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Chemical Sensors Based on Controlled-Release Polymer Systems

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A novel chemical sensor has been developed in which the polymer ethylene–vinyl acetate is used as a controlled-release system to deliver reagents to the sensing region of an optical fiber for a homogeneous competitive immunoassay based on fluorescence energy transfer. A competition reaction is used to enable continuous measurements of the solution antigen concentration. More generally, the technique allows irreversible indicating chemistries to be used in the construction of chemical sensors that can measure continuously for long periods. Although the sensor configuration has not been optimized in all respects, data are presented for a model system in which a fluorescein-labeled antibody and Texas Red–labeled immunoglobulin G (IgG) are used.

IN THE LAST DECADE, CHEMICAL SENSORS have emerged as viable alternatives to traditional methods of analysis. The ideal sensor provides in situ measurements continuously, reversibly, and in real time. The development of a sensor for a particular species depends on the availability of reversible transduction schemes to detect the analyte of interest. Most reversible schemes are based on changes that occur when a chemical compound or membrane interacts with the analyte. The measured properties might be optical or electrical signals, depending on the type of sensor (*1*). In the absence of reversible schemes, irreversible chemistries can be used, such as antibody-based immunoassays, that consume analyte by forming very tight binding complexes with the analyte. These schemes can be used to perform assays or may be used to construct probes, which, by definition, are irreversible and cannot make continuous measurements. However, probes can be adapted to make rapid sequential measurements if reagents are replenished or regenerated. Such probes use pumps to deliver fresh reagent continuously (*2*) or chaotropic reagents to regenerate the binding site of an antibody (*3*). This approach solves some of the problems associated with discrete sampling but does not allow long-term monitoring or true continuous measurements.

To expand the diversity of chemical sensors, we have developed a technique that circumvents the irreversibility of immunoassays and allows us to exploit the specificity and selectivity of antibodies while still preserving continuous measurement capability. To do so, we have adapted a controlled-release delivery system, capable of sustaining a constant release of fresh immunochemicals or any reagent of an irreversible transduction scheme, to perform continuous measurements. Typically, controlled-release systems are biocompatible polymers that release on contact with an aqueous environment by passive diffusion from the polymer matrix [such as ethylene vinyl acetate (*4*)] or hydrolytic breakdown of the polymer structure [such as poly(glycolic acid) (*5*)]. We present preliminary data demonstrating the viability of this idea.

With the advent of hybridoma technology (*6*), it became possible to produce antibodies to virtually any compound. Although the binding between an antibody and antigen is reversible and noncovalent, most immunochemical reactions essentially are irreversible because of the large association constants (K_a), which typically range between 10^5 to 10^9 M^{-1} (*7*). The association constants are composed of large forward (k_1) and small reverse (k_{-1}) rates, ranging from 10^7 to 10^9 M^{-1} s^{-1} and 10^2 to 10^{-4} s^{-1}, respectively. These kinetic parameters make antibodies extremely specific and selective for the analyte of interest.

Fluoroimmunoassays have been developed (*8*) for various proteins (such as antibodies and enzymes), hormones (such as steroids and thyroxine), and drugs (such as digoxin and gentamicin). Immunoassays used clinically fall into two categories. Heterogeneous assays require periods of incubation and washing, while homogeneous assays need no separation step, being based typically on a competition reaction between a fluorescently labeled and an unlabeled antigen (*9*).

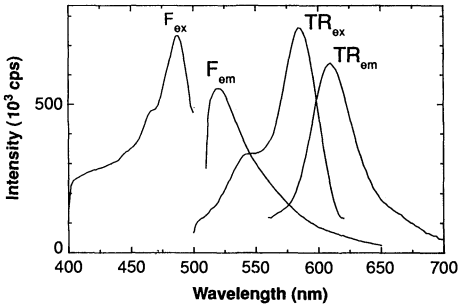

Fig. 1. Excitation and emission spectra of F-Ab to IgG and Texas Red–labeled IgG antigen, showing the overlap of the F-Ab emission spectrum and TR-Ag excitation spectrum.

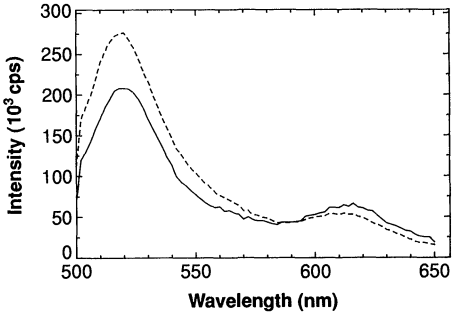

Fig. 2. The dependence of the emission spectra on antigen concentration: spectrum 1 (– – –) high concentration (500 μg/ml); spectrum 2 (——) low concentration (0 μg/ml).

