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Fiber-optic biosensors

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Introduction

Fiber-optic biosensors are devices in which a biocatalyst is immobilized at the distal tip of a fiber-optic sensing device (Arnold, 1984; Arnold and Meyerhoff, 1988; Wangsa and Arnold, 1989). The biocatalyst mediates between this sensing device and the analyte of interest by either forming a detectable species from the analyte or by consuming a detectable co-substrate. Fig. 1 shows the various processes that occur at the sensing tip when a detectable species is produced from the biocatalytic reaction. The analyte or substrate of the biocatalyzed reaction diffuses from the bulk solution to the biocatalytic layer where the substrate is

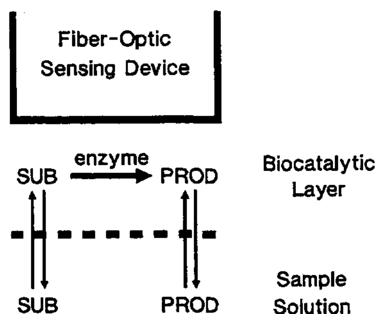

Fig. 1. Schematic representation of a fiber-optic biocatalytic biosensor.

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converted to a detectable species. A steady-state concentration of the detectable species is established and a corresponding steady-state signal is obtained (Carr and Bowers, 1980). The magnitude of this signal is related to the analyte concentration in the bulk solution through a calibration curve.

Various types of fiber-optic biosensors have been developed. These biosensors can be grouped according to the type of species detected by the sensing device. Biosensors based on the detection of oxygen, ammonia and pH have been reported. Individual fiber-optic chemical sensors (FOCSs) for the selective detection of oxygen, ammonia and pH, respectively, are used in the fabrication of these biosensors. In addition, biosensors based on the direct detection of either a chromophoric or luminescent participant in the biocatalyzed reaction have been reported.

Oxygen detection

Considerable effort has gone into the development of oxygen selective FOCSs (Peterson et al., 1984; Lubbers and Opitz, 1983). In general, these devices are based on the dynamic quenching effect of oxygen on various fluorescent dyes. The response of such sensors is given by the Stern-Volmer equation for dynamic quenching:

$$\frac{I}{I_0} = \frac{1}{1 + K p_{O_2}} \tag{1}$$

where I and I_0 correspond to the fluorescence intensities in the presence and absence of oxygen, respectively; K is the quenching constant; and p_{O_2} is the partial pressure of oxygen in the sample. A linear relationship between the measured fluorescence intensity ratio and the oxygen partial pressure has been found experimentally (Peterson et al., 1984; Lubbers and Opitz, 1983).

A biosensor can be constructed by immobilizing an oxidase enzyme at the sensing tip of the oxygen FOCS. For example, the enzyme glucose oxidase catalyzes the oxidation of glucose by oxygen to form gluconic acid and hydrogen peroxide. Fig. 2 shows a schematic diagram for the operation of an oxygen based fiber-optic glucose biosensor. The presence of glucose causes the consumption of oxygen which reduces the extent of fluorescence quenching. A fiber or bundle of fibers supplies the excitation radiation to a layer of indicator solution. A fraction of the radiation emitted by the non-quenched fluorescent indicator is collected by the fibers and guided to a photomultiplier tube (PMT) detector. The resulting fluorescence intensity is related to the sample glucose concentration through a calibration curve (Uwira et al., 1984; Kroneis and Marsoner, 1987; Opitz and Lubbers, 1987, 1988; Trettnak et al., 1988; Shah et al., 1988).

