UC Irvine
UC Irvine Previously Published Works

Title
Fiber-optic chemical sensors for competitive binding fluoroimmunoassay.

Permalink
https://escholarship.org/uc/item/74q6j5wj

Journal
Analytical chemistry, 59(8)

ISSN
0003-2700

Authors
Tromberg, BJ
Sepaniak, MJ
Vo-Dinh, T
et al.

Publication Date
1987-04-01

DOI
10.1021/ac00135a033

Copyright Information
This work is made available under the terms of a Creative Commons Attribution License, availalbe at https://creativecommons.org/licenses/by/4.0/

Peer reviewed

eScholarship.org Powered by the California Digital Library
University of California

Fiber-Optic Chemical Sensors for Competitive Binding Fluoroimmunoassay

Bruce J. Tromberg[1] **and Michael J. Sepaniak***

Department of Chemistry, University of Tennessee, Knoxville, Tennessee 37996-1600

Tuan Vo-Dinh* and Guy D. Griffin

Advanced Monitoring Development Group, Health and Safety Research Division, Oak Ridge National Laboratory, P.O. Box X, Oak Ridge, Tennessee 37831

This paper describes the development of a fiber-optic chemical sensor based on the principle of competitive-binding fluorescence immunoassay. Rabbit immunoglobin G (IgG) is covalently immobilized on the distal sensing tip of a quartz optical fiber. The sensor is exposed to fluorescein isothiocyanate (FITC) labeled and unlabeled anti-rabbit IgG. The 488-nm line of an argon-ion laser provides excitation of sensor-bound analyte. This results in fluorescence emission at the optical fiber's sensing tip. Sensor response is inversely proportional to the amount of unlabeled anti-IgG in the sample. Limits of detection (LOD) vary with incubation time, sample size, and measurement conditions. For 10-μL samples, typical LOD are 25 fmol of unlabeled antibody in a 20-min incubation period. These results indicate that each fiber-optic fluoroimmunosensor can be constructed to perform a single sensitive, rapid, low-volume immunoassay, in in situ or benchtop applications.

Fiber-optic chemical sensors (FOCSs) have been designed to provide simple, rapid, in situ analyses of trace chemicals (*1–8*). FOCSs are characterized by their chemically selective immobilized reagent phase at the fiber's sampling terminus. This reagent phase distinguishes these devices from their less selective physical sensor counterparts (*9–11*).

In order to detect trace amounts of chemicals with high sensitivity and specificity, the principles of solid-phase immunoassay can be applied to FOCS design. This involves the covalent immobilization of receptor molecules (antibody or antigen) to the distal face of a single-strand 600-μm-diameter quartz optical fiber. The fiber and immobilized receptor molecules form a stable, selective fluoroimmunosensor (FIS). Careful selection of the proper immobilization procedure enhances FIS stability by minimizing receptor leakage. Provided there is sufficient immobilized reagent for reasonable sensitivity, this direct attachment of antibody or antigen via organosilanating reagents is preferable to those techniques which utilize membrane or gel-entrapped reagent phases. This is due to the fact that sensor response times are limited by mass transport to the fiber and immunochemical kinetics. Despite their potential for higher loadings, these processes may be slower in membrane or gel systems (*1*, *2*).

Several types of immunoassays can be performed. The simplest involves in situ FIS incubation followed by direct measurement of a naturally fluorescent analyte (*12*). For nonfluorescent materials, in situ incubation is followed by "development" in fluorophor-labeled second antibody. The resulting "antibody sandwich" produces a fluorescence signal that is directly proportional to bound antigen. The sensitivity obtained when using these techniques increases with increasing amounts of immobilized receptor (*13*). A third detection scheme involves competition between fluorophor-labeled and unlabeled antigen. In this case, the unlabeled analyte competes with labeled analyte for a limited number of receptor binding sites. Assay sensitivity therefore increases with decreasing amounts of immobilized reagent (*13*).

The appropriate FIS immunoassay method is determined by the chemical characteristics of the antigen/antibody system of interest. For example, naturally fluorescent antigens and haptens should be analyzed by direct measurement. Nonfluorescent haptens should be detected via competitive binding or sandwich techniques, though the scarcity of hapten epitopes (antibody recognition sites) may diminish the sensitivity of sandwich analyses. An alternate means of gaining sensitivity in direct and sandwich assays entails increasing the absolute amount of immobilized receptor by increasing the fiber surface area. This can be accomplished with small-diameter fibers by utilizing evanescent-wave excitation of biomolecules bound to the circumference of the sensing tip. These sensors are characterized by large exposed surface areas and low evanescent wave penetration depths (*5*, *14*, *15*). Their primary limitation is the fact that evanescently excited fluorescence is coupled less efficiently into the optical fiber than distal-face (vide infra) excitation.

