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Biosensors Based on Polymer Networks Formed by Gamma Irradiation Crosslinking

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ABSTRACT

Water-soluble polymers immobilized by gamma radiation have been investigated as a means of developing electrochemical sensors. Enzyme-based sensors for glucose and lactate have been made by immobilizing glucose oxidase and lactate oxidase, respectively, on platinized graphite electrodes. The enzyme is entrapped in a polymeric network of poly(vinyl alcohol) that is formed by gamma radiation crosslinking. Electrodes coated with poly (*N*-vinylpyrrolidone) and its corresponding monomer and then crosslinked with gamma radiation show an extraction of catecholamines into the polymer film that enhances the analytical signal for their detection by electrochemical oxidation. Poly(dimethyldiallylammonium chloride) spin-coated on a screen-printed electrochemical cell provides sufficient ionic conductivity for the cell to function as a gas sensor for oxygen, which is detected by reduction at a platinum working electrode.

Index Entries: Biosensor; polymer network; gamma irradiation crosslinking; poly(*N*-vinylpyrrolidone); poly(vinyl alcohol); poly(dimethyldiallylammonium chloride); lactate oxidase; oxygen sensor; lactate sensor; neurotransmitter sensor; microelectrode; screen printing; solid-state voltammetry.

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INTRODUCTION

One of the most difficult aspects of sensor development is the achievement of adequate selectivity for the intended application. Polymer films immobilized on electrode surfaces are an effective means of achieving selectivity for an electrochemical sensor. The polymer can impart selectivity by virtue of one or a combination of several mechanisms.

1. Specific interactions between the polymer film and components in the sample can enhance the electrochemical signal for the analyte and diminish it for interferences. Analyte can selectively partition into the film by electrostatic, hydrogen bonding, hydrophobic, or coordination interactions.
2. Restricted diffusion into the polymer based on molecular size can provide useful selectivity for samples in which the size of an interference is larger than the size of the analyte.
3. The polymer can participate indirectly in the achievement of selectivity by providing a matrix for the immobilization of an enzyme or an antibody that interacts with the analyte.

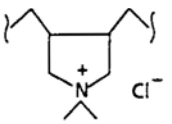
This paper describes some of our research on the use of gamma radiation as a technique for the immobilization of polymers on sensor surfaces and for the entrapment of enzymes in polymer networks to form sensing elements in biosensors.

Water-soluble polymers immobilized by gamma radiation have been investigated as a means of imparting selectivity to electrochemical sensors by means of the mechanisms outlined above (*1–8*). Gamma rays generate radicals in the polymer that cause polymerization and/or crosslinking of the film. The resulting polymer network is insoluble, yet it will swell in water because of its hydrophilic character. The swelling of the network can be controlled by the amount of monomer mixed with the polymer prior to crosslinking and the radiation dosage. The polymers poly(*N*-vinylpyrrolidone) (PNVP), poly(vinyl alcohol) (PVAL), and poly (dimethyldiallylammonium chloride) (PDMDAAC) have been investigated for the purpose of developing electrochemical sensors (Fig. 1).

Some advantages of this technique for preparing polymer-modified electrochemical sensors are

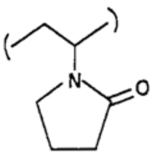
- No "shadowing;"
- No removal of excess reactant(s) or byproduct(s);
- Simplicity of the crosslinking procedure; and
- Formation of a sterile device.

The minimization of shadowing, compared to crosslinking by UV radiation, is owing to the high penetration of gamma rays. This feature could



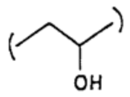
POLY(DIMETHYLDIALLYLAMMONIUM CHLORIDE)

PDM DAAC



POLY(N-VINYL PYRROLIDONE)

PNVP



POLY(VINYL ALCOHOL)

PVAL

Fig. 1. Structures of polymers.

be especially important for the fabrication of sensors that require immobilization of a polymer inside a chamber. Some disadvantages of crosslinking by gamma radiation are

- Requirement of a gamma radiation source;
- Radiation damage to device and membrane components;
- Gas evolution; and
- Radical reaction mechanisms.

