with those first obtained, illustrating the reproducibility and stability of the microdroplet titrator. The results for the titrations of HOAc and NaOH proved to be poorer than those obtained previously, which was expected based on the behavior those systems displayed earlier. With a smaller sample volume, the effects of evaporation of HOAc and the reaction of CO_2 with NaOH would be even more pronounced and would lead to poorer precision.

CONCLUSIONS

The microdroplet titrator used in this study can perform microscale titrations with a high degree of accuracy and precision. Its advantages over other microtitration instruments are that it is directly digital in operation, and thus easily automated, and offers greater precision. The only serious problems with the device are reactions that occur between the small-volume sample solution and the atmosphere and drift in the driving electronics which causes the calibration of the instrument to change. The first problem could be eliminated by designing a sample container that would allow an inert atmosphere to surround the sample solution. The second problem could be solved by electronic feedback from the bimorph to the driving electronics. Therefore, neither of these problems would be difficult to overcome and should not be viewed as serious disadvantages. Also, because the titrator is computer-controlled, other applications for the instrument could be easily implemented by software modification. These applications could include pH-stat experiments, kinetics experiments performed on a microscale, or microsample deposition.

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LITERATURE CITED

(1) Benedetti-Pichler, A. A. "Microtechniques of Inorganic Analysis"; Wiley: New York, 1942.
(2) Pecar, M. *Microchem. J.* **1959**, *3*, 557-563.
(3) Rogers, D. W.; Lillian, D.; Chawla, I. D. *Mikrochim. Acta* **1968** *4*, 722-728.
(4) Knobloch, V.; Mudrova, B. *Mikrochim. Acta* **1970**, *2*, 235-239.
(5) Ceska, M.; Grossmuller, F.; Sjodin, A. V. *Int. J. Appl. Radiat. Isot.* **1971**, *22*, 311-314.
(6) Walsby, J. R. *Anal. Chem.* **1973**, *45*, 2445-2446.
(7) Spokane, R. B.; Brill, R. V. *Anal. Biochem.* **1980**, *109*, 449-453.
(8) Shabushnig, J. G.; Hieftje, G. M. *Anal. Chim. Acta* **1981**, *126*, 167-174.
(9) Shabushnig, J. G.; Hieftje, G. M. *Anal. Chim. Acta* **1983**, *148*, 181-182.
(10) May, K. R. *J. Sci. Instrum.* **1945**, *22*, 187-189.
(11) May, K. R. *J. Sci. Instrum.* **1950**, *27*, 128-131.

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Fluorescence Polarization Immunoassay of Phenytoin Employing a Sulfonamido Derivative of 2-Naphthol-8-sulfonic Acid as a Label

Jeffrey S. O'Neal and Stephen G. Schulman*

College of Pharmacy, Box J-4, J. Hillis Miller Health Center, University of Florida, Gainesville, Florida 32610

The synthesis of a new fluorescent derivative of 5,5-diphenylhydantoin (phenytoin) and its application in a fluorescence polarization immunoassay are described. This fluorescent label undergoes excited-state proton transfer which results in a relatively large shift of the fluorescence spectrum to wavelengths much longer than the wavelength of excitation, thereby decreasing background serum matrix interference. Good precision was obtained for this immunoassay, demonstrating the effectiveness of the label in this system.

Phenytoin (5,5-diphenylhydantoin) is a widely prescribed therapeutic drug for the treatment of epilepsy. The phenytoin plasma concentration range of 9–21 mg/L is considered to be clinically useful, concentrations outside of this range being either toxic or noneffective. It is therefore highly desirable to monitor phenytoin blood concentrations (*1*, *2*). Traditional methods of phenytoin quantitation include gas chromatography (*3*), high-performance liquid chromatography (*4*), radioimmunoassay (*5*), and homogeneous enzyme immunoassay (*6*). Fluorescence immunoassays for phenytoin have also recently been developed, including a double-antibody technique (*7*), a magnetizable solid-phase method (*8*), a reactant-labeled assay (*9*), and a fluorescence polarization immunoassay (*10*).

Fluorescence immunoassays have almost exclusively relied upon derivatives of fluorescein as labeling compounds. Although relatively high molar absorptivities and quantum yields have been achieved, alternative fluorescent labels can potentially provide increased spectroscopic detectabilities and, hence, decreased limits of analyte detection. In this experiment a fluorescent derivative of phenytoin containing a sulfonamido derivative of 2-naphthol-8-sulfonic acid was synthesized and used in a fluorescence polarization immunoassay (FPIA) for phenytoin. This fluorescent label is one of a series of potential derivatizing reagents which fall into the class of hydroxyaromatic sulfonic acids. These probes undergo excited-state prototropic dissociation which is schematically summarized in Figure 1. This process, in hydroxyaromatics, results in large shifts to long emission wavelengths which are highly desirable when the analytical signal originates from a biological matrix.