In this paper, the FIS is exposed to fluorophor-labeled and unlabeled ligand. This results in competition for FIS binding sites. Signal intensity in this competitive binding immunoassay scheme is inversely proportional to analyte (unlabeled ligand) concentration. Though the fiber's diminutive surface area restricts the absolute amount of bound analyte, excellent sensitivity is achieved since an efficient distal-face fluorescence collection geometry is used. In addition, the small amount of immobilized receptor serves as the limiting reagent in the competitive assay. As a result, rapid, nonequilibrium low-volume analyses can be performed. Moreover, factors that alter fluorescence signals, such as quenching, matrix interferences, and self-absorption are relatively insignificant for the FIS. This is partly due to the fact that the sensing terminus is thoroughly washed following analyte incubation and all measurements are made in a controlled matrix.

EXPERIMENTAL SECTION

Materials. Rabbit immunoglobin G (IgG), polyclonal anti-rabbit IgG, polyclonal fluorescein isothyocyanate (FITC) conjugated anti-rabbit IgG, bovine serum albumin (BSA), and ovalbumin were purchased from Cooper Biomedical, Inc., Malvern, PA. Spectrapor cellulose dialysis membrane (MW cutoff = 12000) was acquired from Thomas Scientific, Philadelphia, PA. (3-Glycidoxypropyl)trimethoxysilane (GOPS) was obtained from Aldrich Chemical Co., Milwaukee, WI. Phosphate-buffered saline (PBS), pH 7.4, and all other reagents were supplied by Sigma Chemical Co., St. Louis, MO.

[1] Also Oak Ridge Associated Universities Graduate Fellow, Advanced Monitoring Development Group, Health and Safety Research Division, Oak Ridge National Laboratory.

0003-2700/87/0359-1226$01.50/0 © 1987 American Chemical Society

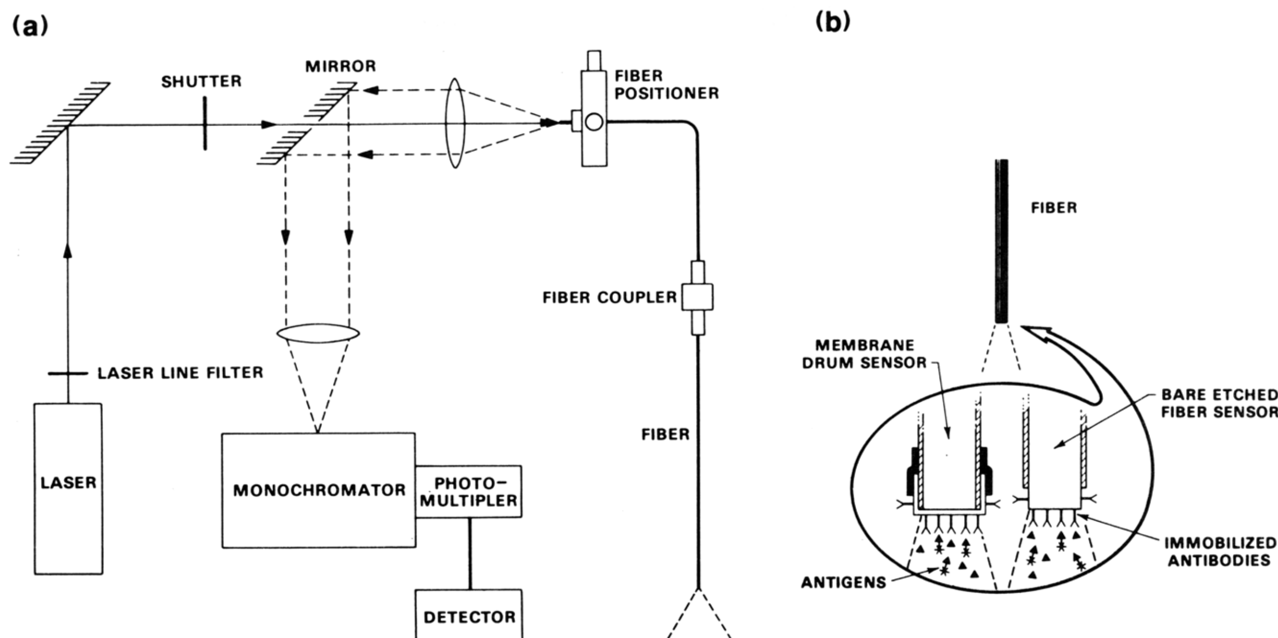

Figure 1. (a) Instrumental apparatus and (b) magnified view of possible fiber-sensing termini.