In addition to causing the desired crosslinking reaction that forms the polymer network, gamma radiation can have undesirable effects. For example, many plastics are degraded by gamma radiation so that they become brittle. Membrane components such as biomolecules can be denatured by exposure to high levels of radiation. The formation of free radicals during the radiation process can cause undesirable chemical reactions to occur in the membrane. Hydrogen is evolved in some crosslinking reactions that can result in the formation of bubbles in the polymer film.

Ionically conducting polymer networks can form the basis for solid-state voltammetric cells. Cells of this type can serve as sensors for species in the gas phase that partition into the polymer film. Sensing can be direct by oxidation or reduction of the species or indirect by measurement of some property of the polymer film that is altered by the species.

IMMOBILIZATION OF ENZYMES

One way to achieve selectivity is by means of a biological reaction that is specific for the target analyte. For this reason, there is considerable interest in biochemical sensors or biosensors (*9*). The response of a biosensor is based on a specific biological reaction that is coupled to a chemical sensor in order to impart the selectivity of the biological reaction to the device. This has been accomplished by the immobilization of biological materials such as enzymes and antibodies onto chemical sensing elements such as electrodes, optical fibers, and piezoelectric materials.

Although biomolecules are sensitive to exposure to high levels of radiation, some enzymes are sufficiently resistant to gamma radiation to be immobilized in a polymer matrix on an electrode. We have demonstrated this for glucose oxidase (*5*), alkaline phosphatase (*7*), and lactate oxidase (*8*) immobilized in PVAL. PVAL can be immobilized on electrode surfaces by gamma radiation crosslinking to form an adherent film that is sufficiently hydrophilic to swell when immersed in water. The swelling gives access to the immobilized enzyme by solution species that are sufficiently small to penetrate the polymer network.

For example, lactate oxidase (LOD) has been immobilized on platinized graphite electrodes by sandwiching a thin enzyme layer between two layers of PVAL. The three layers are individually coated on the electrode, which is then subjected to gamma irradiation. The resulting sensor responds to lactate by electrochemical oxidation of H_2O_2 generated by the enzyme-catalyzed oxidation of lactate that diffuses into the network. The current is proportional to the concentration of hydrogen peroxide at the electrode surface, which is proportional to the concentration of lactate in solution. A diagram of the sensor and the mechanism for response are shown in Fig. 2.

Electrodes of this type show rapid (10–50 s to steady state) hydro-amperometric response to aliquotes of lactate injected into a stirred buffer solution. The linear range of response is 26–1.7 μM with a detection limit of 13 μm.

Maximum response to lactate is obtained in the dosage range of 2–6 Mrad. Lower response at lower dosages is attributed to loss of enzyme owing to incomplete crosslinking of the polymer. Nonirradiated electrodes give a good initial response to lactate, but this initial response decreases rapidly as the polymer and enzyme gradually dissolve from the surface. Lower response at high dosages is attributed to radiation damage to the enzyme. Some response can be obtained for electrodes with dosages as high as 36 Mrad.

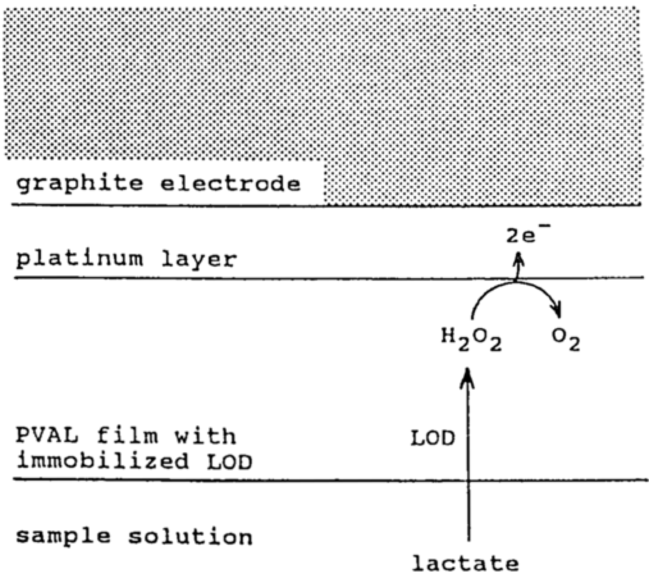


Fig. 2. Schematic diagram of biosensor for lactate.