The large displacements between longest wavelength absorption and longest wavelength fluorescence maxima which accompany excited-state dissociation of hydroxyaromatics are

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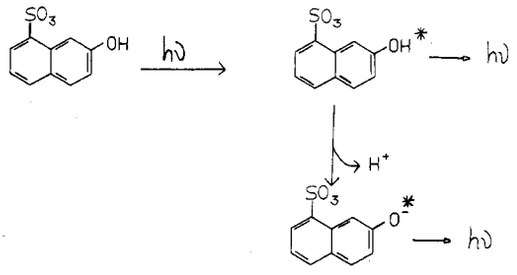

Figure 1. Schematic depiction of 2-naphthol-8-sulfonate excited-state proton transfer.

derived from the large stabilization of the lowest excited singlet state of the conjugate base relative to that of the conjugate acid. This relative stabilization is responsible for the red shifts of the absorption and fluorescence spectra, accompanying dissociation of hydroxyaromatics in general. Because at near neutral pH the emission of the conjugate base originates only from photodissociation of the directly excited conjugate acid and not from direct excitation of the base, the very long wavelength emission of the conjugate base is very much separated from the short wavelength absorption of the conjugate acid, even when the Stokes's shift of the latter is small. By use of the emission of the photodissociated hydroxyaromatic label, not only is interference from scattered incident radiation decreased but emissions from fluorescent molecules of biological origin which tend to lie at considerably shorter wavelengths become less bothersome.

The polarized fluorescence of macromolecules was first observed by Weber (*11*) and its subsequent application to immunological systems was first explored by Dandliker (*12, 13*). The basis for the fluorescence polarization immunoassay (FPIA) is the increase in rotational relaxation time of a small fluorescent molecule upon binding to an antibody, resulting in an increase (positive or negative) in fluorescence polarization. This allows the analysis of the macromolecule-bound fluorophore in the presence of the free fluorophore even when both emit in the same spectral region. competition between labeled and unlabeled drug molecules for a limited number of antibody binding sites allows the production of a standard curve from which unknown concentrations of drug may be extracted. the equation used to calculate fluorescence polarization, P, is

$$P = \frac{I_{\parallel} - I_{\perp}}{I_{\parallel} + I_{\perp}} \quad (1)$$

where $I_{\parallel}$ is the intensity of fluorescence when polarizers placed in the excitation and emission beams have their optical axes parallel with respect to each other and $I_{\perp}$ is the fluorescence intensity when the optical axes of the polarizers are oriented perpendicular to each other.

EXPERIMENTAL SECTION

Reagents. Phenytoin and 4-bromoethylphthalimide (BPI) were obtained in 99% purity from Aldrich Chemical Co. (Milwaukee, WI). Reagent-grade thionyl chloride and sodium acetate were obtained from Fisher Scientific Co. (Fair Lawn, NJ). Technical-grade 2-naphthol-8-sulfonate sodium salt was obtained from Eastman Kodak Co. (Rochester, NY).

Apparatus. Fluorescence polarization measurements were performed by using a Perkin-Elmer MPF 2A fluorimeter equipped with a Hitachi 018-0054 polarization accessory. Low-pressure preparative liquid chromatographic separations were obtained by using a SY-SSR pump from Fluid Metering, Inc. (Oyster Bay, NY) and glass columns from Ace Glass, Inc. (Vineland, NJ). Absorptiometric enzyme immunoassays were carried out by using a Du Pont automated clinical analyzer (Wilmington, DE).

Drug-Label Conjugate Synthesis. Purification of 2-Naphthol-8-sulfonic Acid Sodium Salt. A low-pressure liquid chromatographic system was used with a bentonite/cellulose (5:95) solid phase and a H_2O/isopropanyl alcohol (3:97) mobile phase at 40 psi. The pure sodium naphtholsulfonate was frontally eluted, and collected fractions were tested for purity by using a silica gel thin-layer chromatographic system with a mobile phase consisting of H_2O/ethyl acetate/acetone (12:38:50). The R_f of the pure product is approximately 0.65.