Multimode, fused-silica, 600-μm-diameter optical fibers (numerical aperture = 0.22) were supplied by Math Associates, Westbury, NY. FIS fibers were cut into 0.6-m lengths and terminated with SMA connectors (Math Associates). Both ends were hand-polished with 15-μm, 3-μm, and 0.3-μm lapping film.

Apparatus. A Perkin-Elmer 650-40 spectrofluorometer was used to evaluate the immobilization procedures for protein-binding capacity and stability. All FIS measurements were performed with the instrument shown in Figure 1a. Light from a Spectra-Physics Model 162-A forced-air-cooled argon-ion laser (approximately 10 mW at 488 nm) is directed through a 488-nm laser line filter (Corion Corp., Holliston, MA) and a Uniblitz variable shutter (hand-triggered 1-s aperture). Since the shutter permits the delivery of controlled-duration excitation pulses, FITC photodegradation is minimized and fiber-to-fiber reproducibility is enhanced. Excitation light passes through a mirror with a small hole in the center and is focused by a 25-mm-diameter $f/2$ lens onto the incident end of an $f/2.2$ optical fiber. A portion of the red-shifted FIS fluorescence emission is collected at the distal face. This signal returns to the incident end and diverges at a half angle of ~12.5°. The 25-mm-diameter $f/2.0$ lens collimates the FIS signal. The mirror directs the collimated emission through a 40-mm-diameter $f/3.6$ focusing lens, a 550-nm band-pass filter (Corion Corp., FWHM = 50 nm), and into an Instruments SA H-10 ($f/3.5$) monochromator. Photocurrent generated by an RCA 1P28 photomultipler tube operated at 700 V is detected by a Keithley Model 485 picoammeter. Data are simultaneously collected and stored in the Keithley's memory and on a strip chart recorder.

Procedures. *Surface Silanization with (3-Glycidoxypropyl)trimethoxysilane (GOPS).* Fibers were stripped of approximately 3 mm of cladding The quartz cores were washed in hot 1 M HNO_3 and rinsed in water. A 10% aqueous solution of GOPS was heated to 90 °C and maintained at pH 3 with 1 M HCl (*16*). Fibers were submerged, allowed to react for 2 h, then dried overnight at 105 °C.

Membrane Drum Preparation. Wet cellulose membranes were stretched over the ends of 16-gauge stainless steel tubes and affixed with heat-shrink tubing (Figure 1b). Membranes were treated in 1 mM EDTA and distilled, deionized water prior to activation. Fibers were placed inside the drum assembly.

Surface Activation and Protein Attachment. GOPS-derivatized fibers and membrane assemblies were oxidized in 0.1 M periodic acid for 1 h (*16, 17*). The resulting aldehydic surfaces were washed in water and phosphate-buffered saline (PBS) pH 7.4. Fibers and membranes were then suspended in polypropylene vials containing 1 mL of 3–6 mg/mL protein and allowed to react for 24 h at 4 °C. During this time, glass- and membrane-bound aldehydes formed Schiff bases with protein amino groups. Gentle reduction of the Schiff bases was accomplished by the addition of $NaBH_4$ in PBS for three consecutive 15-min intervals (*16, 17*). Fibers and membranes were rinsed in PBS and stored in 1% ovalbumin/PBS at 4 °C in order to minimize effects of nonspecific binding (*18*). Sensors prepared in this manner were stable for several weeks.

Measurements. *Determination of Optimum Substrate for Immobilization.* In order to determine the extent of protein surface coverage and degree of nonspecific binding, fibers and membranes were prepared with rabbit IgG and BSA reagent phases. Sensors were incubated with 0.1 mg/mL anti-rabbit IgG-FITC for 60 min at room temperature. Following incubation, sensors were rinsed well with PBS and then reacted with 3 M potassium thiocyanate (KSCN) chaotropic reagent. The amount of specifically bound protein was determined by measuring FITC fluorescence in the chaotropic wash at λ_{ex} = 488 nm, λ_{em} = 520 nm with a Perkin-Elmer 650-40 spectrofluorometer.