The temperature dependence of response gives an optimum at 55°C compared to an optimum activity at 35°C for the free enzyme, indicating stabilization by the radiation immobilization process. The sensors are stable for over 80 d when stored in dry form or in phosphate-azide solution at pH 7 and room temperature with continuous stirring.

SELECTIVE PARTITIONING

Electrodes coated with PNVP and its corresponding monomer NVP and then crosslinked with gamma radiation show an extraction of phenolic compounds from aqueous solution into the polymer film (3,4). This characteristic has been used as a means of preconcentrating catechol, caffeic acid, and catecholamine neurotransmitters (dopamine and DOPAC) into the polymer film and thereby enhancing the analytical signal for their detection by electrochemical oxidation. By comparison, the electrode response to ascorbate, which is the main interferent in the detection of neurotransmitters by in vivo monitoring in the brain, is attenuated by the PNVP film. The response of modified electrodes for catechol relative to ascorbate is increased by a factor of 2–5 compared to bare electrodes.

Figure 3 shows voltammograms at a carbon fiber microelectrode of dopamine and of a mixture of dopamine and ascorbate. In the voltammogram of the mixture no distinguishable wave is evident for dopamine. A voltammogram of the same mixture of dopamine and ascorbate recorded at a microelectrode coated with a film of gamma radiation cross-linked PNVP is shown in Fig. 4A. A distinguishable peak for dopamine (DA) is now

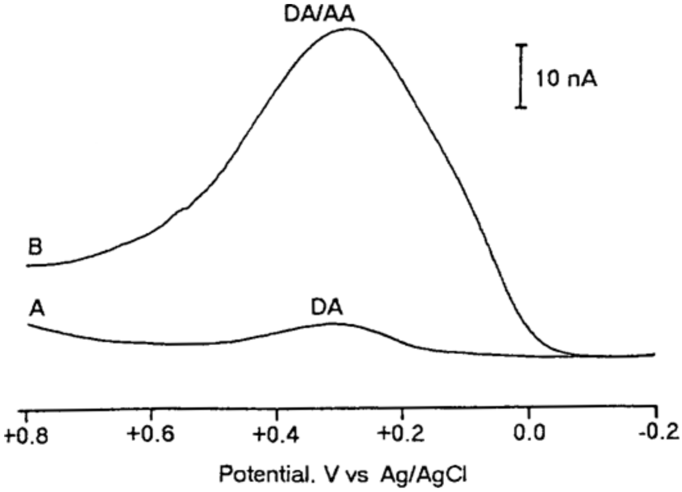


Fig. 3. Differential pulse voltammograms of (A) 1 m*M* dopamine and (B) 1 m*M* dopamine, 10 m*M* ascorbate in 0.02*M* phosphate buffer pH 7.4 at a bare carbon fiber microelectrode (*4*).

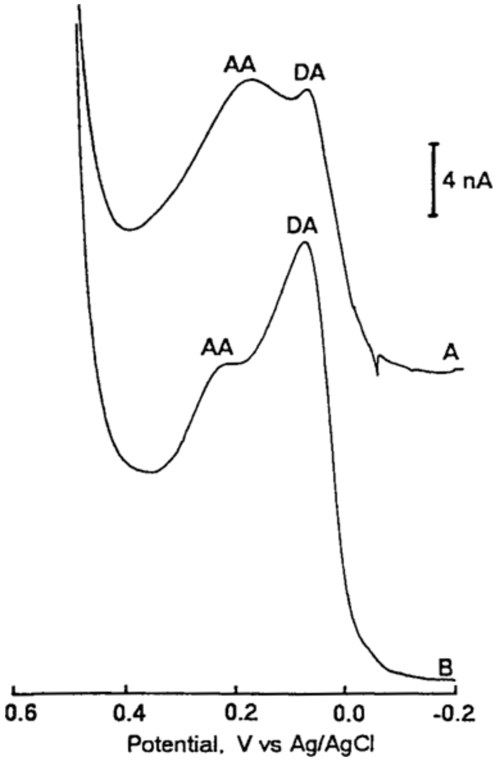


Fig. 4. Differential pulse voltammograms of 1 m*M* dopamine and 10 m*M* ascorbate at a carbon fiber microelectrode modified with a film of PNVP cross-linked with 20 Mrad. (A) First voltammogram after equilibration of film with solution. (B) Voltammogram after oxidative depletion of ascorbate in polymer film at 0.5 V (*4*).