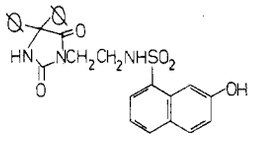

Figure 2. Structure of 2-8-DPH.

Synthesis of 2-Acetoxy-8-naphthalenesulfonic Acid Sodium Salt. 2-Naphthol-8-sulfonic acid sodium salt (2.03×10^{-2} mol, 5 g), 17 g of acetic anhydride (1.67×10^{-1} mol), and 0.5 g of sodium acetate (6.10×10^{-3} mol) in 30 mL of dimethylformamide were stirred for 3 h at 80 °C. After filtration of the reaction mixture 100 mL of petroleum ether/acetone (70:30) was added and the solution kept at 4 °C overnight to cause precipitation. The reaction yield was 78%.

Synthesis of 2-Acetoxy-8-naphthalenesulfonyl Chloride. 2-Acetoxy-8-naphthalenesulfonic acid sodium salt (8.67×10^{-3} mol, 2.5 g) and 2.5 mL of thionyl chloride (3.43×10^{-2} mol) were heated to reflux, while stirring, until no solid material remained. The mixture was diluted with 20 mL of methylene chloride, filtered, and evaporated under partial vacuum to near dryness. Upon cooling the product precipitated (mp 86–88 °C), yield 85%.

Synthesis of 3-(2′-Phthalimidoethyl)-5,5-diphenylhydantoin. Phenytoin (3.96×10^{-3} mol, 1.0 g), 0.548 g of potassium carbonate (3.96×10^{-3} mol), and 1.007 g of BPI (3.96×10^{-3} mol) were added to 12 mL of dimethylformamide, and the mixture was refluxed for 2 h. H_2O (30 mL) was added, and the resulting precipitate was filtered, dissolved in a minimum volume of hot methanol, filtered hot, and small white needles were collected after 3 h from the cooled solution (mp 220–222 °C), yield 90%.

Synthesis of 3-(2-Aminoethyl)-5,5-diphenylhydantoin. 3-(2′-Phthalimidoethyl)-5,5′-diphenylhydantoin (8.58×10^{-3} mol, 3.65 g) and 1.31 mL of 40% aqueous methylamine (1.72×10^{-2} mol) were added to 150 mL of methanol and stirred for 48 h. The solution was then filtered, evaporated under reduced pressure, and redissolved in methylene chloride. The product was extracted into 0.5 M HCl, the aqueous phase was filtered, and aqueous sodium hydroxide was added to give a pH of 13. The product was extracted back into methylene chloride which was evaporated under reduced pressure to give the pure product (mp 148–150 °C), yield 65%.

Synthesis of 3-[2″-Acetoxynaphthyl-8″-sulfonyl)-2′-amino]ethyl-5,5-diphenylhydantoin. 3-(2-Aminoethyl)-5,5-diphenylhydantoin (3.38×10^{-3} mol, 1.00 g), 1.02 g of 2-acetoxy-6-naphthalenesulfonyl chloride (3.39×10^{-3} mol) and 0.470 mL of triethylamine (3.37×10^{-3} mol) were added to 20 mL of acetone, stirring at room temperature for 30 min. This mixture was filtered and the supernatant dried under reduced pressure and chromatographed on 200 g of silica employing a chloroform/methanol (98:2) mobile phase. A fraction between the eluting volumes of 200–500 mL contained the pure product (mp 108–110 °C), yield 60%.

Synthesis of 3-[(2″-Hydroxynaphthyl-8″-sulfonyl)-2′-amino]ethyl-5,5-diphenylhydantoin (2-8-Phenytoin). 3-[2″-Acetoxynaphthyl-8″-sulfonyl)-2′-amino]ethyl-5,5-diphenylhydantoin (1.78×10^{-4} mol, 100 mg) was added to 10 mL of 0.15 M NaOH, stirring for 1 h until dissolved. After filtration the solution was titrated dropwise with concentrated HCl, stirring, to precipitate the product (mp 110–114 °C), yield 95%. ^{1}H NMR ($CDCl_3$) δ 8.8–7.6 (m, 6 H, aromatic-*H*), 7.3 (s, 10 H) 5.3 (s, 1 H, hydroxy-H), 3.6 (d, 2, J = 3 Hz, CH_2-$NHSO_2$ aromatic), 2.9 (d, 2, J = 3 Hz, hydantoin-CH_2). The structure of 2-8-phenytoin is illustrated in Figure 2.

