

See discussions, stats, and author profiles for this publication at: <https://www.researchgate.net/publication/51294596>

Ferrocene-Mediated Enzyme Electrode for Amperometric Determination of Glucose

ARTICLE *in* ANALYTICAL CHEMISTRY · APRIL 1984

Impact Factor: 5.64 · DOI: 10.1021/ac00268a018 · Source: PubMed

CITATIONS

1,136

READS

684

9 AUTHORS, INCLUDING:



Tony Cass

Imperial College London

157 PUBLICATIONS 4,115 CITATIONS

SEE PROFILE



Hugh Allen O Hill

University of Oxford

378 PUBLICATIONS 12,134 CITATIONS

SEE PROFILE



Anthony P F Turner

Linköping University

375 PUBLICATIONS 12,257 CITATIONS

SEE PROFILE

Table III. Apparent Kinetic Parameters for Homogeneous Enzyme Studies

[inosine], M	type III enzyme		type V enzyme	
	K_m , M	V_{max} , mV/ min	K_m , M	V_{max} , mV/ min
0	9.77×10^{-5}	128.7	9.05×10^{-5}	91.0
2×10^{-4}	9.33×10^{-5}	116.0	1.15×10^{-4}	60.5
2×10^{-3}	1.30×10^{-4}	110.0	8.80×10^{-5}	37.7
2×10^{-2}	2.41×10^{-4}	69.2	7.16×10^{-5}	12.1

for each type of enzyme but in an opposite direction for each. The type III enzyme experiences an increase in apparent K_m with increasing inosine concentration, while the type V enzyme shows a slight decrease in apparent K_m at high inosine concentration. The most dramatic homogeneous effect is that of increasing inosine concentration upon the V_{max} values, however. For the type III enzyme, apparent V_{max} is reduced by nearly a factor of 2 but, for the type V enzyme, apparent V_{max} decreases more than 7-fold. Such product inhibition reveals itself through longer response times in the immobilized enzyme electrodes.

The results obtained in this study show that the potentiometric ammonia gas-sensing enzyme electrode does exhibit linear responses to substrate concentrations both above and below the K_m value of the adenosine deaminase enzyme when sufficient enzyme is immobilized at the electrode surface. The BSA-glutaraldehyde cross-link provides for stabilization of the enzyme activity as shown by the observed electrode lifetime. Comparison of the homogeneous kinetic parameters with those obtained from the immobilized study reveals significant changes in the kinetic properties of the enzyme when

it is immobilized, possibly resulting from conformational changes in the enzyme upon exposure to BSA and glutaraldehyde. The magnitude of the effect of addition of inosine on apparent K_m and V_{max} depends upon whether the enzyme is immobilized or free in solution. The apparent K_m for the immobilized enzyme remained essentially constant upon addition of inosine, while the apparent K_m for the homogeneous enzyme did show some variation but in opposite direction for the type III and V enzymes.

In the construction of enzyme electrodes, it is desirable to obtain the highest V_{max} (fastest rate) and lowest K_m (greatest affinity) values possible. From the results of this study carried out at high enzyme levels it is apparent that type III adenosine deaminase would be the best choice for the construction of an immobilized enzyme electrode both from the point of view of apparent K_m and V_{max} values and from the less pronounced product inhibition effect on the type III enzyme compared to the Type V enzyme. Even in the absence of initial inosine, type III enzyme electrodes have faster response times than corresponding electrodes prepared with type V enzyme.

Registry No. Adenosine deaminase, 9026-93-1; adenosine, 58-61-7.

LITERATURE CITED

- (1) Arnold, M. A.; Rechnitz, G. A. *Anal. Chem.* **1982**, *54*, 2315-2317.
- (2) Brady, J. E.; Carr, P. W. *Anal. Chem.* **1980**, *52*, 977-980.
- (3) Mascini, M.; Guilbault, G. G. *Anal. Chem.* **1977**, *49*, 795-798.
- (4) Lineweaver, H.; Bruke, D. J. *Am. Chem. Soc.* **1934**, *56*, 658-666.
- (5) Deng, I.; Enke, C. *Anal. Chem.* **1980**, *52*, 1937-1940.

