Linear Dependence of the Potential of "Wired" Glucose Oxidase Electrodes on the Concentration of Glucose

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Potentiometric assays, unlike amperometric or coulometric ones, do not require accurate definition and knowledge of the area of the measuring electrode or microcell volume. Their potential scales, however, with the logarithm of the concentration of the analyte, while in amperometric, chronoamperometric, or chronocoulometric assays the charge or current scales linearly with the concentration. Here we show that in a potentiometric glucose assay the potential can scale linearly with concentration. Such scaling is realized when the electrode is coated with a resistive, but nevertheless electron-conducting, film where glucose is electrooxidized. The film consists of a redox polymer film that "wires" reaction centers of covalently co-immobilized glucose oxidase to the electrode. After a potential pulse is applied to the "wired" enzyme electrode so that the electrode-bound redox centers are electrooxidized, the floating electrode potential decays to a value that varies linearly with the concentration of glucose.

Introduction

The concentration of glucose and other biochemicals can be monitored electrochemically through potentiometry, amperometry, or coulometry.[1] While amperometry requires knowledge of the area of the electrode and coulometry requires knowledge of the liquid volume analyzed, potentiometry does not require such knowledge. In industrial practice, control of electrode dimensions and microcell volumes is of essence in amperometric and coulometric biosensors, but not in potentiometric ones. The disadvantage of potentiometric devices was that their output scaled with the logarithm of the concentration of the analyte rather than with its concentration. Consequently, the penalty in potentiometry has been the inability to resolve small changes in concentrations.

In this article we show that when a special system and a special electrode are used, the output potential of a glucose monitoring system scales approximately with the glucose concentration, not with its logarithm. The system differs from earlier potentiometric systems in two ways. The working electrode is modified with an electron-conducting redox polymer, within which an enzyme catalyzing the oxidation of glucose is bound, and the system monitors the relaxation of the potential after application of a potential pulse.

Earlier Nagy, Nagy, and Fehér chronopotentiometrically assayed NADH on a glassy carbon electrode modified with Meldola blue. After applying a potential pulse, they measured the rate of relaxation of the potential. They found that the initial slope, measured in V/s, decreased linearly upon increasing the NADH concentration.[2] Tomlinson and Torrance[3] and Harsányi, Toth, and Pungor[4] reported for potentiometric silver electrodes deviations from the classical Nernstian relationship and at very low chloride concentrations observed a linear dependence of the potential on concentration.

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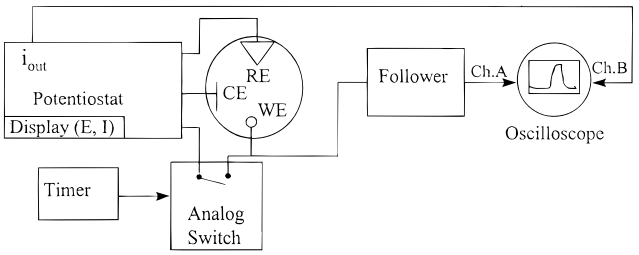

Figure 1. Block diagram, showing the components of the measurement system.

Experimental Section

Materials, Reagents, Buffers, and Electrodes. The materials, reagents, and buffers used were similar to those described, as were the miniature electrodes.[5−7] For sake of brevity the method of their preparation and their structure are not repeated here, but the reader should be aware of the following. The gold electrodes were of 250 μm diameter and were recessed at 90 μm depth in a polyimide sleeve. They were coated with three layers: a transduction or sensing layer, consisting of a water-containing redox polymer in which glucose oxidase was immobilized and through which the reaction centers of the enzyme were electrically connected ("wired") to the electrode; a mass-transport limiting layer; and a biocompatible solution-contacting hydrogel. The glucose oxidase "wiring" polymer was {poly[1-vinylimidazole) osmium(4,4′-dimethylbipyridine)$_2$-Cl]}$^{+/2+}$ where one imidazole in 13 was osmium complexed. The redox polymer was cross-linked with poly(ethylene glycol) diglycidyl ether.

The much larger, 5 mm diameter, printed carbon electrodes on Mylar were prepared with a sensing layer identical with that of the miniature gold electrodes. Their mass transport restricting layer consisted of a Poretics polycarbonate membrane with 0.01 μm pores (Poretics catalog no. 11001).

