CONF-8904254--1

To be published in: Proceedings of the Symposium on Biosensors
University of North Carolina, September 7-9, 1989.

BNL-43112

BNL--43112

DE90 002301

AMPEROMETRIC BIOSENSORS FOR GLUCOSE, LACTATE, AND GLYCOLATE BASED ON OXIDASES AND REDOX-MODIFIED SILOXANE POLYMERS

PAUL D. HALE, TORU INAGAKI, HUNG SUI LEE and TERJE A. SKOTHEIM
Department of Applied Science, Division of Materials Science, Brookhaven National Laboratory, Upton, New York

HIROKO I. KARAN Division of Natural Science and Mathematics, Medgar Evers College, City University of New York, Brooklyn, New York

YOSHI OKAMOTO Department of Chemistry, Polytechnic University, Brooklyn, New York

1 INTRODUCTION

Amperometric biosensors based on flavin-containing oxidases undergo several steps which produce a measurable current that is related to the concentration of substrate. In the initial step, the substrate converts the oxidized flavin adenine dinucleotide (FAD) or flavin mononucleotide (FMN) into the reduced form ($FADH_2$ or $FMNH_2$). Because these cofactors are located well within the enzyme molecule, direct electron transfer to the surface of a conventional electrode does not occur to a measurable degree. A common method of facilitating this electron transfer is to introduce oxygen into the system because it is the natural acceptor for the oxidases; the oxygen is reduced by the $FADH_2$ or $FMNH_2$ to hydrogen peroxide, which can then be detected electrochemically. The major drawback to this approach is the fact that oxidation of hydrogen peroxide requires a large overpotential, thus making these sensors susceptible to interference from electroactive species. In order to lower the necessary applied potential, several non-physiological redox couples have been employed to shuttle electrons between the flavin moieties and the electrode. For example, sensors based on the ferrocene/ferricinium redox couple [1,2] and on electrodes consisting of conducting salts such as TTF-TCNQ (tetrathiafulvalene-

---

tetracyanoquinodimethane) [3,4] have previously been reported. Electron relays have also been attached directly to the enzyme molecule in order to facilitate electron transfer [5,6]. More recently, these studies have been extended to include systems where the mediating redox species are covalently attached to polymers such as poly(pyrrole) [7], poly(vinylpyridine) [8], and poly(siloxane) [9]. The present paper describes the development of amperometric biosensors based on flavin-containing enzymes and this latter family of polymeric mediators.

2 REDOX-MODIFIED SILOXANE POLYMERS

The ferrocene-modified poly(siloxane) relay systems are shown schematically in Figure 1. Although these materials are insoluble in water, their high degree of flexibility allows the ferrocene moieties to achieve close contact with the flavin centers of the enzyme, and thus serve as efficient electron transfer mediators from the reduced enzyme to the electrode. The homopolymer is comprised of approximately 35 subunits (m=35, n=0), with each containing a bound ferrocene moiety. In the copolymers, m:n ratios of approximately 1:1 and 1:2 were used, and the polymer subunits were randomly distributed (i.e. random block copolymers). Steps were taken to ensure that no low molecular weight species (which could act as freely diffusing electron transfer mediators) were present. Thin layer chromatography and high-performance liquid chromatography showed that no oligomeric materials were present in the purified materials.

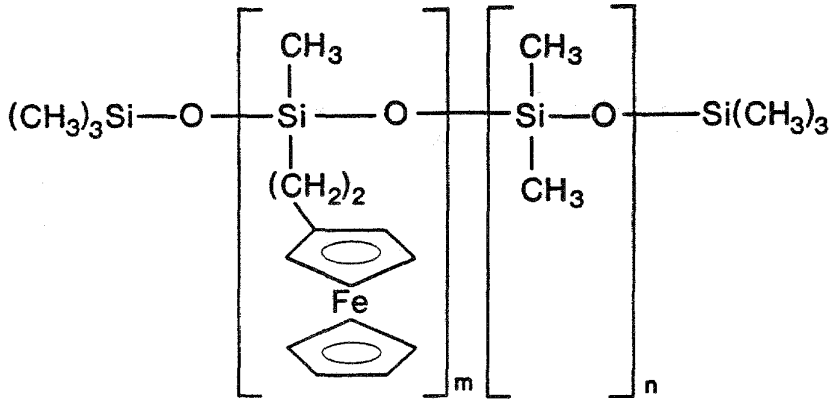

FIGURE 1 Schematic diagram of the ferrocene-modified siloxane polymers used as electron relay systems in oxidase-based amperometric sensors.