The rate of protein loss following covalent attachment (leakage) was determined by immobilizing anti-rabbit IgG-FITC on five identically prepared membranes and fibers. Each sensor was suspended in polypropylene vials containing 1 mL of PBS. Samples were shaken at room temperature for approximately 2.5 h. During this time, FITC fluorescence from successive vials was measured at regular intervals.

Competitive Binding Assays. All measurements were performed with GOPS-derivatized fibers. FIS reagent phases consisted of rabbit IgG. Sample solutions contained varying amounts of anti-rabbit IgG. Two types of competitive binding assays were investigated.

The first method involved simultaneous incubation of the FIS in a mixture of labeled and unlabeled analyte. Dose–response curves were obtained for 1-mL stirred, 1-mL unstirred, and 10-μL solutions. Each solution in these three categories contained different amounts of anti-rabbit IgG along with 31 pmol (1-mL solutions) or 15.5 fmol (10-μL solutions) of anti-rabbit IgG-FITC. Blank solutions consisted of pure FITC labeled antibody in PBS.

A separate FIS was used for each sample. Data points on each dose–response curve were obtained by using identically prepared fibers. All fibers were rinsed well in 20 mL of PBS after the appropriate incubation period. Measurements were performed following rinsing in 1 mL of fresh PBS. Excitation was provided by the 488-nm laser line. Fluorescence signals were detected at 550 nm with an 8-nm bandwidth. Background signals were obtained for each fiber prior to incubation in 1 mL of fresh PBS. All signals were collected during the 1-s period the shutter was open. Peak intensities (I) were recorded in the picoammeter memory (data read rate was 3 points/s) and on the strip chart

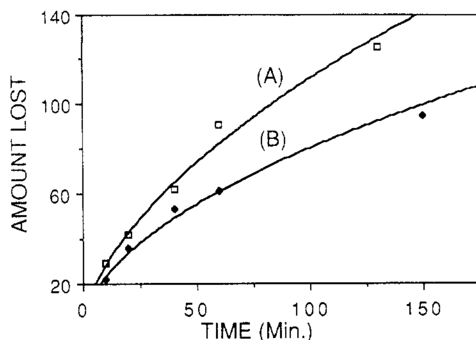

Figure 2. Amount of protein lost (ng/cm^2) vs. time (min) for (A) membrane-drum and (B) GOPS-derivatized fiber sensors.

recorder. Instrumental noise (typically 0.3 nA) was determined by measuring the recorder signals for unincubated sensors in PBS. Percent bound labeled analyte (%B) was determined by using eq 1 (*19*):

$$\%B = (I_{\text{sample}} - I_{\text{background}})/(I_{\text{blank}} - I_{\text{background}}) \quad (1)$$

A second competitive binding method involved a 2-min preincubation of the FIS in varying concentrations of 1 mL of stirred, unlabeled analyte. Fibers were then rinsed and incubated for 2.0 min in 1 mL of stirred anti-rabbit IgG-FTIC (0.5 mg/mL). Measurements were performed following rinsing in 1 mL of fresh PBS. Signal detection procedures for this preincubation assay were identical with those described for the simultaneous-incubation technique.

RESULTS AND DISCUSSION

Fiber-optic chemical sensors used in fluoroimmunoassays are based on highly specific antibody–antigen interactions. Just as in other ligand-binder competitive assays, FIS analyses require competition between labeled and unlabeled analyte for a limited number of receptor binding sites. All measurements involve separation of free labeled ligand from a receptor-bound fraction. The FIS competitive assay can therefore be classified as a heterogeneous solid-phase fluoroimmunoassay.

Optimal measurement conditions for heterogeneous solid-phase competitive assays are usually found when matrix interferences are virtually eliminated and receptor binding sites nearly saturated by labeled ligand (*19*). This requires that the solid surface be stable and easily washable and that the amount of immobilized receptor be maintained as low as reasonably possible for adequate detectability. Both the membrane-drum and GOPS-derivatized fibers were evaluated with regard to these criteria.