Apparatus. The system's block diagram is shown in Figure 1. Its elements were a home-built miniature low-power poten-

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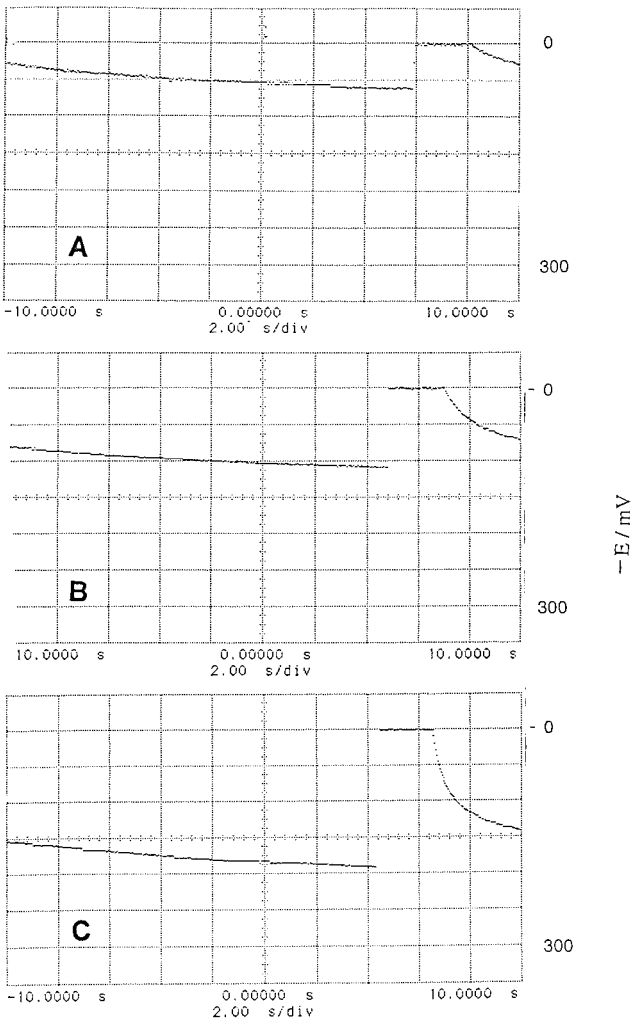

Figure 2. Time dependence of the potential of the working electrode. The switch was opened, and the potential was allowed to float for $\tau =$ 20 s; next the switch was closed, and 300 mV vs Ag/AgCl was applied for $t = 2$ s; then the circuit was again opened for $\tau = 20$ s. Glucose concentrations (A) 0 mM, (B) 8 mM, and (C) 16 mM. 37° C; 0.15 M NaCl; pH 7.4, 0.02 M phosphate buffer; solution stirred and air-exposed.

tiostat with a display, a follower, a timer-activated analog switch (SCL 4066), a standard three-electrode electrochemical cell, and an oscilloscope for continuous monitoring of the current and the potential (HP 54501A). Chronoamperograms were recorded while the switch was closed. The follower-sensed potential of the working electrode was virtually zero, the working electrode being connected to a current-to-voltage converter. All potentials were measured versus ground. Chronopotentiograms were recorded after opening the switch.

Results

Figure 2 shows the observed chronopotentiograms at (A) 0, (B) 8, and (C) 16 mM glucose concentrations. The cycles of the chronopotentiograms involved two steps, repeated as necessary: in the first step of $\tau = 20$ s duration the switch was open, and the working electrode potential was allowed to float; in the second step the switch was closed, and a potential of +300 mV versus the Ag/AgCl reference electrode was applied for $t = 2$ s; the switch was then opened again, and the cycle was repeated. As seen in Figure 2, both the decay characteristics of the potential after opening the switch and the potential observed 20 s after the switch was opened depended on the concentration of glucose. The potential measured 20 s after

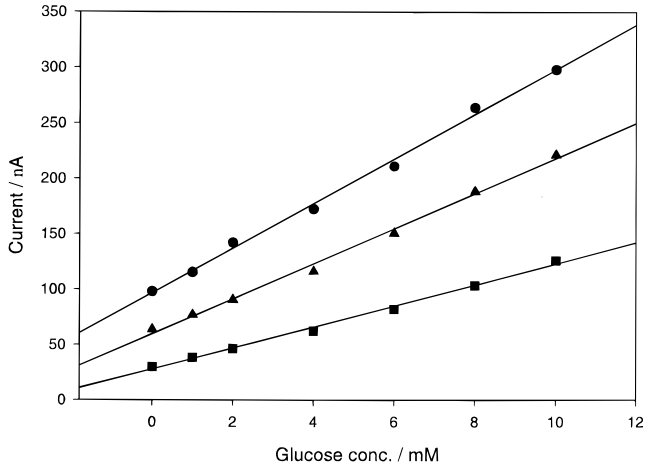

Figure 3. Dependence of the current measured at the end of the period during which the 300 mV (Ag/AgCl) potential was applied on the glucose concentration. The currents following application of $t = 1$, 2, and 5 s long potential pulses are shown. Conditions as in Figure 2.