RECEIVED for review October 5, 1983. Accepted December 23, 1983. We gratefully acknowledge the support of NSF Grant CHE-8025625.

Ferrocene-Mediated Enzyme Electrode for Amperometric Determination of Glucose

Anthony E. G. Cass,¹ Graham Davis, Graeme D. Francis, and H. Allen O. Hill*

Inorganic Chemistry Laboratory, University of Oxford, Oxford OX1 3QR, United Kingdom

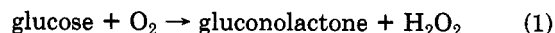
William J. Aston, I. John Higgins, Elliot V. Plotkin, Lesley D. L. Scott, and Anthony P. F. Turner

Biotechnology Centre, Cranfield Institute of Technology, Bedford MK43 0AL, United Kingdom

An amperometric enzyme electrode for the analysis of glucose is described. The electrode uses a substituted ferrocene ion as a mediator of electron transfer between immobilized glucose oxidase and a graphite electrode. A linear current response, proportional to the glucose concentration over a range commonly found in diabetic blood samples (1-30 mM), is observed. Data are presented on the influence of oxygen, pH, and temperature upon the electrode. Results with clinical plasma and whole blood samples show good agreement with a standard method of analysis.

In the condition diabetes mellitus, the determination of blood glucose levels rapidly, conveniently, precisely, and

economically is important for its diagnosis and effective management. The routine analysis of glucose in a variety of physiological fluids is one of the most frequent operations in a clinical chemistry laboratory. Many protocols exist for glucose analysis and have been recently reviewed (1). Those most commonly employed, at present, are based upon the use of the enzyme glucose oxidase to catalyze the following reaction:



Though the product of the reaction, hydrogen peroxide, may be determined by a number of methods, potentiostatically controlled electrochemical oxidation at a platinum electrode has attracted the most interest. This combination of enzymatic catalysis and current measurement at a controlled potential is a feature of the amperometric enzyme electrode. These devices have been reviewed by Carr and Bowers (2). Despite the emphasis on amperometric determination of

¹ Present address: Centre for Biotechnology, Imperial College, London SW7 2AZ, UK.

hydrogen peroxide (2), there are some disadvantages in using a dioxygen-coupled glucose assay for in vitro monitoring in whole blood and plasma: variations in oxygen tension of the sample may introduce fluctuations into the electrode response; at low oxygen tension the upper limit of linearity for the current response may be reduced. In commercial analyzers, this is usually circumvented by predilution of the plasma into oxygenated buffer.

An enzyme electrode that can determine glucose in undiluted whole blood or plasma samples may find many novel applications. To this end, we have sought an alternative amperometric detection method, based on glucose oxidase, that is not dependent on oxygen as the mediator of electron transfer. Electron acceptors for glucose oxidase that have been described previously include hexacyanoferrate(III) (3), and a range of organic dyes (4): the former is not readily entrapped at an electrode; the latter, though widely used in spectrophotometric measurements, have a number of disadvantages for electrochemical use including ready autooxidation, instability in the reduced forms, and pH-dependent redox potentials. A compound was required that combined the well-behaved electrochemistry of hexacyanoferrate with the variations available through structural modification of the organic dyes. Electrochemical investigation, using dc cyclic voltammetry, showed ferrocene (bis(η^5 -cyclopentadienyl)iron) and its derivatives (5, 6) in their oxidized, ferricinium ion forms, to be efficient electron acceptors for soluble glucose oxidase. In addition, these molecules fulfill many of requirements necessary for incorporation into an enzyme electrode configuration (7). We report here on the construction and in vitro testing of a ferrocene-mediated glucose enzyme electrode.

EXPERIMENTAL SECTION

Reagents. Glucose oxidase (EC 1.1.3.4 type 2, from *Aspergillus niger*, mol wt 186 000), supplied by Boehringer Mannheim, had an activity of 274 IU mg⁻¹. The concentration of glucose oxidase is expressed in terms of the molarity of catalytically active FAD. This was determined spectrophotometrically by using a Pye Unicam SP8-200, employing a molar extinction coefficient of 1.31×10^4 L mol⁻¹ cm⁻¹ at 450 nm (8). D-Glucose (AnalaR) was from BDH; ferrocene and its derivatives were from Strem Chemical Co. All solutions were prepared from Aristar grade reagents (BDH) in high-purity water (Millipore); supporting electrolyte was 0.1 M K₂HPO₄ adjusted to the required pH with HClO₄; glucose solutions were stored overnight to allow equilibration of α - and β -anomers.