Antisera. Rabbit anti-phenytoin-3-valeryl-BSA was obtained from Miles-Yeda, Ltd. (Rehovot, Israel). Antisera stock solutions were prepared by diluting the antisera 1:10 into a pH 7.5, μ = 0.10 phosphate buffer containg 0.1% sodium azide.

Table I. Spectroscopic Data of 2-8-DPH and a Fluorescein Derivative of Immunoglobulin G (F-IgG) (*14*)

	2-8-DPH	F-IgG
absorption max, nm	337	492
fluorescence max, nm	480	518
molar absorptivity, L mol^{-1} cm^{-1}	5100	72 000
fluorescence quantum yield	0.298	0.82

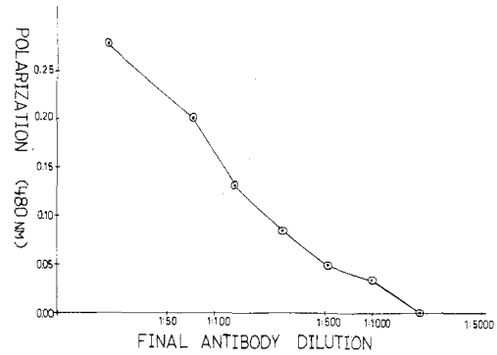

Figure 3. Variation in fluorescence polarization of 7.5×10^{-7} M 2-8-DPH with varying dilutions of rabbit anti-DPH.

RESULTS AND DISCUSSION

Characterization of 2-8-Phenytoin. Excitation and emission maxima, molar absorptivity at the absorption wavelength maximum, and fluorescence quantum yield of 2-8-phenytoin are summarized and compared with those properties of a fluorescein derivative in Table I. The ground-state pK_a of 2-8-phenytoin was determined spectrophotometrically to be 8.22 ± 0.02.

Antibody Dilution. Solutions of 7.5×10^{-7} M 2-8-phenytoin in $\mu = 0.10$, pH 7.4 phosphate buffer were titrated with an antiserum stock solution and the polarization values calculated for the various increments of the antiserum solution. The values obtained are illustrated in Figure 3. An optimal final antibody dilution of 1:100 was used in all experiments.

FPIA Protocol. Human serum or normal pooled human serum spiked with phenytoin (75 μL) was added to 75 μL of a 1% aqueous solution of sodium dodecyl sulfate. The surfactant was added to reduce nonspecific serum protein binding of small molecules, as described by Kobayashi et. al. (*15*). After a 20-min incubation period 2 mL of 7.0×10^{-7} M phenytoin in pH 7.5, $\mu = 0.10$, phosphate buffer followed by 20 μL of 1:10 diluted antibody solution was then added. The fluorescence polarization was then calculated, using excitation and emission maxima of the fluorophor, 337 and 480 nm. All incubations and measurements were carried out at 30 °C.

Standard Curve. A typical standard curve is given in Figure 4, showing the variation of polarization with serum phenytoin concentration.

Precision and Recovery. Within-day and between-day assay precisions are listed in Table II. Recovery data for this assay plotted as the amount of phenytoin added vs. the amount of phenytoin found yielded a straight line of $F = 1.02A - 0.145$, where F is the amount found and A is the amount added to pooled normal human serum for concentrations of 5, 10, 15, 20, 25, and 30 mg/L phenytoin. The standard errors of the slope and intercept are, respectively, 0.02 and 0.031.

Correlation Studies. Patient serum samples containing phenytoin were obtained from the Clinical Research Center at Shands Teaching Hospital at the University of Florida and analyzed by an absorptiometric enzyme immunoassay (EIA) method and by the FPIA method. A plot of the phenytoin concentrations determined by the FPIA method against those determined by the EIA method for the 26 samples yields a straight line where $F = 1.04E - 1.06$, where F is the amount calculated by the FPIA method and E is that calculated by the absorptiometric enzyme immunoassay method. Standard errors of the slope and intercept, respectively, are 0.12 and 0.10.

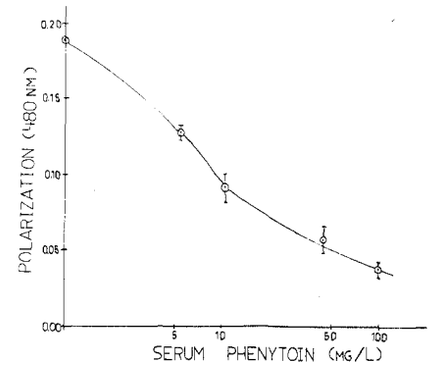

Figure 4. Fluorescence polarization immunoassay standard curve illustrating the change in polarization of 2-8-DPH with increasing concentrations of unlabeled phenytoin.