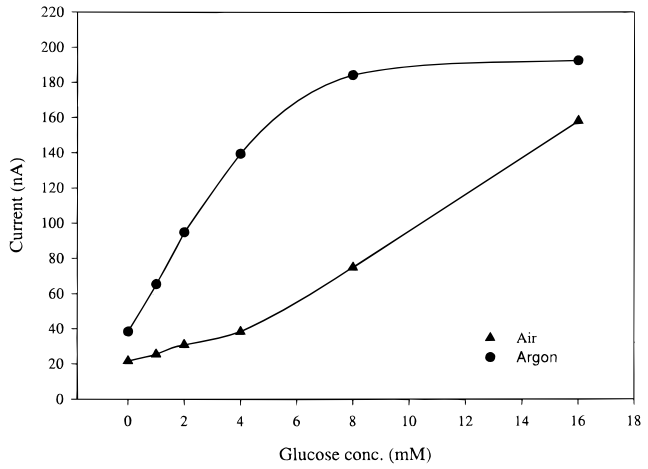

Figure 4. Effect of deoxygenation of the solution on the amperometric calibration curves. Conditions as in Figure 3; applied potential pulses of $t = 1$ s duration.

opening the switch did not change significantly when the transiently applied potential was raised from 300 to 400 mV vs Ag/AgCl.

Figure 3 shows three amperometric calibration curves. The currents were measured at the end of the 1, 2, or 5 s periods during which the switch was closed, a 300 mV (Ag/AgCl) potential being applied. The currents depended on the oxygenation of the solutions, increasing when the solution was swept with argon (Figure 4).

Figure 5 shows for 25 mM glucose concentration on a log/log chart the dependence of the current (i) on the periods τ and t. For a diffusion-controlled process $\log i \propto -0.5 \log \tau$. The theoretical −0.5 slope is shown as a dashed line at the bottom of the figure.

Figure 6 shows the dependence of the potential difference (absolute value vs ground) on the glucose concentration. The potential was measured after the electrode was poised at 300 mV (Ag/AgCl) for 1, 2, or 5 s (switch closed) and then allowed to float for 50 s (switch opened). The values obtained for 1, 2, or 5 s pulses were similar, the potential decreasing linearly with glucose concentration. The effect of deoxygeneration on the potentiometric calibration curve is shown in Figure 7. The effects of deoxygenation on the potentiometric and amperometric calibration curves were similar (Figure 4). The relationship

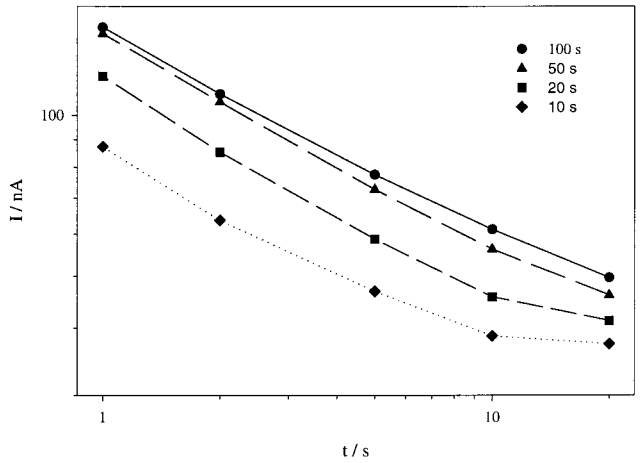

Figure 5. Dependence of the current at the end of the potential pulse on its duration (t) and on the intervals (τ) between the pulses. Note that both the ordinate and the abscissa are logarithmic. The dashed line at the bottom represents the ideal relationship for a diffusion-controlled process ($\log i \propto -0.5 \log t$). Conditions as in Figure 2.