Figure 2 indicates that, following immobilization, the amount of protein lost due to rinsing was slightly higher for the membrane-drum FIS. In order to determine the extent of nonspecific binding to the FIS, two types of proteins, rabbit IgG and bovine serum albumin (BSA), were immobilized on membranes and fibers by using the previously described procedures. After incubating membranes and fibers in anti-IgG-FITC and washing in PBS, sensors were reacted with 3 M KSCN in order to disrupt the noncovalent antigen–antibody bonds. Table I shows that BSA membranes and fibers interacted relatively poorly with anti-IgG-FITC. This result was expected since anti-IgG should not specifically recognize BSA. The extent of nonspecific interaction, however, was greater for fiber surfaces (~13%) than for membrane surfaces (~1%). This may have been due, in part, to slightly more effective PBS rinsing of the membranes.

By assuming the anti-rabbit IgG-FITC to be approximately univalently bound to immobilized rabbit IgG and the 3 M KSCN chaotropic rinse to be reasonably effective, GOPS derivatization yields protein loadings ~3 μg/cm^2. This corresponds to 8 × 10^{-9} g or 50 fmol of immobilized rabbit IgG

Table I. Optimum Substrate for Immobilization[a]

type of surface	immobilized reagent	
	IgG	BSA
cellulose membrane	0.77	0.06
quartz optical fiber	2.9	0.43

[a] Anti-IgG-FITC lost from surface (μg/cm^2) following wash in 3 M KSCN.

(MW ~1.6 × 10^5) on the distal end of a 600-μm-diameter FIS. This procedure was repeated several times and FIS loadings were consistently 18–50 fmol (1–3 μg/cm^2). These values agree well with previously reported 1 μg/cm^2 antibody loadings on quartz surfaces when using (aminopropyl)triethoxysilane (APTS) derivatization (*14*). Since the membrane-drum sensors were slightly less stable, more cumbersome to construct, and yielded lower protein loadings, GOPS-derivatized fibers were used in the FIS competitive binding experiments.

In order to determine whether a single FIS or multiple sensors were necessary for data collection, FIS signal reproducibility was investigated. Five identically prepared fibers with rabbit IgG reagent phases were incubated for 5 min in 1-mL stirred solutions of 3.1 × 10^{-9} M anti-rabbit IgG-FITC. The relative standard deviation for measured signals was 10.2%. Repeated exposure of similarly prepared sensors to 3 M KSCN chaotropic reagent followed by rinsing in PBS and reincubation in 3.1 × 10^{-9} M anti-rabbit IgG-FITC failed to restore signals to their original levels. In fact, after one chaotropic wash, FIS response was typically 30–40% poorer. After three washes with 3 M KSCN, most fibers failed to bind specific antibody. Because of these results, each data point on the competitive binding dose–response curves was obtained by using a separate, identically prepared FIS. In spite of the reasonably reproducible between-fiber response, efforts are in progress to identify effective, nondestructive chaotropic reagents for convenient, single-fiber use.

Simultaneous-incubation dose–response curves are illustrated in Figure 3. General curve shapes concur with expected FIS competitive binding behavior. Samples containing greater amounts of unlabeled analyte exhibit lower signals due to the fact that proportionally more labeled analyte is displaced from the limited number of FIS binding sites. The 10-μL and 1-mL unstirred samples exhibit significant curvature. This is primarily attributable to three factors: (1) the heterogeneous range of avidities of the polyclonal anti-rabbit IgG antibodies, (2) possible differences in the avidities of the FITC-labeled and unlabeled antibodies (due to labeling), and (3) the randomly oriented immobilized rabbit IgG binding sites (*20*). Regions of optimal detectability are on the steepest portions of the curves. As with most competitive assays, this covers a narrow interval of analyte concentrations and results in linear dynamic ranges (LDR) of approximately 1 order of magnitude, at best. Limits of detection (LOD) were obtained by determining the amount of unlabeled analyte at two RSD units below $I = I_B$ (i.e., %B ~ 80). This corresponds to 8 pmol, 13 pmol, and 25 fmol for the 1-mL unstirred, 1-mL stirred, and 10-μL drop samples. It should be emphasized that these LOD are strongly dependent on a number of factors, including incubation time, extent and reproducibility of fiber surface coverage, instrumentation, and immunochemical response. As a result, day to day LOD may fluctuate significantly.