The competitive fluorimmunoassay that we adapted to a controlled-release system is based on a fluorescence energy-transfer mechanism (*10*). When the immunocomplex (F-Ab:TR-Ag) forms between fluorescein-labeled antibody (F-Ab) and Texas Red–labeled IgG (TR-Ag) (Eq. 1)

$$\text{F-Ab} + \text{TR-Ag} \rightarrow \text{F-Ab:TR-Ag} \quad (1)$$

$$\text{F-Ab} + \text{Ag} \rightarrow \text{F-Ab:Ag} \quad (2)$$

the fluorophors are within the critical distance of 10 nm required for nonradiative energy transfer to occur (*11*). Upon excitation, fluorescein molecules transfer their energy to Texas Red nonradiatively, thereby quenching the fluorescein and enhancing the Texas Red intensities. This fluorophor combination was chosen because of the moderately efficient overlap between the emission spectrum of the energy donor, fluorescein, and the excitation spectrum of the energy acceptor, Texas Red, while allowing sufficient resolution of the Texas Red emission peak (Fig. 1). In addition, the dyes are commercially available in reactive forms readily conjugated to proteins (Molecular Probes, Eugene, OR) and have been used in

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other applications (*12*). If an immunocomplex (F-Ab:Ag) forms between F-Ab and unlabeled IgG (Ag) (Eq. 2), no energy transfer occurs because Texas Red is not present to accept energy from fluorescein. Therefore, the amount of energy transfer that occurs is proportional to the concentration of unlabeled antigen. The fluorescence intensity dependence on the presence of unlabeled antigen is shown in Fig. 2. As the sensor is exposed to a high concentration of unlabeled antigen (500 μg/ml), less energy transfer occurs, resulting in larger fluorescein and smaller Texas Red intensities (spectrum 1). In contrast, low concentrations of unlabeled antigen (0 μg/ml) result in a large amount of energy transfer, since most of the F-Ab is bound to TR-Ag, producing smaller fluorescein and larger Texas Red intensities (spectrum 2).

The sensor is constructed by incorporating F-Ab and TR-Ag into two EVA copolymers (Fig. 3) (*13*). The release rate of F-Ab from the EVA polymer is shown in Fig. 4. During the first 2 days, an initial fast-release rate is observed, followed by a slower sustained-release rate. The initial fast rate is attributed to surface diffusion of F-Ab from the polymer plug in the reservoir. The sustained release rate that occurs after an initial incubation period of 2 days may result from the formation of an interconnected pore network (*4*) and is free of surface effects. In the sensor configuration, immunoreagents are released continuously from the polymer reservoirs into the reaction chamber. Because of concentration gradients, unlabeled Ag diffuses into the reaction chamber from the bulk solution until a steady state is reached and competes with the TR-Ag for the available binding sites on the F-Ab. Eventually, the bound species diffuse out of the reaction chamber. However, since there is a continuous release of reagents, a constant concentration of reagents is maintained in the reaction chamber. The fluorescence intensity changes of the competition reaction are monitored with an optical fiber (*14*) and are used to calculate the amount of unlabeled antigen in the sample solution (*15*).

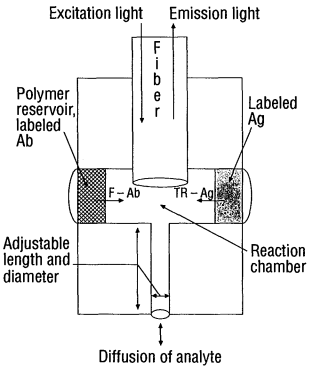

Fig. 3. Continuous-release fluoroimmunosensor configuration. The overall physical dimensions of the sensor's configuration are 15 mm by 12 mm by 10 mm. The volumes of the polymer reservoirs and reaction chamber are 100 mm^3 and 40 mm^3, respectively. Typically, the dry weight percentages of antibody-labeled polymer and antigen-loaded polymer were 1.5% and 8%, respectively.

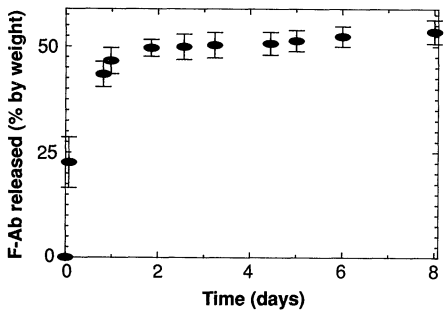

Fig. 4. After 2 days, a constant release rate is achieved from the F-Ab–containing polymer. Results are the mean ± SD for five different individual polymer slabs.