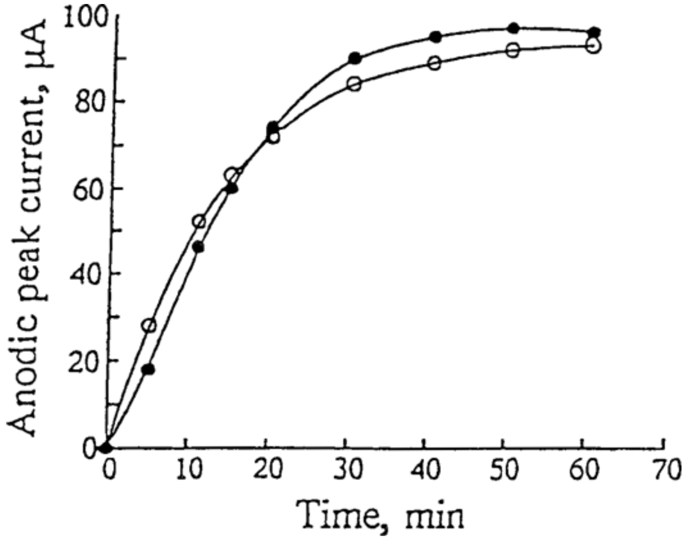


Fig. 5. Time course of anodic peak current at a PNVP/ascorbate oxidase coated electrode: ○—1 m*M* catechol; ●—1 m*M* catechol and 1 m*M* ascorbate (*10*).

evident because of extraction of dopamine and partial rejection of ascorbate (AA). Since the electrochemistry of dopamine is reversible whereas ascorbate is irreversibly oxidized, oxidative depletion of ascorbate in the film by cycling the electrode potential further enhances the selectivity for catechol species in the presence of ascorbate, as shown in Fig. 4B. The potential was held at 0.5 V for 15 min to oxidize ascorbate in the film, stepped to −0.2 for a few minutes to regenerate the reduced form of dopamine in the film by electrochemical reduction, and then scanned in the positive direction to detect the dopamine by oxidizing it again.

Additional discrimination against ascorbate can be achieved by immobilizing the enzyme ascorbate oxidase in the PNVP membrane (*10*). The ascorbate diffusing into the membrane is enzymatically converted into an electroinactive product before it reaches the electrode surface. Similar responses are obtained for catechol alone and catechol in the presence of ascorbate, as shown in Fig. 5.

SOLID-STATE VOLTAMMETRIC SENSOR

Since PDMDAAC is an ionomer, it is an ionic conductor by virtue of the mobility of the chloride ion in films of the polymer that are partially hydrated. We have explored the use of PDMDAAC as the basis for solid-state electrochemical sensors (*11–13*). This type of sensor consists of an electrochemical cell in which the usual liquid electrolyte is replaced with a solid polymer that is ionically conducting.

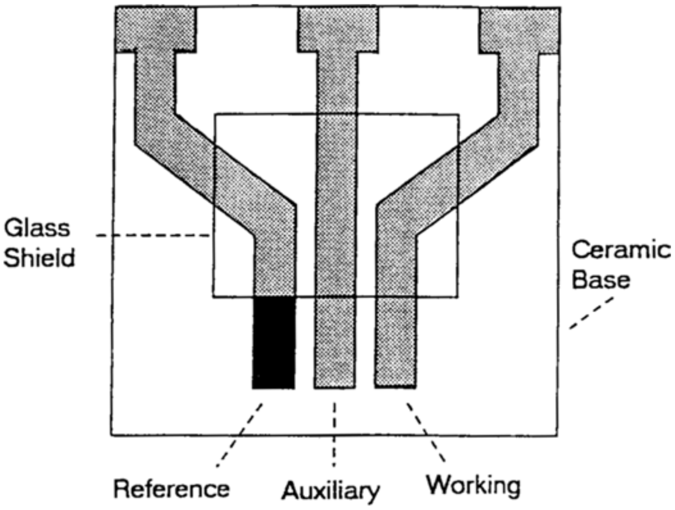


Fig. 6. Schematic diagram of screen-printed electrochemical cell. Shaded areas are strips of platinum, dark area is Ag/AgCl coated on platinum.