The following modified Stern-Volmer equation describes the response of such oxygen-based fiber-optic biosensors:

$$\frac{I}{I_0} = \frac{1}{1 + K p_{O_2} - K' \,[\mathrm{Sub}]} \tag{2}$$

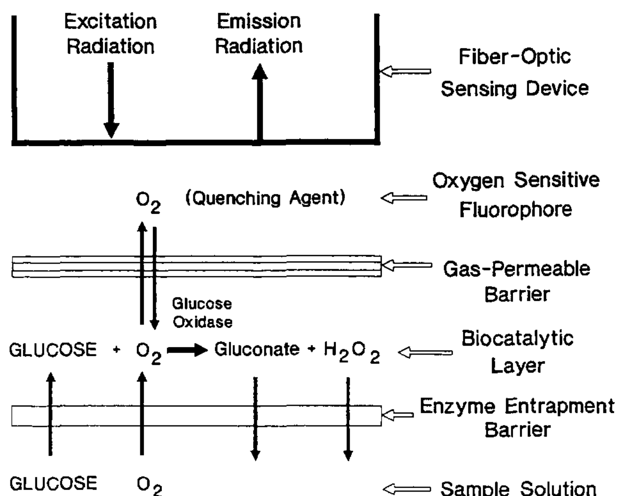


Fig. 2. Diagram of a fiber-optic glucose biosensor.

where [Sub] is the concentration of the enzyme substrate (i.e., glucose) and K' is an empirical proportionality constant which accounts for various diffusional processes and enzyme kinetic parameters. This equation shows that the response is dependent on both the concentration of the enzymatic substrate (analyte) and the partial pressure of oxygen. For samples in which the oxygen level is not constant, a correction for any variations in the oxygen level is required. A reference oxygen FOCS has been proposed for this purpose (Wolfbeis, 1987).

Similar fiber-optic biosensors have been reported for lactate (Lubbers et al., 1981; Voelkl et al., 1981), xanthine (Voelkl et al., 1981), ethanol (Wolfbeis, 1987; Lubbers et al., 1987; Voelkl et al., 1980, 1981; Wolfbeis and Posch, 1988) and hydrogen peroxide (Posch and Wolfbeis, 1989). For these sensors, lactate oxidase, xanthine oxidase, alcohol oxidase and catalase, respectively, have been coupled with fiber-optic oxygen sensors based on fluorescence quenching.

Ammonia detection

Fiber-optic biosensors have also been constructed by coupling a deaminating enzyme with a fiber-optic ammonia gas sensor. The enzyme catalyzes a reaction which generates ammonia from the analyte of interest and the generated ammonia is detected by the sensor. The ammonia FOCS is fabricated with an indicator solution trapped between an optical fiber detection system and a gas-permeable membrane (Arnold and Ostler, 1986; Rhines and Arnold, 1988). This indicator solution contains a pH indicator dye with a suitable acid dissociation constant. Ammonia in the sample crosses the membrane and alters the pH of the indicator solution. This pH change is measured optically by monitoring the non-protonated form of the pH indicator dye. Both fluorescence and absorbance detection modes have been used. A

comparison of these modes reveals that fluorescence detection is preferred for most situations (Rhines and Arnold, 1989).

The first ammonia-based fiber-optic biosensor was for the measurement of urea (Arnold, 1988; Rhines and Arnold, 1989). Urease catalyzes the following reaction:

$$\text{Urea} \rightarrow 2NH_3 + CO_2. \quad (3)$$

A urea biosensor is constructed by immobilizing urease at the sensing tip of the ammonia FOCS. The generation of ammonia is detected and the resulting optical signal is related to the concentration of urea in the sample solution. This fiber-optic urea biosensor has been found to be capable of measuring urea in human serum samples (Rhines and Arnold, 1989).

Presently, the only other report of a fiber-optic biosensor based on ammonia detection is that for glutamate (Arnold, 1989). This biosensor uses glutamate oxidase to generate ammonia from glutamate. Many other biosensors can be envisioned based on this sensor concept, however. Indeed, many examples of similar biosensors based on ammonia detection with the commercially available ammonia gas-sensing electrode have been reported (Arnold, 1983; Turner et al., 1987; Arnold and Meyerhoff, 1988; Guilbault, 1988). In addition, the same overall strategy can be used to prepare biosensors based on the detection of enzymatically generated carbon dioxide. Several reports of carbon dioxide gas-sensing FOCSs have appeared (Vurek et al., 1983; Wolfbeis et al., 1988). Many biosensors based on coupling a suitable decarboxylating enzyme with the carbon dioxide gas-sensing FOCS can be envisioned, but to date, no such biosensor has been reported.