Biological Samples. Heparinized whole blood and plasma samples from human diabetics were supplied by the Metabolic Unit, Guy's Hospital, London. Plasma samples were analyzed with a Yellow Springs Instruments glucose analyzer.

Apparatus. Dc cyclic voltammetry experiments were performed with a two-compartment cell that had a working volume of 1 mL. In addition to the 4-mm pyrolytic graphite disk working electrode, the cell contained a 1-cm² platinum gauze counter-electrode and a saturated calomel electrode as reference. All potentials are referred to the saturated calomel electrode (SCE). For dc cyclic voltammetry, an Oxford Electrodes potentiostat was used with a Bryans X-Y 26000 A3 chart recorder. The potentiostatically controlled steady-state current measurements were made with either a cell designed to monitor up to seven enzyme electrodes with a working volume of 100 mL with separate compartments for the counterelectrode and the reference electrode or a cell of similar design with a 1-mL volume. The working compartments of both cells could be stirred during operation with a magnetic stirrer bar. Current-time curves were recorded with a Bryans y-t BS-271 recorder. The temperature of the electrochemical cells during experiments was controlled to within ± 0.5 °C with a Churchill thermocirculator.

Construction of the Glucose Enzyme Electrode. Graphite foil 1 mm thick, supplied by Union Carbide, was the base sensor. Electrodes were constructed by cutting the graphite into 4 mm diameter disks and sealing into glass tubes with epoxy resin. Connection to the external circuit was by a wire attached with

Table I. Rates of Glucose Oxidase Oxidation Measured at pH 7 and 25 °C

ferrocene derivative	E_0 /mV vs. SCE	$10^{-5}k_s$, L mol ⁻¹ s ⁻¹
1,1'-dimethyl	100	0.77
ferrocene	165	0.26
vinyl	250	0.30
carboxy	275	2.01
1,1'-dicarboxy	285	0.26
(dimethylamino)methyl	400	5.25

silver Araldite to the back of the electrode. The electrodes were then heated at 100 °C in air for 40 h and allowed to cool and 15 μ L of 1,1'-dimethylferrocene (0.1 M in toluene) was deposited onto the surface of the electrode and air-dried. Covalent attachment of the glucose oxidase to the oxidized graphite surface was achieved by a method similar to that described by Bourdillon (9). The electrodes were placed in 1 mL of a solution of water-soluble 1-cyclohexyl-3-(2-morpholinoethyl)carbodiimide *p*-methyl-toluenesulfonate from Sigma Chemical Co. (0.15 M in 0.1 M acetate, pH 4.5) for 80 min at 20 °C, washed with water, and then placed in a stirred solution of acetate buffer (0.1 M, pH 9.5) containing glucose oxidase (12.5 mg mL⁻¹) for 90 min at 20 °C. After washing, the electrodes were covered with a polycarbonate membrane (Nucleopore, 0.03 μ m) and stored in buffer containing 1 mM glucose at 4 °C.

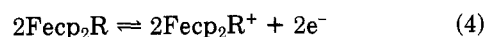
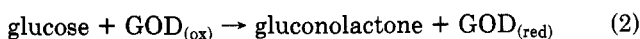
Enzyme Electrode Pretreatment. After fabrication and prior to experiments, the electrode response was stabilized by continuous operation of the electrode under potentiostatic control at 160 mV in 7 mM glucose over a 10-h period. Thereafter the electrodes were found to give a more stable response during 50 h of further operation. All electrodes that had been modified with glucose oxidase had undergone this preconditioning process.

RESULTS AND DISCUSSION

Ferricinium Ion as an Oxidant for Glucose Oxidase.