We have developed prototype sensors constructed of three Pt electrodes screen-printed onto a 1×1×0.025 in. ceramic plate as shown in Fig. 6. In order to create the electrochemical sensing area, the tips of these electrode fingers are masked off from the rest of the material by a doubly screen-printed glass film. A reference electrode is created by coating one of the electrode tips with Ag/AgCl. The other two are left as bare Pt to act as the working and auxiliary electrodes. The sensor is coated with polymer by spin-coating with a solution of PDMDAAC to cover all three electrodes. The polymer film is then immobilized by gamma radiation crosslinking.

Atmospheric oxygen can be detected by reduction at this PDMDAAC-coated electrochemical sensor. Cyclic voltammograms obtained when the sensor is exposed to different concentrations of oxygen are shown in Fig. 7. Figure 7A shows the voltammogram obtained when the sensor is placed in an atmosphere of 100% oxygen. The reduction wave for oxygen appears at approximately −0.8 V vs Ag/AgCl. Figure 7B demonstrates the disappearance of the reduction wave upon purging the atmosphere with argon.

Figure 8 shows the amperometric response of the sensor at a potential of −0.8 V vs Ag/AgCl to various concentrations of oxygen. The apparent steady-state response time of the sensor is less than 30 s, which in these measurements was limited by how fast the surrounding atmosphere could be changed to a new concentration of oxygen.

The calibration curve for the above data is shown in Fig. 9 along with calibration curves for sensors with other polymer film thicknesses. This figure demonstrates the effect of film thickness on the response of the sensor. The sensitivity of the sensor can be increased by decreasing the polymer film thickness. This is a result of decreasing the diffusion layer and thus increasing the slope of the concentration-distance profile. Each

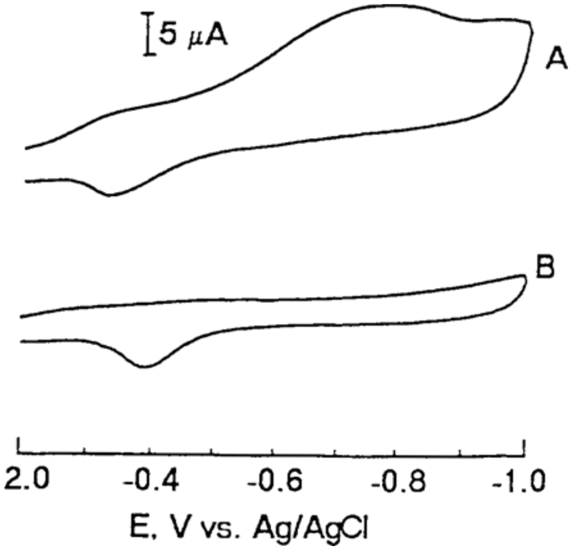


Fig. 7. Cyclic voltammograms of PDMDAAC coated Pt/ceramic sensor in (A) pure oxygen and (B) pure argon. Ag/AgCl reference. Scan rate = 100 mV/s (13).

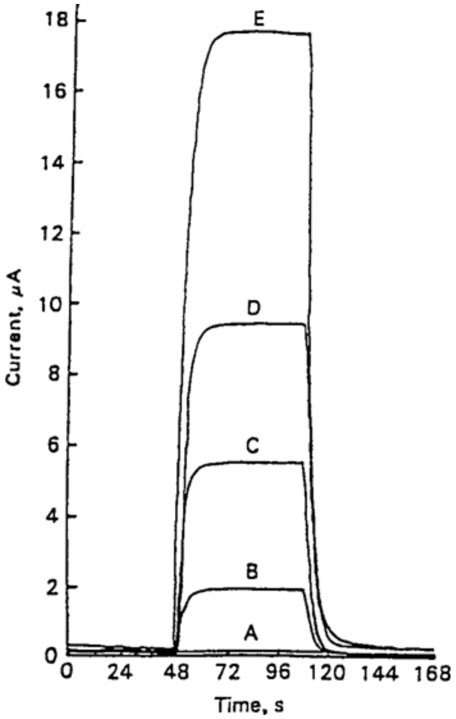


Fig. 8. Amperometric response of PDMDAAC coated Pt/ceramic sensor to different concentrations of oxygen in oxygen/argon mixtures. E = −0.8 V vs Ag/AgCl. (A) 0%, (B) 10%, (C) 30%, (D) 50%, (E) 100% (13).