The effect of sample stirring on the FIS was also investigated. Signal levels attained with 5-min, stirred 1-mL sample incubations (Figure 3b) were approximately the same as those produced with 20-min, unstirred, 1-mL samples. This is a good indication that for the high-avidity antibodies, which have

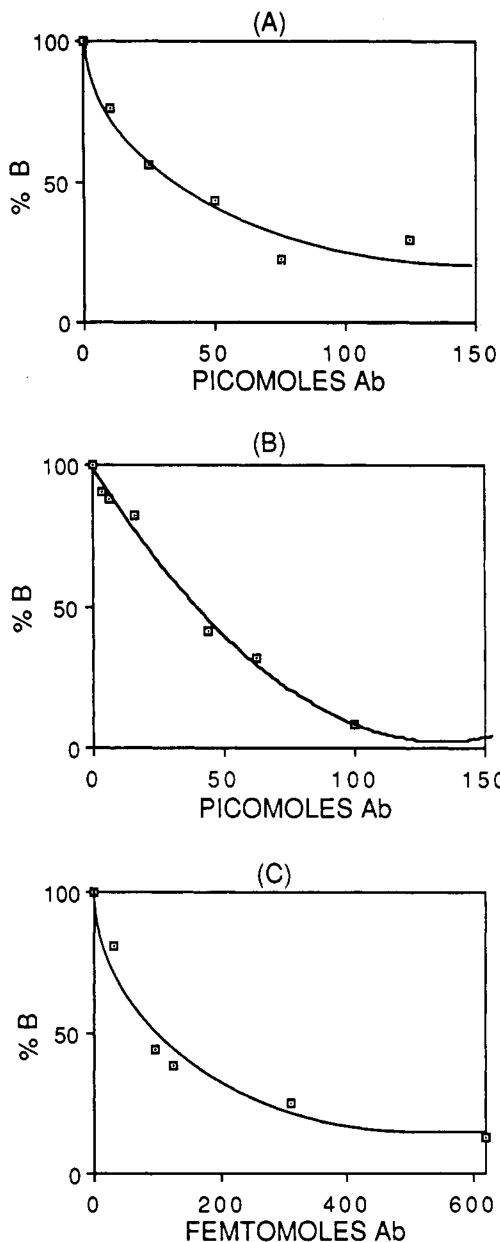

Figure 3. Simultaneous-incubation FIS dose response: percent bound (%*B*) labeled anti-IgG vs. amount of unlabeled anti-IgG (Ab) for (A) 1.0-mL unstirred solutions, 20-min incubations; (B) 1.0-mL stirred solutions, 5-min incubations; (C) 10-μL drops, 20-min incubations. Relative standard deviation per data point = 10.2%.

relatively rapid reaction kinetics, the FIS response rate is limited by the rate of antibody transport to the fiber surface. Recently reported experimental evidence supports this notion (*20, 21*).

An additional feature of the stirred 1 mL sample dose–response data is that it displays less curvature and greater LDR than data from the unstirred solutions. Since an absence of curvature in the competitive binding dose response usually indicates a relatively homogeneous antibody population, the rapid rate of supplying the FIS with antibody during forced convection (stirring) may increase the proportion of high-avidity antibodies which react with the IgG on the fiber surface.

The second competitive binding approach, preincubation, yielded a dose response which is illustrated in Figure 4. LOD obtained with this measurement technique was approximately 100 pmol for 1-mL solutions. This value is strongly influenced by incubation times and concentration of labeled antibody. Selection of the appropriate incubation time in 0.5 mg/mL

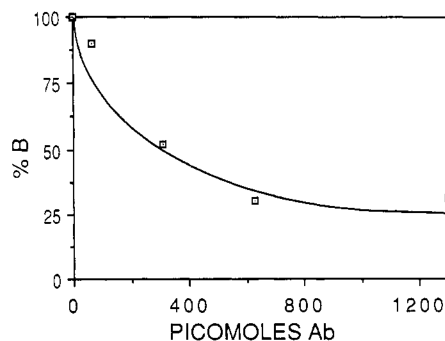

Figure 4. Preincubation FIS dose response: percent bound (%*B*) labeled anti-IgG vs. amount of unlabeled anti-IgG (Ab) for 1.0-mL stirred solutions, 2-min incubations. Relative standard deviation per data point = 10.2%.

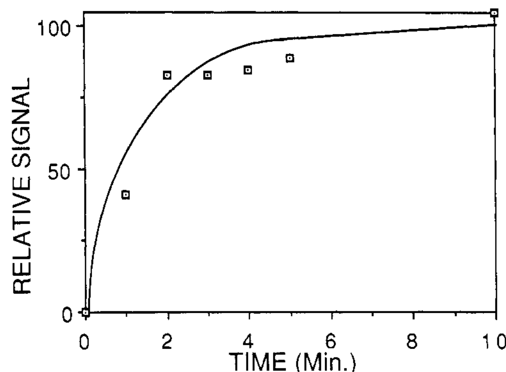

Figure 5. Relative signal vs. time (min) for FIS in 0.5 mg/mL stirred anti-IgG-FITC.

labeled antibody was accomplished by recording the sensor's time-dependent response in a 1.0-mL stirred solution. These results, illustrated in Figure 5, demonstrate that saturation of FIS binding sites is nearly complete after only 2 min.