3 GLYCOLATE BIOSENSORS

Modified carbon paste for the glycolate sensors was made by thoroughly mixing 50mg of graphite powder with a measured amount of the ferrocene-containing polymer (the latter was first dissolved in chloroform), resulting in approximately 45μmole of ferrocene per gram of graphite powder. After evaporation of the solvent, 5mg of glycolate oxidase (3.8 units/mg) and 10μl of paraffin oil were added, and the resulting mixture was blended into a paste. The paste was packed into a 1.0ml plastic syringe which had previously been partially filled with unmodified carbon paste, leaving approximately a 2mm deep well at the base of the syringe. The resulting surface area of the electrode was 0.025cm^2. Electrical contact was achieved by inserting a silver wire into the top of the carbon paste. The mediating ability of the ferrocene-modified siloxane polymers can be seen in Figure 2, which shows cyclic voltammograms for the glycolate biosensors in pH 8.0 phosphate buffer solution before and after addition of glycolate.

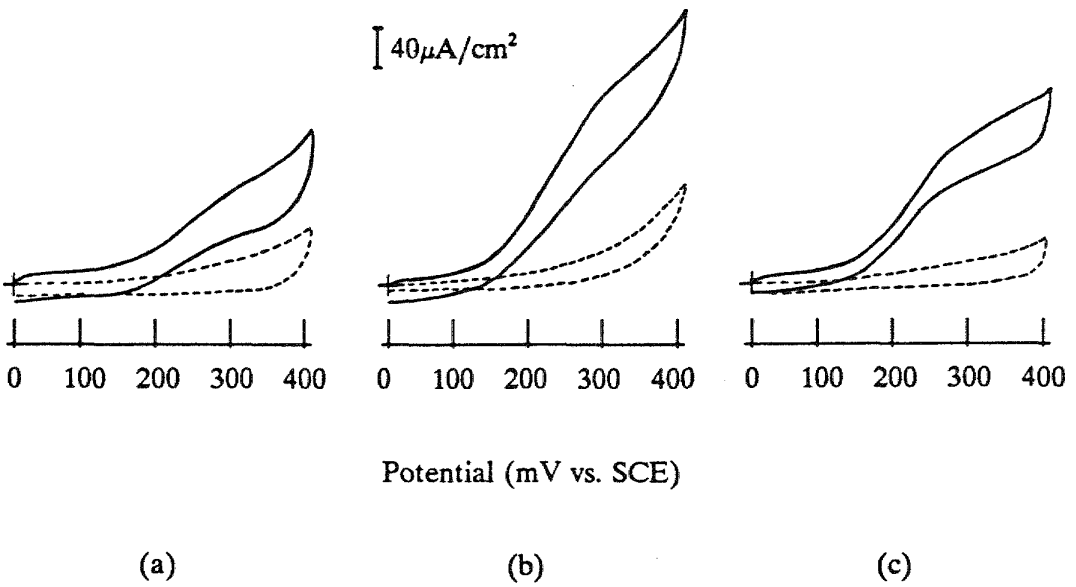

FIGURE 2 Cyclic voltammograms for the ferrocene-modified polysiloxane/glycolate oxidase/carbon paste electrodes (scan rate: 5mV/s) in pH 8.0 phosphate buffer (with 0.1M KCl) solution with no glycolate present (dashed line) and in the presence of 0.1M sodium glycolate (solid line). The electrode in (a) contained the ferrocene-modified homopolymer, while those in (b) and (c) contained the copolymers with m:n ratios of 1:1 and 1:2, respectively.

Calibration curves are shown in Figure 3 for glycolate biosensors containing the ferrocene-modified siloxane homopolymer, the 1:1 copolymer, and the 1:2 copolymer. As in the cyclic voltammetry experiments, the three polymeric relay systems display similar electron transfer mediation characteristics. The apparent Michaelis-Menten constants (K_M) for the sensors may be determined from electrochemical Eadie-Hofstee plots; K_M values range from 2 to 5mM for these sensors.

