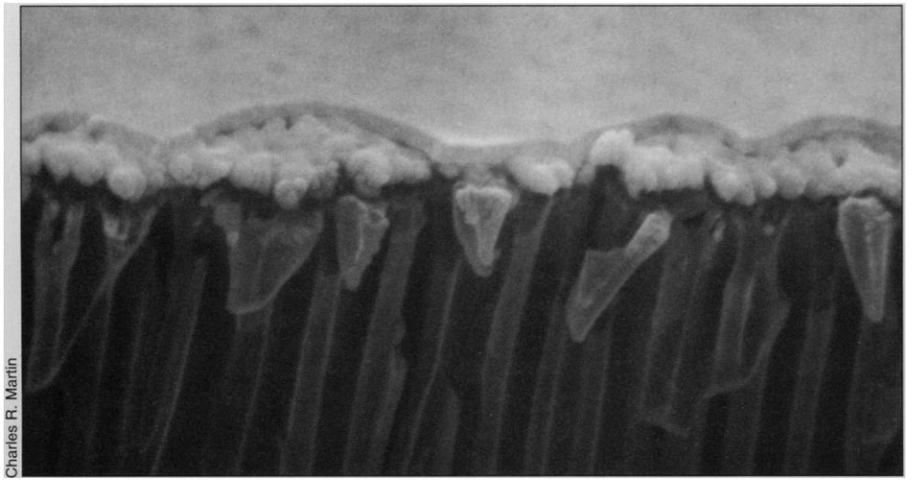


Charles R. Martin

Ultraslim. *A scaffolding of aluminum oxide supports an exquisitely thin polymer membrane, one approach to creating a more sensitive probe for glucose.*

Polymer Scientists Work to Beef Up Biosensors

Tiny enzyme- or antibody-based probes that sense substances from glucose to pesticides find their future—in plastics

IF BIOSENSORS—THE LITTLE ELECTRICAL OR optical probes that analyze solutions and body fluids—were human laboratory workers, their ears would be burning. "Slow-working, easily confused, insensitive, unadaptable to new situations, apt to contaminate the working space," are some of the comments researchers have been making about them for 20 years, even as biosensors have become a mainstay of home and laboratory testing for substances such as glucose, sodium ions, and dissolved oxygen. And in those two decades, while all manner of revolutions have come and gone in laboratories, the gap between existing sensors and the keen, versatile helpers scientists and clinical technicians dream of has remained wide. Until now.

Researchers at dozens of laboratories throughout the world are modifying biosensors with specialized polymers. The result may be a new generation of ultra-fast, sensitive, stable, versatile, and long-lasting devices. If so, whole new worlds will open to biosensors. Durable, long-lived ones might act as environmental sentinels, warning of groundwater contamination. Others might do yeoman service in basic research, sensitively tracing biochemical substances such as neurotransmitters. Perhaps the most common theme in biosensor research is the quest for an improved glucose sensor—keen, long-lived, and able to trace changing concentrations of the sugar in real time. Such sensors would be a boon for clinical laboratories and industrial fermentation processes, and miniaturized versions might even be implanted in the bodies of diabetics to monitor blood-sugar levels. "There's a huge market for [glucose] devices," says biosensor investigator Charles R. Martin, professor of chemistry at Colorado State University.

The basic design of biosensors isn't likely to change. Many common models consist of an electrode tipped with enzymes, antibodies, or other reagents that interact chemically with analytes—the substances being analyzed. In some designs, the reagents are enclosed in a semipermeable membrane; in others they are adsorbed onto electrode surfaces. In a typical enzyme-based system, the enzyme oxidizes the analyte, releasing electrons that are ferried to the electrodes by other molecules known as electron transfer agents. The strength of the electric signal indicates the analyte concentration. In some sensors an optical fiber takes the place of the electrode and the signal is not electric current but light, generated when enzymes or antibodies form fluorescent complexes with the analytes.

