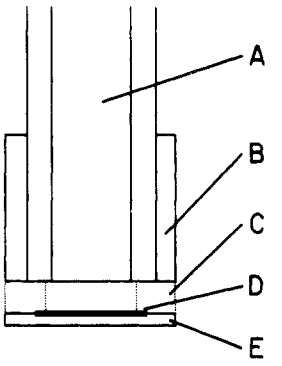

Figure 2. Construction of FOCS: (A) bifurcated fiber optic bundle, (B) PTFE sleeve, (C) 2-mm-long spacer, (D) PTFE membrane with immobilized ionophore, and (E) PTFE support for membrane.

Condensation of 4 with *4-Methylumbelliferone.* Product **4** (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 **5** as a colorless oil [^{1}H NMR ($CDCl_3$) δ 2.4 (3 H, s), 2.9 (4 H, s), 3.5–4.0 (20 H, m), 4.15 (2 H, s), 6.1 (1 H, s), 6.8 (1 H, d), 7.4 (1 H, d), 8.0 (1 H, s); FAB-MS 846 (M^+), 601, 440, 231, 173].

Apparatus. All spectroscopic measurements were made using an Oriel (Stratford, CT) modular spectrophotometer, configured as described earlier.[3] 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.

Probe Construction and Procedure. The probe construction is shown in Figure 2. A previously-swollen piece of PTFE membrane (FHUP 047 00, Millipore) was placed in the PTFE housing and was immersed in a 0.50 mM solution of **5** (in HPLC-grade methanol from Fisher, Fair Lawn, NJ) for several hours. The probe was washed with deionized water and placed in 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.

RESULTS AND DISCUSSION

Recently, interest has grown in the development of optical sensors for metal ions. One class of compounds which may provide an appropriate chromogenic metal-selective reagent for development of such a sensor is crown ethers. The ability of crown ethers to complex alkali and alkaline earth ions has led to their use in a variety of applications,[16,17] including ion chromatography, phase-transfer systems, and ion-selective electrodes. Covalent attachment of a chromophore to the crown ether skeleton has allowed the spectrophotometric determination of metal ions.[18,19] FOCS can be developed by immobilization of such a modified crown ether at the tip of an optical fiber.

In 1987, Alder et al. reported a fiber optic sensor based on an immobilized chromogenic crown ether, a "crowned" nitrophenylazophenol.[20] The reagent was adsorbed on ground Amberlite XAD-2 resin (a styrene/divinylbenzene copolymer), which was trapped at the end of an optical fiber by a porous PTFE membrane. The probe responded reversibly to aqueous potassium ions in the concentration range 10^{-3}–10^{-1} M and gave a K^+/Na^+ selectivity ratio of 6.4. The response times ranged from 2 to 7 min, depending on the change in concentration. The same group later reported that the probe was actually more selective for the more highly-charged calcium ion, with a Ca^{2+}/K^+ selectivity ratio of 8.3.[21] This probe construction was used with a number of different chromogenic crown ethers to produce optical sensors for potassium ions.[22]

The fluorogenic crown ether **5** was synthesized in order to evaluate the feasibility of using fluorocarbon-based immobilization of fluoroionophores in fiber optic sensors. A perfluorinated alkyl chain was substituted on one of the nitrogens of the diazacrown ether, and 4-methylumbelliferone (a fluorogenic tag) was attached to the other. Monoacylation of the diazacrown ether skeleton with perfluorooctanoyl chloride proved to be impractical, producing only the disubstituted species. However, a monosubstituted carbamate could easily be prepared.[15] 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.

Diazacrown ethers with two pendant chromogenic side arms have been shown to exhibit selectivity for divalent alkaline earth metal ions over monovalent alkali metals.[23] Similar monoazacrowns with a single chromogenic side arm are also selective for divalent cations.[24,25] This suggests that the diazacrown **5** with a single chromogenic side arm would also be selective for alkaline earth metal ions. Association of the diaza-crown **5** with a metal ion results in the loss of a proton from the methylumbelliferone moiety. Deprotonation of umbelliferone induces a change in the fluorescence intensity which is proportional to the concentration of metal in solution.[26,27]

Calibration curves for Ca^{2+} and Mg^{2+} in the 0–5 mM range are shown in Figure 3. The probe exhibits similar responses to the two divalent cations. The detection limit for Ca^{2+} is 0.1 mM as determined by the Ca^{2+} concentration that gives a signal that is 3-fold higher than the peak-to-peak noise level of the blank. This detection limit is 2.5-fold better than that reported by Ashworth et al. using chromogenic crown