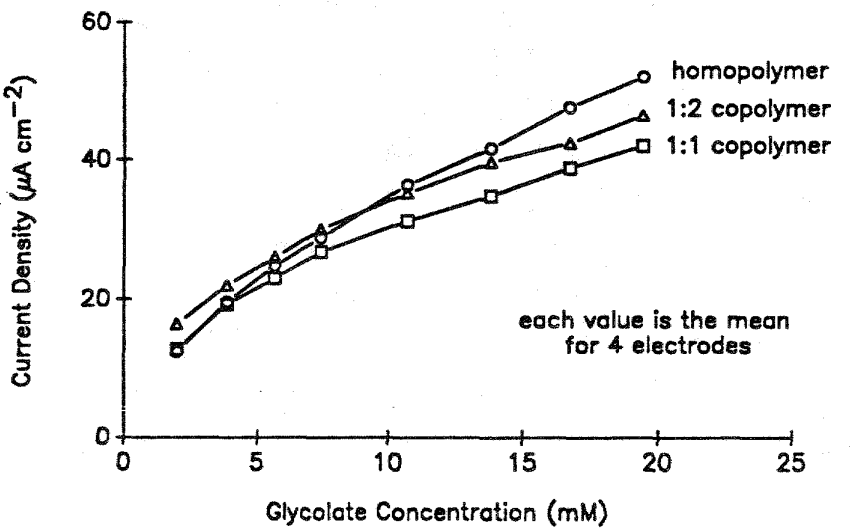

FIGURE 3 Calibration curves for the ferrocene-modified poly(siloxane)/glycolate oxidase/carbon paste electrodes at E=300mV (vs. SCE).

4 GLUCOSE BIOSENSORS: STABILITY OF THE RESPONSE

Previous studies [9] have demonstrated that these ferrocene-modified siloxane polymers work very well as electron relay systems in glucose oxidase-based biosensors. Because the relays are not free to diffuse out of the device, the sensors maintain a response to glucose over long periods of time. Figure 4 shows typical glucose calibration curves for a carbon paste electrode containing the ferrocene-modified siloxane homopolymer and glucose oxidase after storage in pH 7 phosphate buffer solution. Even after one year the sensor displays an adequate response to the addition of glucose. The decrease in response over time is most likely due to loss of enzyme into the storage solution; a simple enzyme immobilization (e.g. cross-linking) would further improve the long-term stability.

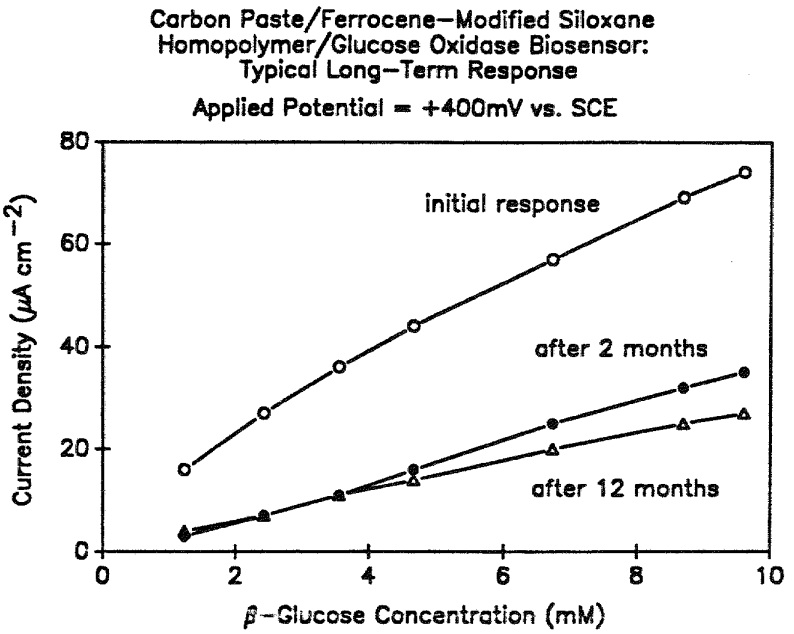

FIGURE 4 Typical glucose calibration curves for the ferrocene-modified siloxane homopolymer/glucose oxidase/carbon paste electrode after storage in pH 7 buffer.