Detection of pH

Fiber-optic biosensors have been reported based on pH changes in the micro-environment of the biocatalytic layer. A fluorescent pH indicator dye is co-immobilized with the biocatalyst at the distal tip of an optical fiber. An example is the fiber-optic penicillin biosensor (Goldfinch and Lowe, 1984; Kulp et al., 1987; Fuh et al., 1988; Yerian et al., 1988). Penicillinase catalyzes the cleavage of the β-lactam ring of penicillin to produce penicillinoic acid. The production of an acid lowers the pH which is measured as a change in the relative concentrations of the protonated and non-protonated forms of the immobilized pH indicator dye. The acid dissociation constant for the dye must be compatible with the pH profile for the biocatalytic activity. Besides penicillin, pH-based fiber-optic biosensors have been reported for glucose (Goldfinch and Lowe, 1984; Trettnak et al., 1989) and urea (Goldfinch and Lowe, 1984).

Fiber-optic biosensors based on pH measurements suffer from the same drawbacks as do biosensors based on pH electrodes (Arnold and Meyerhoff, 1988). The major problem is the pH dependency of the biocatalytic activity. Because the ability of the enzyme to catalyze a reaction is pH dependent, the amount of effective biocatalytic activity at the sensor tip can change during sensor operation. The overall sensor response may or may not be repeatable depending on the pH stability

of the immobilized enzyme. The sensor response is also strongly dependent on the buffer capacity of the sample solution. High buffer capacity provides low sensitivity over a wide dynamic range; whereas, low buffer capacity provides high sensitivity over a narrow dynamic range. A difference in the buffer capacity between the standards and the sample cannot be tolerated. Parameters that alter the acid dissociation constant of the indicator dye, such as ionic strength and temperature, can also alter the sensor's response. Finally, pH-based fiber-optic biosensors are susceptible to interference by endogenous quenching agents.

Chemi- and bio-luminescence reactions

The fiber-optic biosensors described above are based on coupling the suitable biocatalytic reaction with a FOCS that responds to either a product or co-substrate of the catalyzed reaction. Biosensors have also been developed where the biocatalytic reaction either consumes or produces an optically measurable species. Here, the detected species from the biocatalyzed reaction is measured directly with the fiber-optic sensing device.

Freeman and Seitz (1978) reported the first such fiber-optic biosensor for the measurement of hydrogen peroxide. This biosensor uses peroxidase at the distal tip of a fiber-optic bundle. The peroxidase catalyzes a chemiluminescence reaction between hydrogen peroxide and luminol. A fraction of the light produced by this reaction is measured with a PMT detector. The fiber-optic bundle serves as a conduit through which the light travels from the biosensor tip to the detector.

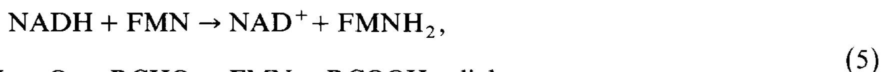
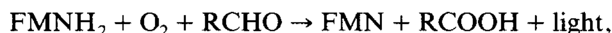
A similar device has been reported with the peroxidase enzyme immobilized directly on the face of a solid-state photodiode detector (Aizawa et al., 1984). This second system has been extended for the measurement of glucose by co-immobilizing glucose oxidase and peroxidase. The glucose oxidase produces hydrogen peroxide from glucose which is detected through the peroxidase-catalyzed chemiluminescence reaction. A similar system has been reported where the chemiluminescence detection of hydrogen peroxide produced from glucose is used for a fiber-optic glucose biosensor (Abdel-Latif and Guilbault, 1988). In this last system, the chemiluminescence generated from the reaction of bis(2,4,6-trichlorophenyl) oxalate with hydrogen peroxide is used in combination with glucose oxidase. A micellar environment is used to enhance the chemiluminescence reaction.