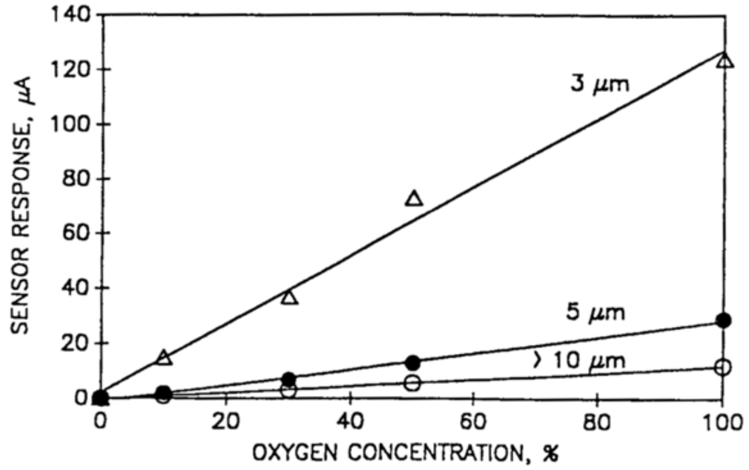


Fig. 9. Calibration curve for atmospheric oxygen at Pt/ceramic sensor coated with different thicknesses of PDMDAAC (*13*). E = −0.8 V vs Ag/AgCl.

point on the calibration curves is the average of three repeated measurements. The standard deviation for the repeated measurements was less than 1%, demonstrating reasonable precision among trials. The response to oxygen remained linear with respect to concentration over the range of 0–100% for approx 23 d. After this time, the sensor continued to respond to oxygen, however, the precision was poorer.

ACKNOWLEDGMENTS

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REFERENCES

1. De Castro, E. S., Huber, E. W., Villarroel, D., Galiatsatos, C., Mark, J. E., Heineman, W. R., and Murray, P. T. (1987), *Anal. Chem.* **59,** 134–139.
2. Huber, E. W. and Heineman, W. R. (1988), *Anal. Chem.* **60,** 2467–2472.
3. Coury, Jr., L. A., Birch, E. M., and Heineman, W. R. (1988), *Anal. Chem.* **60,** 553–560.
4. Coury, Jr., L. A., Huber, E. W., Birch, E. M., and Heineman, W. R. (1989), *J. Electrochem. Soc.* **136,** 1044–1049.
5. Galiatsatos, C., Ikariyama, Y., Mark, J. E., and Heineman, W. R. (1990), *Biosens. & Bioelect.* **5,** 47–61.
6. Birch, M. E., Coury, Jr., L. A. and Heineman, W. R. (1990), *Anal. Chem.* **62,** 1123–1130.
7. Hajizadeh, K. (1990), Ph. D. Dissertation, University of Cincinnati.

8. Hajizadeh, K., Halsall, H. B., and Heineman, W. R. (1991), *Anal. Chim. Acta* **243,** 23–32.
9. Turner, A. P. F., Karube, I., and Wilson G. S. (1987), *Biosensors: Fundamentals and Applications,* Oxford University Press, New York.
10. Imato, T., Morioka, H., and Heineman, W. R. (1992), 4th International Meeting on Chemical Sensors, Tokyo, Japan, September 13–18.
11. Tieman, R. S., Heineman, W. R., Johnson, J., and Seguin, R. (1992), *Sens. & Actuators B* **8,** 199–204.
12. Tieman, R. S., Igo, D. H., Heineman, W. R., Johnson J., and Seguin, R. (1991), *Sens. & Actuators B* **5,** 121–127.
13. Tieman, R. S. and Heineman, W. R. (1992), *Anal. Lett.* **25,** 807–819.