A number of ferrocene derivatives, Table I, with a range of redox potentials, 100–400 mV vs. SCE, were investigated by dc cyclic voltammetry as possible oxidants for glucose oxidase. These experiments were performed in argon-saturated quiescent solutions. Under the experimental conditions used in this study and over the complete range of potential scanned (–100 to 500 mV) and range of potential scan rates ($\nu = 1$ –100 mV s⁻¹), ferrocene and its derivatives gave voltammograms consistent with a reversible one-electron redox agent at a pyrolytic graphite electrode ($\Delta E_p = 60$ mV; $i_p/\nu^{1/2} = \text{constant}$; $D_0 = 3 \times 10^{-6}$ cm² s⁻¹). Under the same conditions neither glucose nor glucose oxidase exhibited any observable electrochemistry.

Figure 1a, shows a voltammogram of ferrocenemonocarboxylic acid (Fecp₂R) in the presence of glucose alone. Upon addition of glucose oxidase (GOD) to the solution a striking change in the voltammogram occurs, Figure 1b. No peaks are observed and a large catalytic current flows at oxidizing potentials. This behavior is particularly apparent at the slower scan rates and is indicative of the regeneration of ferrocene from the ferricinium ion by the enzyme glucose oxidase in its reduced form. The latter is maintained in this reduced state by the presence of substrate. The following scheme describes the reaction sequence:



The rate of reaction between the reduced form of the enzyme and the oxidized ferrocene (eq 3) can be derived from an analysis of the cyclic voltammograms provided that the heterogeneous electron transfer reaction (eq 4) is fast (diffusion controlled) compared to the rate of the homogeneous reaction

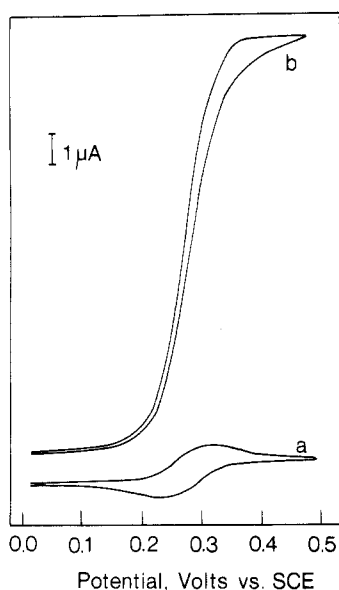


Figure 1. (a) Dc cyclic voltammogram of ferrocene monocarboxylic acid (0.5 mM) at pH 7 and 25 °C, in the presence of D-glucose (50 mM) at a scan rate of 1 mV s⁻¹. (b) As for (a), but with the addition of glucose oxidase (10.9 μM).

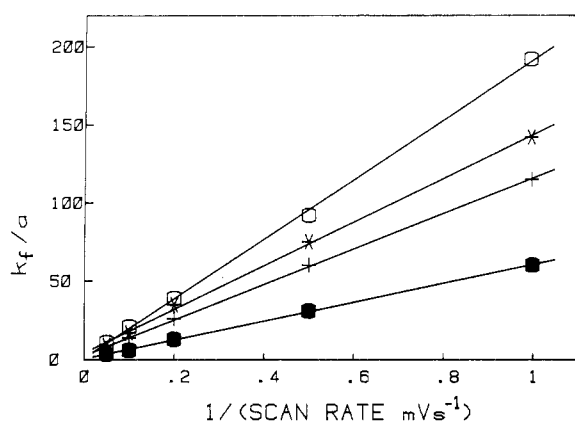
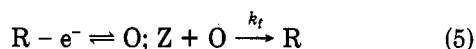


Figure 2. Kinetic parameter k_f/a as a function of $1/\nu$ for various glucose oxidase concentrations: (●) 10.9, (+) 20.6, (*) 29.3, (○) 37.2 μM.