5 LACTATE BIOSENSORS

Preliminary studies indicate that the poly(siloxane) relay systems can efficiently mediate the electron transfer from both lactate oxidase and lactate monooxygenase to a carbon paste electrode. A typical lactate calibration curve is shown in Figure 5 for a carbon paste electrode containing a dimethylferrocene-modified siloxane polymer (m:n ratio of 1:2) and lactate monooxygenase. The use of dimethylferrocene as the polymer-bound mediator allows this sensor to be operated efficiently at lower applied potential values than the sensors described above. The lactate sensors were constructed in a manner similar to the glycolate sensors described in Section 3. The constant potential experiment in Figure 5 was performed in a pH 7.0 phosphate buffer solution containing 0.1M KCl, which was deaerated by N_2 bubbling for 10min. Work is presently underway to better characterize the lactate biosensors based on the poly(siloxane) relay systems and both lactate monooxygenase and lactate oxidase.

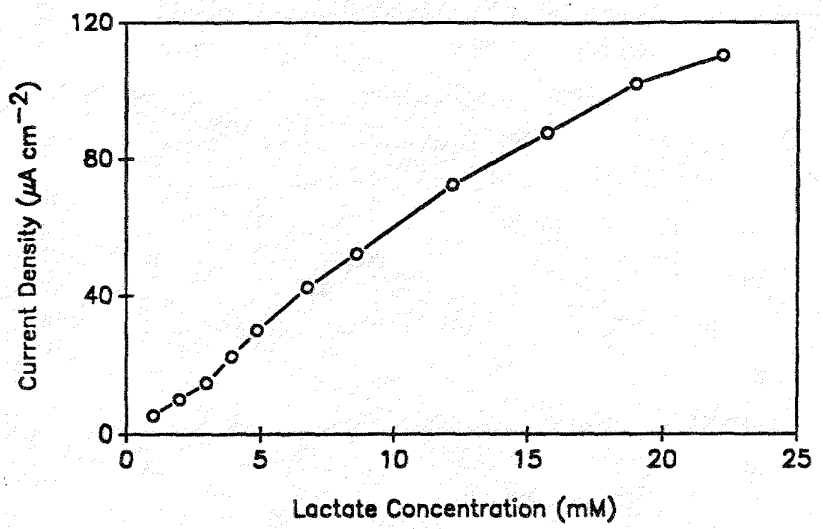

FIGURE 5 Typical lactate calibration curve for the dimethylferrocene-modified siloxane (1:2) copolymer/lactate monooxygenase/carbon paste electrode (E=200mV vs. SCE).

ACKNOWLEDGMENT

This research has been sponsored by the U.S. Department of Energy, Division of Materials Science, Office of Basic Energy Science (DE-AC02-76CH00016).

REFERENCES

1. A.E.G. Cass, G. Davis, G.D. Francis, H.A.O. Hill, W.J. Aston, I.J. Higgins, E.V. Plotkin, L.D.L. Scott and A.P.F. Turner, Anal. Chem., 56, 667 (1984).
2. J.M. Dicks, W.J. Aston, G. Davis and A.P.F. Turner, Anal. Chim. Acta, 182, 103 (1986).
3. W.J. Albery, P.N. Bartlett and D.H. Craston, J. Electroanal. Chem., 194, 223 (1985).
4. P.D. Hale and R.M. Wightman, Mol. Cryst. Liq. Cryst., 160, 269 (1988).
5. Y. Degani and A. Heller, J. Phys. Chem., 91, 1285 (1987).
6. Y. Degani and A. Heller, J. Am. Chem. Soc., 110, 2615 (1988).
7. N.C. Foulds and C.R. Lowe, Anal. Chem., 60, 2473 (1988).
8. Y. Degani and A. Heller, J. Am. Chem. Soc., 111, 2357 (1989).
9. P.D. Hale, T. Inagaki, H.I. Karan, Y. Okamoto and T.A. Skotheim, J. Am. Chem. Soc., 111, 3482 (1989).