The most important feature of the preincubation assay is that it can be used in situ. For example, sensors could be placed in vivo for a specified period of time, removed, and "developed" in a high concentration of labeled analyte. Standardization would be difficult, since preincubation conditions for calibration solutions would have to be identical with samples. This would entail simulating in vivo matrices and mass-transport conditions.

Simultaneous incubation, on the other hand, could not be used for in vivo measurements. Due to its extremely low sample volume requirements, rapid response times, and single sample manipulation step, this type of assay may be useful only for benchtop analyses.

A major drawback to both types of assays is the fact that multiple fibers must be used. As a result, measurement error can often be attributed to imprecision in fiber derivatization and biomolecule immobilization. At present, our work indicates that fiber-to-fiber relative standard deviation is about 10%. It is hoped that continued attention to the variables that control the extent of fiber surface coverage will minimize the effects of this source of measurement imprecision. Additional factors which influence FIS reproducibility and detectability include the cumbersome manual SMA coupling procedure, instrumental noise, and optical background. The use of high-speed fiber-optic switches is currently being investigated in order to minimize these effects and further facilitate rapid, simultaneous, multisite analyses.

In conclusion, the results presented in this paper demonstrate that antibody-based fiber-optic chemical sensors can be developed to perform simple, rapid immunoassays in microliter volumes of analyte. Assay sensitivity varies in a manner that is consistent with competitive binding mea-

surements. Though linear dynamic ranges (LDR) are poor, excellent sensitivity and detectability are achievable over a narrow interval of analyte concentrations. We are presently investigating antibody-sandwich and direct assays in order to extend FIS applications. Manipulation of these techniques, along with the preincubation competitive assay, will allow full exploitation of the in situ measurement possibilities offered by fiber-optic sensor instrumentation.

ACKNOWLEDGMENT

The authors thank C. J. Wust of the University of Tennessee for his valuable advice and R. N. Compton of Oak Ridge National Laboratory for his loan of laboratory equipment and space.

Registry No. GOPS, 2530-83-8.

LITERATURE CITED

(1) Munkholm, C.; Walt, D. R.; Milanovich, F. P.; Klainer, S. M. *Anal. Chem.* **1986**, *58*, 1427-1430.
(2) Seitz, W. R. *Anal. Chem.* **1984**, *56*, 16A-34A.
(3) Milanovich, F. P. *Environ. Sci. Technol.* **1986**, *20*, 441-442.
(4) Arnold, M. A. *Anal. Chem.* **1985**, *57*, 565-566.
(5) Andrade, J. D.; Vanwagenen, R. A.; Gregonis, D. E.; Newby, K.; Lin, J. N. *IEEE Trans. Electron Devices* **1985**, *ED-32*, 1175-1179.
(6) Peterson, J. I.; Fitzgerald, R. V.; Buckhold, D. K. *Anal. Chem.* **1984**, *56*, 62-67.
(7) Zhujun, Z.; Seitz, W. R. *Anal. Chim Acta* **1984**, *160*, 47-55.
(8) Schultz, J. S.; Mansouri, S.; Goldstein, I. J. *Diabetes Care* **1982**, *160*, 47-55.
(9) Ross, I. N.; Mbanu, A. *Opt. Laser Technol.* **1985**, Feb., 31-35.
(10) Tromberg, B. J.; Eastham, J. F.; Sepaniak, M. J. *Appl. Spectrosc.* **1984**, *38*, 38-42.
(11) Schwab, S. D.; McCreery, R. L. *Anal. Chem.* **1984**, *56*, 2199-2204.
(12) Vo-Dinh, T.; Griffin, G. D.; Ambose, K. R.; Sepaniak, M. J.; Tromberg, B. J. *Proceedings of the Tenth International Symposium on Polycylic Aromatic Hydrocarbons*; Battelle: Columbus, OH, in press.
(13) Dakubu, S.; Ekins, R.; Jackson, T.; Marshall, N. J. In *Practical Immunoassay: The State of the Art*; Butt, W. R., Ed.; Marcel Dekker: New York, 1984; Chapter 4.
(14) Sutherland, R. M.; Dähne, C.; Place, J. F.; Ringrose, A. S. *Clin. Chem. (Winston-Salem, NC)* **1984**, *30*, 1533-1538.
(15) Hirschfeld, T. E. U.S. Patent 4 447 546, May 8, 1984.
(16) Sportsman, J. R.; Wilson, G. S. *Anal. Chem.* **1980**, *52*, 2013-2018.
(17) Sanderson, C. J.; Wilson, D. V. *Immunology* **1971**, *20*, 1061-1065.
(18) Thorell, J. I.; Larson, S. M. *Radioimmunoassay and Related Techniques*; C. V. Mosby: St. Louis, MO, 1978; Chapter 5.
(19) Aloisi, R. M. *Principles of Immunodiagnostics*; C. V. Mosby: St. Louis, MO, 1979; Chapter 9.
(20) P. C. Rodgers In *Practical Immunoassay: The State of the Art*; Butt, W. R., Ed.; Marcel Dekker: New York, 1984; Chapter 10.
(21) Nygren, H.; Stenberg, M. *J. Colloid Interface Sci.* **1985**, *107*, 561-566.
(22) Stenberg, M.; Elwing, H.; Nygren, H. *J. Theor. Biol.* **1982**, *98*, 307-320.