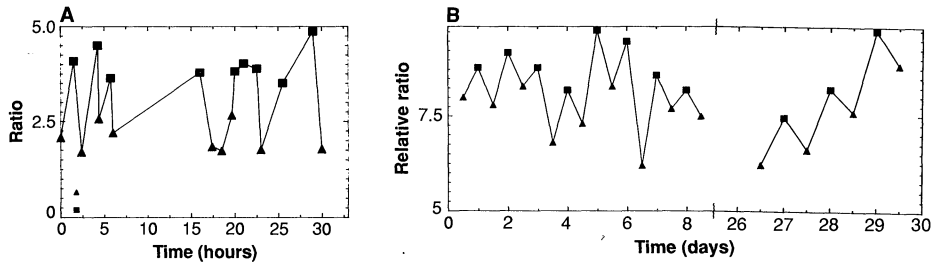

Fig. 5. Response of two different sensors to 0 μg/ml and 500 μg/ml of IgG: (**A**) during the intervals days 1 to 8 and 15 to 30 (in the interval days 9 to 14, the sensor was stored in distilled water at room temperature); and (**B**) during the interval days 1 to 8 and days 26 to 29 (in the interval days 9 to 25, the sensor was stored in distilled water at room temperature). The results in (A) and (B) represent typical data from $n = 5$ and $n = 2$ sensors tested, respectively.

Figure 5 shows the response of two different sensors to the concentrations 0 μg/ml and 500 μg/ml of unlabeled Ag during a short and long time period, respectively. In Fig. 5A, a sensor was cycled between the two concentrations from hours 1 to 8 and 15 to 30; in the interval hours 9 to 14, the sensor was stored in distilled water. To test the operational lifetime of a sensor, the reservoirs were filled with polymer (~70 mg dry weight) and monitored every 12 hours, during days 1 to 8 and 26 to 28 (Fig. 5B). During the days 9 to 25 interval, the sensor was stored in distilled water at room temperature and continued to release reagents. The values were calculated with Eq. 3 (*15*). These data show that the sensors respond to the two concentrations reversibly, indicating that continuous release of immunoreagents from the EVA polymer allows a competitive reaction to occur between the labeled and unlabeled antigen in the reaction chamber. In Fig. 5A, there is a clear difference between the two concentrations. The measurement of the 0 μg/ml ($n = 9$) and 500 μg/ml ($n = 9$) concentrations averaged ratio values over the 30-hour period of approximately 2 and 4, respectively. A typical sensor can be designed to last for 30 days, depending upon the degree-of-loading (*13*) and amount of polymer packed into the reservoirs. In Fig. 5B, the difference in the values for the two concentrations varied significantly and probably is due to large changes in the relative concentrations of the two labeled species. These changes likely exceed the tolerances for the competition reaction and cannot be compensated by using the measurement in Eq. 3. Even with this limitation, the sensor responds reversibly and can be used to monitor trends in concentration.

We developed a sensor based on a fluoroimmunoassay that can be used over extended periods where the response time is not critical. The sensor's optimization is dependent on the indicating chemistries used and the physical configuration of the sensor. Response time is dictated by the rate of diffusion of the analyte from the bulk solution to the reaction chamber or by the release rate. It is also important to assure that diffusion of the F-Ag:Ab or F-Ab:TR-Ag complexes are at a rate where a sufficient signal can be obtained. Analyte diffusion can be controlled by the size of the hole and the length of the channel through which it must travel to reach the interior of the reaction chamber. These dimensions can be tailored to the diffusion characteristics of a specific analyte. A high molecular weight analyte requires large holes and a short path, whereas a low molecular weight analyte requires small holes and a long path. The reaction time of the released reagents with the analyte must be faster than the diffusion of the analyte out of the reaction chamber, or the free analyte would diffuse out of the chamber before the reaction is complete, abrogating the measurement.

Although several groups have developed

immunosensors based on the specificity of antibodies (*16*), most of these sensors rely on exposure of the antibody to subsaturating levels of antigen or to chaotropic agents to restore binding site activity, allowing the sensor to be used more than once. These methods of restoring activity provide discontinuous measurements and less than quantitative recovery. Miller *et al.* (*17*) have developed a reversible sensor based on a similar energy-transfer fluoroimmunoassay that relies on an antibody having a high off-rate, k_{-1}, enabling the sensor to respond quickly and reversibly. However, most antibodies have very slow off-rates, making this approach difficult to generalize unless an exhaustive search for a rapid off-rate monoclonal antibody is undertaken for each antigen. With our method this is not necessary—existing immunoassays can be used and coupled to optical fibers through a controlled-release system. The most immediate applications are likely to be in the monitoring of pollutants at toxic waste sites, groundwater aquifers, and agricultural areas where there is pesticide runoff, since antibodies to these analytes are becoming available commercially (Biodesign Inc., Kennebunkport, Maine).