Table II. Precision of Phenytoin Analysis by FPIA[a]

	precision	
phenytoin concn, mg/L	within-day[b]	between-day[c]
5	5.16	8.22
10	6.72	9.01
15	3.82	5.78
20	3.61	6.28
25	3.30	5.50
30	12.51	15.20

[a] Percent relative standard deviation. [b] Ten replications same day. [c] Three replications on five separate days.

CONCLUSIONS

The labeled drug employed in this immunoassay system has been shown to be sufficiently fluorescent for highly reliable results to be obtained. Large shifts of the fluorescence spectra to wavelengths much longer than the wavelength of excitation effectively eliminate Rayleigh scattering and background serum fluorescence interferences. Additionally, short wavelength excitation offers the possibility of excitation via low-cost nitrogen lasers, further decreasing label detection limits for commerical instrumentation. Further work in this area should result in derivatives possessing larger molar absorptivities and flourescence quantum yields, while still yielding large shifts to longer fluorescence emission wavelengths.

Registry No. BPI, 574-98-1; phenytoin, 57-41-0; 2-acetoxy-8-naphthalenesulfonic acid sodium salt, 92241-04-8; 2-naphthol-8-sulfonic acid sodium salt, 832-85-9; 2-acetoxy-8-naphthalenesulfonyl chloride, 92241-05-9; 3-(2′-phthalimidoethyl)-5,5-diphenylhydantoin, 20000-09-3; 3-(2-aminoethyl)-5,5-diphenylhydantoin, 14046-88-9; methylamine, 74-89-5; 3-[(2″-acetoxynaphthyl-8″-sulfonyl)-2′-amino]ethyl-5,5-diphenylhydantoin, 92220-76-3; 3-[(2″-hydroxynaphthyl-8″-sulfonyl)-2′-amino]ethyl-5,5-diphenylhydantoin, 92241-06-0.

LITERATURE CITED

(1) Kutt, H.; McDowell, F. *J. Am. Med. Assoc.* **1968**, *203*, 167–170.
(2) Kutt, H.; Penry, J. K. *Arch. Neurol. (Chicago)* **1974**, *31*, 283–288.
(3) Richens, A. "Clinical Pharmacology of Anti-Epileptic Drugs"; Schneider, H., Janz, D., Gardner-Thorpe, C., Meinardi, H., Sherwin, A. L., Eds.; Springer: Berlin, 1975; pp 293–303.
(4) Kabra, P. M.; Stafford, B. E.; Marton, L. J. *Clin. Chem.* **1977**, *23*, 1284–1288.
(5) Paxton, J. W.; Rowell, F. J.; Ratcliffe, J. G. *J. Immunol. Methods* **1976**, *10*, 317–327.
(6) Booker, H. E.; Darcey, B. A. *Clin. Chem.* (*Winston-Salem, N.C.*) **1975**, *21*, 1766–1768.
(7) Kurtz, M. J.; Billings, M.; Koh, T.; Olander, G.; Tyner, T.; Weaver, B.; Stone, L. *Clin. Chem.* (*Winston-Salem, N.C.*) **1983**, *29*, 1015–1019.
(8) Kamel, R. S.; Landon, J.; Smith, D. S. *Clin. Chem.* (*Winston-Salem, N.C.*) **1980**, *26*, 1281–1284.

(9) Wong, R. C.; Burd, J. F.; Carrico, R. J.; Buckler, R. T.; Thoma, J.; Boguslaski, R. C. *Clin. Chem. (Winston-Salem, N.c.)* **1979**, *25*, 686–691.
(10) McGregor, A. R.; Crookall-Greening, J. O.; Landon, J.; Smith, D. S. *Clin. Chim. Acta* **1978**, *83*, 161–166.
(11) Weber, G. *Biochem. J.* **1952**, *51*, 145–156.
(12) Dandliker, W. B.; Halbert, S. P.; Florim, M. C.; Alonso, R.; Schapiro, H. C. *J. Exp. Med.* **1965**, *122*, 1029–1048.
(13) Dandliker, W. B.; Alonso, R.; Meyers, C. Y. *Immunochemistry* **1967**, *4*, 295–302.
(14) The, T. H.; Feltkamp, T. R. *Immunology* **1970**, *18*, 865–873.
(15) Kobayashi, Y.; Miyai, K; Tsubota, N.; Watanabe, F. *Steroids* **1979**, *34*, 829–834.