(16) Takagi, M.; Nakamura, H. *J. Coord. Chem.* **1986**, *15*, 53–82.
(17) Forrest, H.; Pacey, G. E. *Talanta* **1989**, *36*, 335–340.
(18) Lohr, H. G.; Vogtle, F. *Acc. Chem. Res.* **1985**, *18*, 65–72.
(19) Takagi, M.; Ueno, K. *Top. Curr. Chem.* **1984**, *21*, 39–65.
(20) Alder, J. F.; Ashworth, D. C.; Narayanaswamy, R. *Analyst* **1987**, *112*, 1191–1192.
(21) Ashworth, D. C.; Huang, H. P.; Narayanaswamy, R. *Anal. Chim. Acta* **1988**, *213*, 251–257.
(22) Al-Amir, S. M. S.; Ashworth, D. C.; Narayanaswamy, R.; Moss, R. E. *Talanta* **1989**, *36*, 645–650.
(23) Katayama, Y.; Fukuda, R.; Iwasaki, T.; Nita, K.; Takagi, M. *Anal. Chim. Acta* **1988**, *204*, 113–125.
(24) Fery-Forgues, S.; Le Bris, M.-T.; Guette, J.-P.; Valeur, B. *J. Chem. Soc., Chem. Commun.* **1988**, 384–385.
(25) Wickstrom, T.; Dale, J.; Lund, W.; Buoen, S. *Anal. Chim. Acta* **1988**, *211*, 223–229.
(26) Blair, T. L.; Desai, J.; Bachas, L. G. *Anal. Lett.* **1992**, *25*, 1823–1834.
(27) Fink, D. W.; Koehler, W. R. *Anal. Chem.* **1970**, *42*, 990–993.

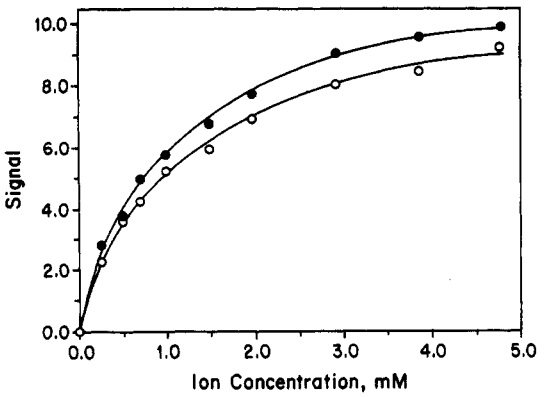

Figure 3. Calibration curves for Ca^{2+} (●) and Mg^{2+} (○). "Signal" refers to percent change in fluorescence intensity.

ethers immobilized on XAD-2 resin,[21] and it is 100-fold better than reported by Kawabata et al. using a chlortetracycline fluoroionophore immobilized on an ion exchange membrane.[28] This may be attributed to a stronger association of the Ca^{2+} with the fluoroionophore **5**. The response can be reversed by immersing the probe in an EDTA solution.

The probe gives a response time of less than 1 min in all our studies. 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.[21] The response time of the latter type of probe is limited by the time required for diffusion of the analyte across the microporous membrane and to the region of the probe where the transduction 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.

The introduction of multiple or branched perfluorinated carbon chains may help to prevent leaching of the reagent off the membrane and improve the lifetime of the probe. Indeed, de Miguel et al. report that multiple-stranded or branched-chain perfluoroalkyl groups are more retained on a perfluorinated stationary phase than singly-stranded analogues.[29] The signal of our probe decreased by about 35% in a 24-h period. This may be due to a combination of leaching of the reagent off the membrane and/or decomposition of the fluoroionophore. However, the same membrane could be regenerated by immersion in the original reagent solution. The reproducibility of the calibration curve, as determined by relative standard deviations of triplicate data points, is typically about 25%. This relatively low reproducibility can be attributed to the factors mentioned above. Incorporation of multiple-stranded perfluoroalkyl groups in the fluoroionophore used should also improve the reproducibility of the sensor response.

In conclusion, 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 which responds to Ca^{2+} and Mg^{2+} with response times of less than 1 min.

ACKNOWLEDGMENT

This work was supported in part by grants from the U.S. Department of the Interior (through the Kentucky Water Resources Research Institute) and the National Science Foundation (EHR-9108764).

RECEIVED for review August 12, 1992. Accepted December 7, 1992.

(28) Kawabata, Y.; Tahara, R.; Imasaki, T.; Ishibashi, N. *Anal. Chim. Acta* **1988**, *212*, 267–271.
(29) de Miguel, I.; Exbrayat, S.; Samain, D. *Chromatographia* **1987**, *24*, 849–853.