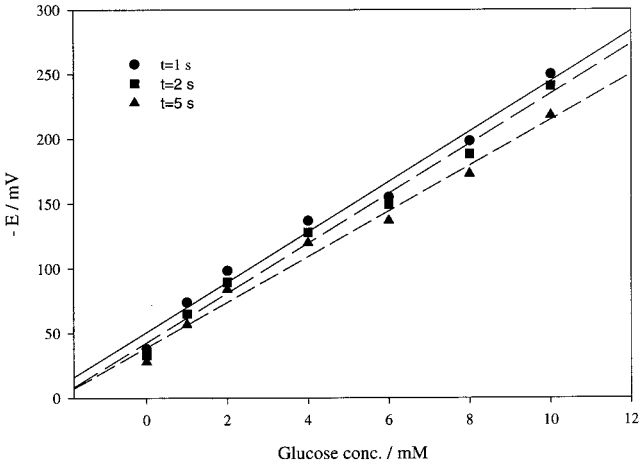

Figure 6. Dependence of the potential, measured at the end of an open-circuit period of 50 s, on glucose; 300 mV (Ag/AgCl) potential pulses were applied for $\tau = 1$, 2, or 5 s. Conditions as in Figure 2.

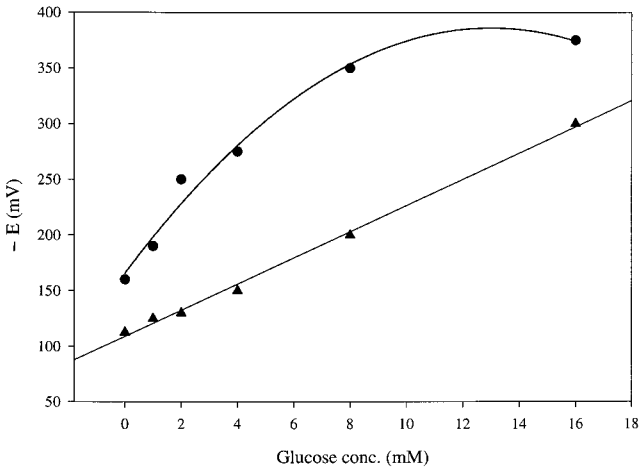

Figure 7. Effect of deoxygenation of the solution on the potentiometric calibration curve. Conditions as in Figure 6; $t = 1$ s.

between the measured currents and potentials is shown in Figure 8. The potentials varied linearly with the currents, both in air and under argon.

To establish that the apparently linear dependence of the potential on the current was not unique to the 0.25 mm diameter

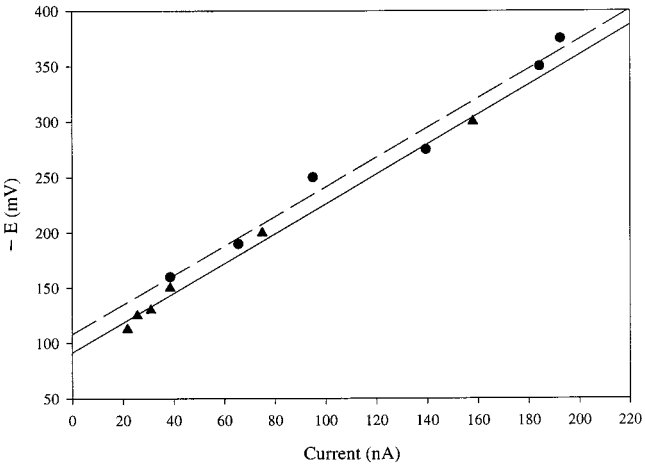

Figure 8. Dependence of the potential at the end of the $\tau = 50$ s open-circuit period following a $t = 1$ s potential pulse on the current at the end of the 1 s pulse. Conditions as in Figure 2.