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15. The concentration of unlabeled antigen can be solved by equating the equilibrium equations for reactions 1 and 2

$$K_{eq1} = [\text{F-Ab:Ag}]/([\text{F-Ab}]/[\text{Ag}])$$

$$K_{eq2} = [\text{F-Ab:TR-Ag}]/(\text{F-Ab}][\text{TR-Ag}])$$

and solving for the antigen concentration.

$$[\text{F-Ab}] = [\text{F-Ab:Ag}]/[\text{Ag}]K_{eq1}$$

$$[\text{F-Ab}] = [\text{F-Ab:TR-Ag}]/[\text{TR-Ag}]K_{eq2}$$

Equating the above expressions and solving for [Ag] yields

$$[\text{Ag}] = \frac{[\text{F-Ab:Ag}][\text{TR-Ag}]K_{eq1}}{[\text{F-Ab:TR-Ag}]K_{eq2}}$$

This expression may be rewritten in terms of fluorescence intensities to yield Eq. 3

$$[\text{Ag}] = \frac{([I_{480}/I_{520}][I_{570}/I_{610}])}{([I_{480}/I_{610}] - 0.06[I_{570}/I_{610}])}$$

where I_x is the intensity at x nanometers. The term $0.06[I_{570}/I_{610}]$ is used to correct for the direct excitation of TR-Ag at 480 nm and is determined experimentally.

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High Winds of Neptune: A Possible Mechanism

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Neptune receives only 1/900th of the earth's solar energy, but has wind speeds of nearly 600 meters per second. How the near-supersonic winds can be maintained has been a puzzle. A plausible mechanism, based on principles of angular momentum and energy conservation in conjunction with deep convection, leads to a regime of uniform angular momentum at low latitudes. In this model, the rapid retrograde winds observed are a manifestation of deep convection, and the high efficiency of the planet's heat engine is intrinsic from the room allowed at low latitudes for reversible processes, the high temperatures at which heat is added to the atmosphere, and the low temperatures at which heat is extracted.

VOYAGER OBSERVATIONS OF CLOUD motions (*1*) showed that Neptune has some of the fastest winds measured on any planet. The strength of Neptune's circulation, despite the small amount of energy received either from its interior or from the sun, was a surprise. Several conceptual models of the general circulation of the giant planets have been proposed (*2*). However, they do not readily provide a simple explanation as to why the highest winds occur on Neptune when the apparent source of energy is so small. Neptune gains only about 1/900th of the solar energy per unit area received by the earth, but the zonal winds in tropical latitudes are more than ten times faster than on the earth.

In this report, we present new measurements of the winds using cloud motions and a new conceptual model based on fundamental principles of angular momentum and energy that offers a plausible mechanism for the exceptionally strong equatorial subrotation or retrograde winds found on Neptune and Uranus. Our model is easiest to illustrate by examination of Neptune's motions in an inertial or absolute frame of reference (Fig. 1).

Gaseous planets are generally assumed to have a core where the fluid rotates as a solid object. Thus, deep within the atmosphere of Neptune we assume that there is a core region where the rotation period equals Neptune's day [assumed to be given by the measurements of the radio period (*3*), 16.11 hours]. Because more energy is received from the interior of the planet than from the sun, deep convective motions develop in the atmosphere as energy is transferred upward from the core region. The upper boundary of the core region represents a surface across which absolute angular momentum is exchanged between the interior and outer gaseous regions of the planet. This same surface as a lower boundary to the free atmosphere determines the vertical extent over which reversible isentropic processes may occur.

An estimate of the upper boundary of the core region can be obtained from the observed cloud motions and a parameter called the potential radius. The potential radius is the perpendicular distance from the axis of rotation at which the relative zonal motion of a given cloud feature vanishes through virtual radial displacement under angular momentum conservation, that is, where the periods of rotation for the planet and the displaced cloud become equal. For all latitudes where the rotation period of a cloud exceeds that of the interior, the potential radius is closer to the spin axis, whereas for all latitudes where the rotation period is less than that of the deep interior, the potential radius is located farther from the spin axis.

We have calculated the meridional distribution of the potential radii for the measured cloud motions (*4*) (Fig. 2). In the

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