RECEIVED for review November 12, 1986. Accepted January 12, 1987. This work has been supported by the National Institutes of Health (GM 34730) and the Office of Health and Environmental Research, U.S. Department of Energy, under Contract Number DE-AC05-84OR21400 with Martin Marietta Energy Systems, Inc.

CORRESPONDENCE

On-Line Mass Spectrometric Detection for Capillary Zone Electrophoresis

Sir: Mikkers, Jorgenson, and co-workers (*1, 2*) have reported on the use of capillary zone electrophoresis (CZE) for high-resolution separations of amino acids, peptides, proteins, and complex salt mixtures. This technique has been shown to provide separation efficiencies of up to 10^6 theoretical plates, often in less than 20 min (*3*). Capillary zone electrophoresis is particularly useful in the separation of ionized and partially ionized species in aqueous solvents, although nonaqueous solvents have also been used (*4*). In CZE separation occurs in a capillary tube filled with a buffer and immersed in buffer reservoirs at each end (Figure 1). The sample is typically introduced as a sample plug by electromigration from a separate sample reservoir (*2*). Electroosmotic flow in the capillary is caused by the migration of ions from the diffusive layer of the electrical double layer at the capillary surface, under the influence of an electrical field imposed tangentially to the surface, causing the ions to migrate toward the oppositely charged electrode (*5*). The resulting bulk electroosmotic flow can be sufficiently fast so that positively charged ions, neutral species, and negatively charged ions elute in short times, with the separation due to differences in the electrophoretic mobilities of the analytes.

Detection of the eluting species in CZE is usually performed by on-line fluorescence or UV absorbance detection. Such detection techniques have been adequate for species that fluoresce, absorb, or are amenable to derivatization with fluorescing or absorbing chromophores (*1, 2*). However these detectors impose difficult cell volume and sample size limitations if high separation efficiencies are to be realized. These limitations constitute a major drawback in the use of CZE for the separation and identification of complex mixtures. The ideal detector for CZE would provide universal detection, selectivity, and sensitivity without degrading separation efficiency.

We have developed a viable alternative to CZE detection based on mass spectrometric interfacing. A capillary zone electrophoresis–mass spectrometry (CZE–MS) interface obviously requires a substantial departure from the conventional CZE arrangement; it is clear that the interface design and ionization method are crucial to success. The liquid flow rate in CZE ($\sim$1 μL/min) is highly compatible with conventional mass spectrometers even if the total column effluent was introduced directly. The direct liquid introduction interfaces developed for LC–MS suffer from orifice plugging at low flow rates and the thermal degradation of high mass–low volatility components (*6, 7*). Thermospray ionization, though attractive, has not been shown to be effective for liquid flow rates below a few tenths of a mL/min. Therefore, our evaluation of the requirements for a mass spectrometer interface suggested an approach that incorporates the electrospray ionization technique developed by Dole et al. (*8*) and the more recent work reported by Fenn and co-workers (*9*). In this communication, we report the successful development of CZE–MS instrumentation for the separation and analysis of ionic species in aqueous solutions.

EXPERIMENTAL SECTION

Apparatus. A schematic of the CZE-MS instrument is given

 © 1987 American Chemical Society