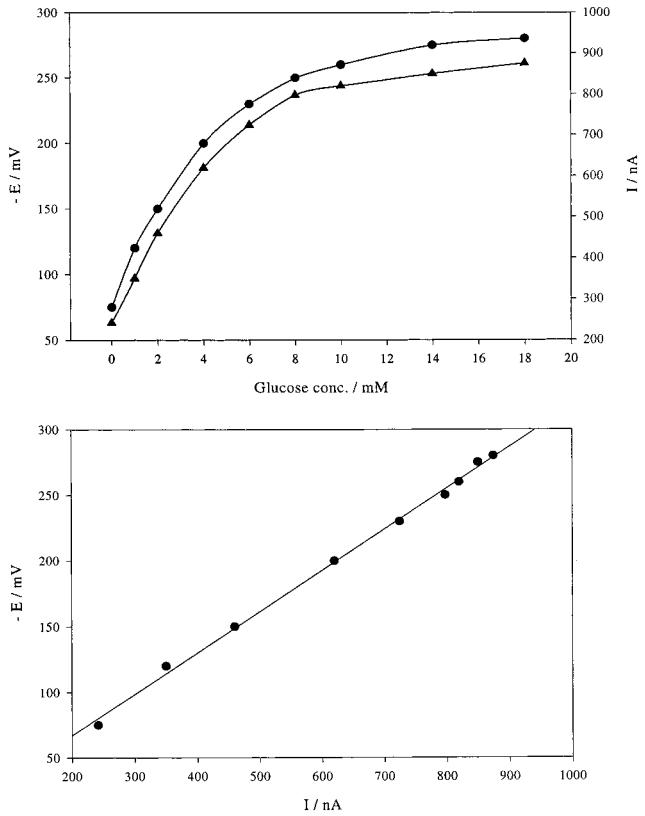

Figure 9. Dependence of the current and of the potential on the glucose concentrations and of the potential on the current for the 5 mm diameter printed carbon electrode. $\tau = 50$ s; $t = 1$ s. Currents were measured at end of the potential pulse. Conditions as in Figure 2.

recessed gold electrode, the interdependences of the potential, current, and glucose concentrations were determined also for a 5 mm diameter printer carbon electrode on Mylar with a sensing layer identical with that of the miniature gold electrode but with a Poretics polycarbonate membrane. Although the structure, size, and mass transport restricting membranes were quite different for the two electrodes, the results were similar; their potentials varied linearly with glucose concentration (Figure 9).

The experiments of Figure 10 show, for comparison, the classical behavior of a conventional 3 mm diameter platinum electrode, to which a redox polymer film was not applied in $K_3Fe(CN)_6$ solutions of different concentrations. Figure 10a shows the dependence of the current, measured at the end of τ