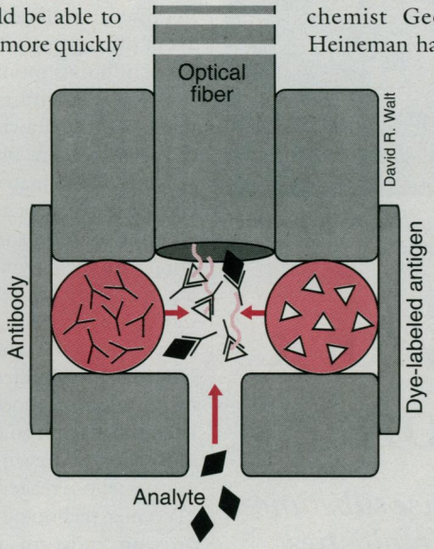
Key to the success of a biosensor is its ability to hold onto the reagent while exposing it to the molecules of interest—and that's just where many of today's designs falter. One problem is reagent leakage, which causes a gradual decline in sensitivity and has discouraged researchers from pursuing implantable designs. Other probes don't leak, but their sensitivity suffers because they admit the analyte molecules too slowly. Still others are sensitive but not selective, responding to unwanted molecules as well as to the molecules of interest. And in sensors that rely on irreversible reactions like the binding of a fluorescent antibody to the analyte, the reagent often gets used up too quickly to allow for continuous monitoring.

Polymer scientists are now overcoming many of those drawbacks by focusing not on the active reagent but on the packaging: the polymer films, matrixes, and reservoirs that enclose the reagent and govern its interaction with the solution and the electrode. By tinkering with the polymers, researchers are finding that they can tether the reagents more tightly while speeding the migration of solution through the sensor. They can screen out unwanted molecules to make the sensors more selective, boost the flow of current from the reagent to the electrode to increase sensitivity, and even extend the lifetimes of sensors whose reagent is used up too quickly. "Polymer technology will be the key to improving most of today's enzyme-based biosensors," says Paul D. Hale, senior scientist at Moltech Corp., a small Stony Brook, New York, firm that is at work on improved sensors for glucose and cholesterol.

Moltech is taking advantage of the ability of polymers to trap and immobilize sensor molecules. Instead of having a leak-prone reservoir of reagents, the company's prototypes trap both the sensor enzyme and the electron transfer agent within the polymer itself. As Hale explains, the ferrocene molecules that serve as the electron transfer agent are covalently bonded to the backbones of such polymers as polysiloxanes and poly(ethylene oxide). The sensor enzyme—glucose oxidase or cholesterol oxidase—is also locked up in the molecular matrix, having been mixed with the precursor molecules before they link up into polymer chains. But because the plastic is porous, the solution to be analyzed can migrate into the sensor, coming into intimate contact with the enzyme.

The result is a sensor that still works after a year of service, long after conventional glucose sensors with untethered molecules have become unusable. Besides appealing to clinical laboratories, Hale thinks the new sensors might also find a home in the biotechnology industry, where they could be used to monitor nutrient levels in fermentation vats over long periods. And, according to Hale, they might serve as the sensor element in a future implantable insulin-delivery system for diabetics.

Colorado State's Martin is tracking the same game—an improved glucose sensor—by a very different path. His prototype sensors, tipped with the usual polymer reservoir, are more conventional than Moltech's at first glance. But the reservoir has walls so exquisitely thin—a film of sulfonated poly(dimethyl siloxane) just 40 to 100 nanometers thick, more than 10 times thinner than a typical semipermeable membrane—that the glucose molecules should be able to enter the reservoir much more quickly than in ordinary glucose sensors. On its own a film so thin would be impossibly fragile, but Martin's is built on a scaffolding of highly porous aluminum oxide, which is permeated with the sensor molecules. Martin expects that his new devices will be sensitive enough to monitor changes in glucose concentrations in real time—precisely what is needed in automated insulin-delivery systems—though he acknowledges that "we don't yet have any data to report" on response times.



A slow leak. *Controlled-release polymer reservoirs prolong the life of a fluorescent probe.*

The sensitivity and stability that Hale and Martin are striving toward are the crucial qualities for glucose sensors. For the sensors used in neurobiology research, keen discrimination is the key attribute. Biosensors that pick up minute quantities of neurotransmitters such as dopamine have been opening a window on neurochemical events in the brains of experimental animals. Unfortunately, according to chemist William R. Heineman of the University of Cincinnati, current biosensors respond not only to the neurotransmitters of interest but also to the ascorbate ions always present in brain tissue, yielding spurious signals.