Bioluminescence based fiber-optic biosensors have also been reported (Arnold, 1988; Blum et al., 1988). Either firefly luciferase or bacterial luciferase is used as the biocatalytic activity. A biosensor for adenosine triphosphate (ATP) has been constructed by immobilizing firefly luciferase at the tip of a fiber-optic bundle (Blum et al., 1988). The following biocatalytic reaction is used:

$$\text{ATP} + \text{luciferin} + O_2 \rightarrow \text{AMP} + \text{oxiluciferin} + PP_i + CO_2 + \text{light}, \qquad (4)$$

where AMP represents adenosine monophosphate and PP_i represents inorganic pyrophosphate. Luciferin is a required co-substrate. In addition, biosensors for reduced nicotinamide adenine dinucleotide (NADH) have been reported based on

the combined activities of NADH : FMN oxidoreductase and bacterial luciferase (Arnold, 1988; Blum et al., 1988):

$$\begin{aligned} NADH + FMN &\rightarrow NAD^{+} + FMNH_2, \\ FMNH_2 + O_2 + RCHO &\rightarrow FMN + RCOOH + \text{light}, \end{aligned} \tag{5}$$

where FMN and $FMNH_2$ are the oxidized and reduced forms of flavin mononucleotide, respectively, and RCHO and RCOOH represent a long chain aldehyde (i.e., decyl aldehyde) and its corresponding acid, respectively. Again, the light generated from either the firefly or bacterial luminescence reaction is measured through a fiber-optic bundle/PMT detector arrangement.

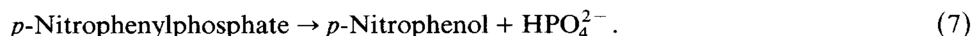
Biosensors for a wide variety of biologically important compounds can be constructed by coupling either an ATP- or NADH-producing enzymatic reaction with these bioluminescence fiber-optic sensors. One such example is the sensor for glutamate which combines glutamate dehydrogenase with the bacterial luciferase reaction scheme (Stuever and Arnold, 1988). Glutamate dehydrogenase catalyzes the following reaction:

$$\text{Glutamate} + NAD^{+} \rightleftharpoons \alpha\text{-Ketoglutarate} + NH_4^{+} + NADH. \tag{6}$$

The formation of NADH leads to the production of light and the intensity of this light can be related to the concentration of glutamate in the sample through a calibration curve.

Absorbance measurements

The concept of fiber-optic biosensors based on the enzymatic formation of a chromophoric species has also been demonstrated (Arnold, 1985). In this system, the enzyme alkaline phosphatase is immobilized at the common end of a bifurcated fiber-optic bundle. Incident radiation from a suitable source is supplied at the sensor tip through one arm of the bifurcated bundle. The other arm transmits back-scattered radiation from the sensor tip to a PMT detector. Alkaline phosphatase is used to catalyze the following reaction:



$$p\text{-Nitrophenylphosphate} \rightarrow p\text{-Nitrophenol} + HPO_4^{2-}. \tag{7}$$

In basic solutions, the reaction product, *p*-nitrophenol, strongly absorbs 405 nm radiation. *p*-Nitrophenylphosphate in the sample solution diffuses to the biocatalytic layer where the chromophore *p*-nitrophenol is generated. This chromophore absorbs a fraction of the radiation which is detected as a decrease in intensity at the detector. An effective absorbance can be calculated based on the incident and resulting radiation intensities. The effective absorbance is linearly related to the concentration of *p*-nitrophenylphosphate in the sample.