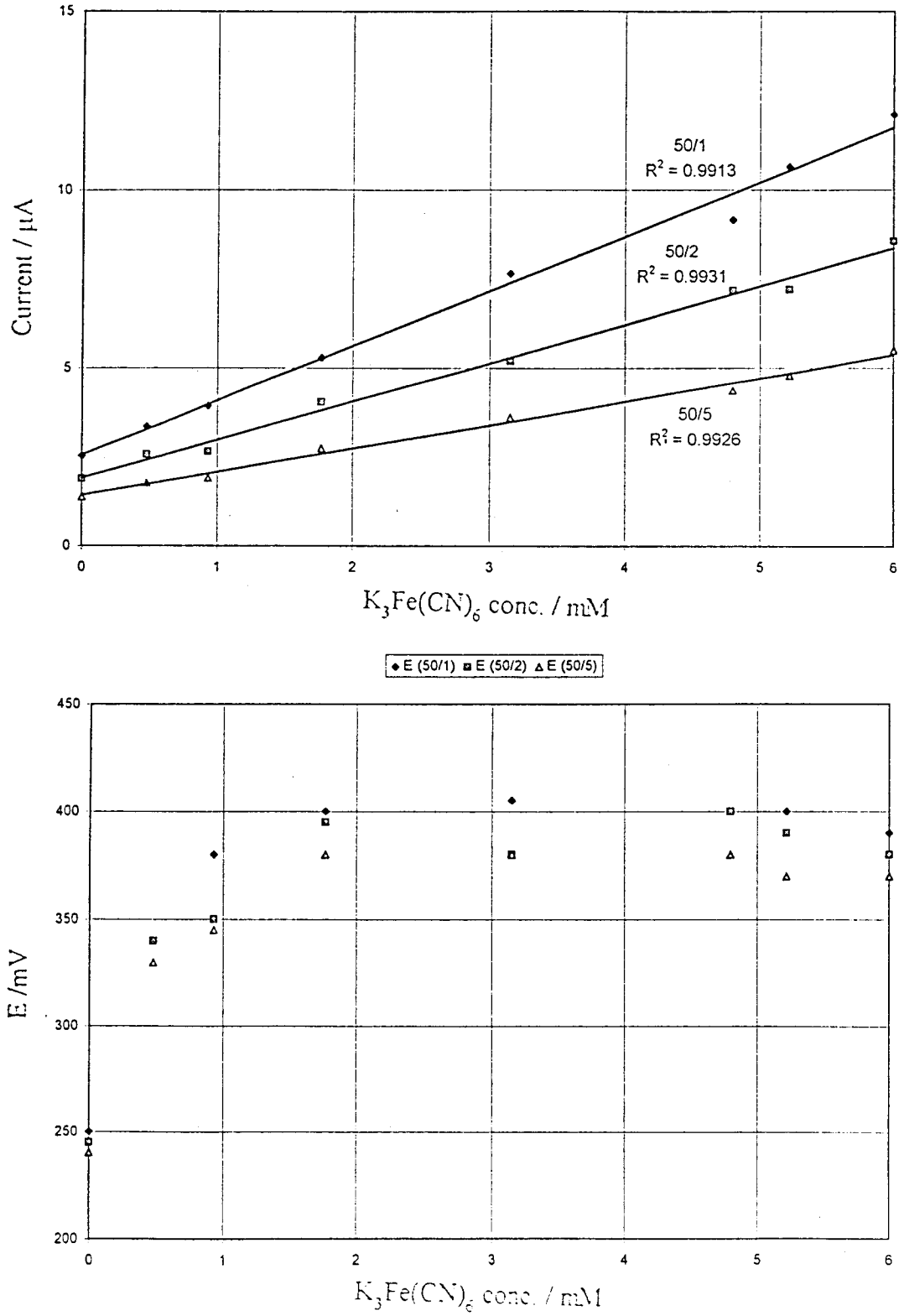

Figure 10. Dependence of the current and of the potential on the concentration of $K_3Fe(CN)_6$. $\tau = 50$ s; $t = 1$ s; 100 mV potential pulses. A 3 mm diameter Pt electrode was used.

$= 1$ s 100, 200, or 300 mV (Ag/AgCl) potential pulses, on the concentration of $K_3Fe(CN)_6$; Figure 10b shows the potentials at the end the $t = 50$ s open-circuit potential periods.

Discussion

When the redox polymer film on the electrode was in the reduced state, after being exposed at open circuit to a glucose solution, closing the circuit and poising the electrode at an oxidizing potential resulted in the flow of a large, rapidly decaying electrooxidation current. The decay characteristics of the current resembled those modeled by a modified Cottrell equation.[8,9]

Figures 8 and 9 show that for redox polymer-based enzyme electrodes, but not for an electrode which is not polymer-modified (Figure 10), the potential to which the electrode relaxed following application of a potential pulse varied linearly with

the current, decreasing as the current increased. The linear dependence held for electrodes of different structure (Figures 8 and 9). Because the current scaled linearly with the glucose concentration (in deoxygenated solutions and at concentrations below the apparent Michaelis constant of the enzyme electrode), the potential also varied linearly with the glucose concentration.

The observed linear dependence of the measured potential on the current or glucose concentration can be explained through the following model: when a resistive film, to which electrons are delivered upon the electrooxidation of glucose and through which electrons diffuse, is applied to an electrode, the current/potential relationship can be controlled either by the electrode kinetics, i.e., the flux of electrons between the electrode and the film, or by the flux of electrons through the film, i.e., the resistance of the films to electron transport. The flux of electrons between the film and the electrode is, as usual, well represented by the Butler−Volmer or Tafel equations, the current increasing exponentially with the overpotential. This is the case for the uncoated Pt electrode in a $K_3Fe(CN)_6$ solution (Figure 10). The flux of electrons through the redox polymer film obeys, however, Ohm's law, the current increasing linearly with the potential across the resistive film. According to the model, the condition for linear dependence of the potential on the current, which is the rate of electron generation through glucose electrooxidation, is that the transport of electrons between the electrode and the polymer be fast relative to the rate of electron transport through the polymer. Also according to the model, the potential to which the redox polymer film relaxes (after application of a pulse of sufficient potential to electrooxidize remote redox centers) is that potential where the outbound and inbound electron currents are equal. In an electrode coated with a redox polymer connecting reaction centers of glucose oxidase to an electrode, the inbound current is (in an oxygen-depleted solution and below the apparent Michaelis constant of the electrode) twice the glucose flux, the electrooxidation reaction being glucose $\rightarrow$ gluconolactone $+ 2H^+ + 2e^-$. At equilibrium the electrode poises itself at a sufficiently reducing potential to create a countercurrent of outbound electrons, equaling the inbound, glucose-generated current. The outbound electron current, when dominated not by the overpotential for electron transport from the electrode to the redox polymer but by the resistance of the polymer, is defined by Ohm's law.

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