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Alterations in Potentiometric Response of Glucose Oxidase Platinum Electrodes Resulting from Electrochemical or Thermal Pretreatments of a Metal Surface

James F. Castner[1] and Lemuel B. Wingard, Jr.*

Department of Pharmacology, School of Medicine, University of Pittsburgh, Pittsburgh, Pennsylvania 15261

A linear correlation has been observed between the log (glucose concentration) and measured potentials at bare Pt- and at enzyme/Pt-indicating electrodes. A comparison study of five different methods of electrode pretreatment was undertaken to evaluate the Nernstian behavior of the sensors. The potentiostatically controlled double-layer and the platinic chloride-treated electrodes exhibited the greatest differences in potentiometric response between the metal and the enzyme/metal electrodes. Sensors fabricated with flame-oxidized and with potentiostatic-oxidized or reduced Pt surfaces demonstrated little difference in Nernstian response between the two types of indicating electrodes. Variations in pH, ionic strength, and oxygen concentration had only minor effects. The mechanism of the enzyme electrode appears to be associated with the products of the biocatalytic reaction; the electrochemical reaction of glucose at the bare metal electrode appears to be dependent on the Pt surface chemistry.

The development of new types of biocatalytic surfaces coupled to potentiometric electrodes is an area of analytical chemistry that has been extremely active during the past few years. Enzymes, microbial cells, special membranes, and immunoproteins have been incorporated into a variety of electrode configurations to impart selectivity for specific drugs, endogenous compounds, antibodies, or ions (*1*, *2*). These electrodes respond to the selected materials potentiometrically.

There is a need for obtaining continuous in vivo measurements of glucose levels for the clinical management of ambulatory insulin-dependent diabetic patients. The development of a small potentiometric enzyme electrode system has been proposed as one approach to meet this clinical need. Such an electrode should respond over a range of about 2.7–22.2 mM glucose (50–400 mg/100 mL) with a mean of roughly 6.6 mM (120 mg/100 mL) for placement in the circulatory system. Alternatively, the electrode could be implanted in a nonvascular part of the body, so long as the glucose concentration responded quickly to changes in blood glucose levels (*3*). Nonvascular placement would reduce the clotting complications caused by contact of blood with foreign materials.

An electrode consisting of glucose oxidase immobilized on platinum (*4*), with or without added catalase (*5*), was shown in vitro to produce a potentiometric response to glucose. The proposed overall reaction sequence at this electrode was as follows.

$$\beta\text{-D-glucose} + O_2 \xrightarrow{\text{glucose oxidase}} \text{D-glucono-}\delta\text{-lactone} + H_2O_2 \quad (1)$$

$$\text{D-glucono-}\delta\text{-lactone} + H_2O \rightarrow \text{D-gluconic acid} \quad (2)$$

$$H_2O_2 \xrightarrow{\text{catalase}} H_2O + {}^1/_2O_2 \quad (3)$$

The hydrogen peroxide produced in reaction 1 had a major influence on the magnitude of the observed Nernstian-like response (*6*, *7*). It was recognized that a potentiometric response was generated when bare platinum metal was placed in solutions of different concentrations of glucose (*6*). However, this glucose-generated potentiometric response was enhanced markedly by the presence of glucose oxidase immobilized on the platinum surface (*4–6*). The difference in response between the enzyme electrodes and bare platinum electrodes was at least 10 times greater for glucose than for possible interfering endogenous materials at normal physiologic levels, with the exception of glycine (only 7 times greater for glucose) (*5*, *8*). In previous studies the platinum support was cleaned by soaking the metal in 20–50% nitric acid plus heating it until white-hot in a natural gas flame. Preliminary results showed that the potentiometric response was influenced by the type of pretreatment that the platinum surface was given (*7*). The characteristics of the oxide film present on the surface of the platinum metal may have been influenced by the surface pretreatment; however, the detailed electrochemistry of platinum oxide films is still not completely understood (*9*, *10*).

The present study was undertaken to examine the relationships between platinum surface pretreatments and potentiometric response elicited from (1) a glucose oxidase enzyme electrode and (2) a non-enzyme bare platinum indicator electrode for the analytes glucose and hydrogen peroxide. The

[1]Present address: E. I. du Pont de Nemours & Co., Inc., Biomedical Products, Wilmington, DE 19898.

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