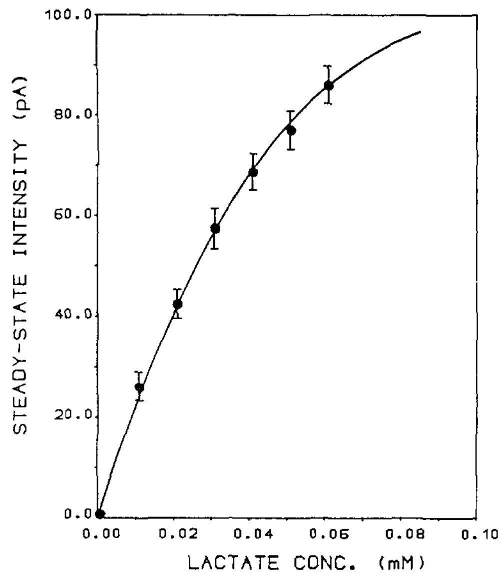


Fig. 3. Response curve for the lactate biosensor based on the fluorometric detection of NADH production.

Detection of NADH fluorescence

Fiber-optic biosensors have also been developed based on the fluorescence of NADH. An example of this is the development of biosensors for lactate and

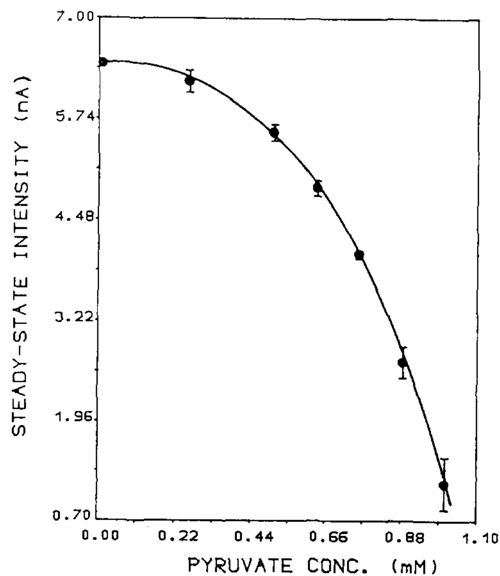


Fig. 4. Response curve for the pyruvate biosensor based on the fluorometric detection of NADH consumption.

pyruvate based on immobilized lactate dehydrogenase (Arnold, 1987; Wangsa and Arnold, 1988). Lactate dehydrogenase catalyzes the following reaction:

$$\text{Lactate} + \text{NAD}^{+} \rightleftharpoons \text{Pyruvate} + \text{NADH}. \qquad (8)$$

Biosensors can be fabricated in either a NADH production or consumption mode. In the NADH production mode, NAD^{+} is added to the sample and the production of NADH at the tip of a fiber-optic sensing device is measured fluorometrically. Fig. 3 shows a typical lactate calibration curve which is generated in the NADH production mode. The pyruvate biosensor, on the other hand, requires the presence of NADH in the sample solution and the consumption of NADH is detected as a decrease in the measured fluorescence intensity. Fig. 4 shows a pyruvate calibration curve. As expected, a decrease in intensity is observed for higher pyruvate concentrations. For both systems, narrow band-pass interference filters are used to select 350 ± 10 nm radiation for excitation and 450 ± 10 nm radiation for emission.

Fiber-optic biosensors based on NADH fluorescence have also been reported for glucose (Narayanaswamy and Sevilla, 1988) and bile acids (Klainer and Harris, 1988). Many other NADH-based fiber-optic biosensors are possible because of the existence of a wide variety of selective dehydrogenase enzymes (Bielka et al., 1979).

A wide variety of fiber-optic biosensors is currently being developed and these biosensors promise to supply new analytical devices for the continuous measurement of important biological compounds. Presently, the field of fiber-optic biosensors is in an exciting stage of rapid growth and major advances can be anticipated within the next few years.

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