Heineman and his colleagues are working on a remedy to this biosensor befuddlement. By bathing certain polymers in gamma rays, Heineman causes their molecular chains to cross link, forming a mesh that admits other molecules only up to a certain size—an effect that may help to screen out the confounding ascorbate ions. By varying the amount of cross linking, and hence the gauge of the mesh, Heineman can also tailor the membranes to discriminate broadly among various classes of neurotransmitter molecules.

But Heineman is showing that cross-linked polymers can do more than just passively screen molecules. He has found that one cross-linked polymer, poly(N-vinyl pyrrolidone), actively attracts catecholamines, a class of neurotransmitters common in the brain and other parts of the nervous system. The combination of filtering and active attraction, Heineman reports, results in electrodes that are two to five times better than unmodified sensors at discriminating catecholamines from ascorbate ions. Working with biochemist George P. Kreishman, Heineman has traced the attraction between his polymers and the neurotransmitters to hydrophobic regions on both classes of molecules. He thinks that knowledge should help him fine tune polymers to be even more discriminating, even to the point of distinguishing between individual neurotransmitters. "Ultimately, you'd like to be able to detect singular events, for example, the firing of a single synapse," says Heineman. That vision might eventually be realized with implantable probes that could detect minute quantities of a specific neurotransmitter in real time.

Chemist Adam Heller at the University of Texas at Austin is exploring another way for polymers to play an role in biosensors: as "wires" that efficiently channel free electrons from the enzyme to the electrode. Heller's wires, developed with Brian Gregg, a photochemist at the Solar Energy Research Institute in Golden, Colorado, consist of a polymer made of poly(vinyl pyridine) linked to osmium-containing molecules. The long polymer chains link up with both the enzyme molecules and the electrode surfaces, in effect establishing bridges across which the osmium atoms can shuttle electrons in a kind of bucket brigade. The dense network of polymeric wires serves to concentrate current from a vast number of enzyme molecules onto the electrode. As a result, says Heller, a molecular-wired glucose biosensor can produce much more current for its size than a conventional sensor.

That opens the possibility of tiny devices that pack a lot of analytical clout. In fact, Heller has used the wire technology to create glucose biosensors with diameters one-tenth that of a human hair. And miniaturization is one of the key requirements for implantable systems.

Heller says he is developing an implantable glucose-monitoring and insulin-dispensing system for hospitalized "fragile" diabetics (those whose insulin levels swing wildly) based on polymer-wire sensors. He is now collaborating with groups in the United States and Germany that are already testing his sensors in animals. Commercialization of the bedside insulin unit may come "within 5 years," Heller predicts.

One of the few biosensor researchers with his eyes on applications outside biomedicine is Tufts University chemistry professor David R. Walt. He's busy trying to improve sensors that measure toxic organic chemicals in the environment by releasing fluorescently labeled antibodies that bind to the toxins. The lifetime of such sensors is inevitably limited—they just run out of antibodies—but it could be lengthened if the labeled antibodies could be enclosed in a protective polymer reservoir and released in a steady trickle.

Walt's sensors have antibody reservoirs made of the controlled-release polymer ethylene vinyl acetate. As the antibodies slowly diffuse out into a reaction chamber, they encounter molecules of dye-labeled antigen diffusing into the chamber from an adjacent reservoir. The resulting dye-antibody complexes fluoresce, but as the unlabeled target molecules enter the chamber from the outside they take up binding sites on the antibodies. Thus the level of fluorescence drops depending on the concentration of target molecules surrounding the probe.

Walt pictures his polymeric sensors in the role of environmental sentinels. "You might use them to measure concentrations of pesticide runoff at farms," he says, "or at waste dump sites that have been cleaned and sealed, where you want to make sure none of the toxic chemicals leach into the groundwater." Such applications are more than a gleam in Walt's eye. He reports that his recently developed polymer-based biosensor for the pesticide atrazine should be ready for field trials within 6 to 8 months.

From field trials to the market is a long road, of course. For all of these new sensors, materials and production costs will have to come down, and implantable sensors will confront the additional hurdles that face any medical device. But Walt nonetheless predicts that polymer-bolstered biosensors will be having a "real impact" in industrial process monitoring by the end of the decade. In a more visionary mode, Heller prophesies that implantable versions of the new sensors, linked to microprocessors and drug delivery systems, "will create a really new world in the way we treat disease." ■ GORDON GRAFF

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