between ferricinium ion and glucose oxidase (eq 3), and that there is a sufficient excess of substrate to ensure that the enzyme is essentially fully reduced (eq 2). The theory developed by Nicholson and Shain (10), analyzed reactions that conform to such conditions on the basis of the following reaction scheme:



where, O and R refer to the respective redox forms of ferrocene, Z is the reduced enzyme, and $k_f = k[Z]$ is the pseudo-first-order rate constant. As with related studies (11), we have analyzed our data making use of Figure 14 of ref 10, which equates the experimentally derived parameter, i_k/i_d (the ratio of the kinetic to diffusion controlled current) to the kinetic parameter $(k_f/a)^{1/2}$. In Figure 2, the data are plotted for a series of glucose oxidase concentrations as k_f/a , where $a = nF\nu/RT$, against $1/\nu$. Under pseudo-first-order conditions the curves should be linear. From the slope of each curve in Figure 2, which equals $k_f nF/RT$, a scan-rate independent pseudo-first-order rate constant is obtained at each glucose oxidase concentration. A plot of the first-order rate constant as a function of glucose oxidase concentration is linear. From the slope $k_f/[GOD]$, the second-order homogeneous rate constant for the reaction between ferricinium monocarboxylic

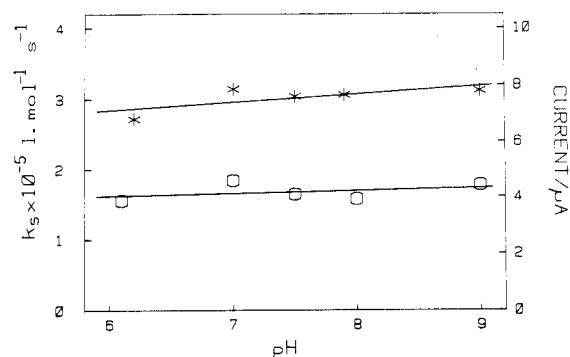


Figure 3. (*) Effect of pH on glucose enzyme electrode, steady-state current measured in glucose (10 mM) at 25 °C. (○) Effect of pH on the homogeneous kinetics of soluble glucose oxidase, conditions as for Figure 1b.

acid and glucose oxidase, eq 3, was obtained: $k = 2.01 \times 10^5$ L mol⁻¹ s⁻¹ (pH 7.0, $I = 0.1$).

Kinetic data obtained by using this technique, presented in Table I, show that all ferrocene derivatives investigated act as rapid oxidants for the enzyme glucose oxidase. While the theory (10) as applied here is suitable for analyzing these high rates of reaction, it assumes that the diffusion coefficients for both reactants are equal. This may introduce a small error into the absolute value of the rate constants. The observed rates for the ferricinium ions are lower than that for the reaction of reduced glucose oxidase with its natural redox partner, oxygen. Using a stopped-flow method, a value of $k = 1.5 \times 10^6$ L mol⁻¹ s⁻¹ (at pH 7.0 and 25 °C) was obtained (12).

Effect of pH on Soluble Glucose Oxidase. Assays of glucose oxidase activity with oxygen as the acceptor are optimal at pH 5.5, whereas with other nonphysiological acceptors broad maxima around pH 7.5 are observed (4). The effect of pH on the electrochemically determined second-order rate constants for the ferrocene derivatives was investigated over the range pH 6.0–9.0. Figure 3 shows the pH dependence of the rate of reaction for glucose oxidase with ferricinium monocarboxylic acid. As with all ferrocenes, Table I, the rate constants are essentially independent of pH. This result was important in relation to the development of a glucose enzyme electrode since it indicates that its performance might be independent of pH over the physiologically relevant range. In plasma for example it is often the case that variations within the limits pH 6.7–8.2 occur. This can result from the addition of heparin, an anticoagulating agent which acts by binding calcium ions thus liberating protons, or through the loss of dissolved carbon dioxide.

Effect of Temperature on Soluble Glucose Oxidase. To determine the activation energy for the reaction between ferricinium ion and reduced glucose oxidase the variation in the catalytic current, as measured by cyclic voltammetry, was recorded in the range 5–55 °C. The temperature range was limited for two reasons: thermal inactivation of the enzyme occurs above 55 °C and the saturated calomel reference electrode is reliable only in the region –10 to +60 °C. Since the diffusion coefficient of the mediator is dependent upon temperature (13), an appropriate correction was made in the calculation of the rate constant. Figure 4 shows a plot of $\log_e k$ vs. $1/T(K)$ from which an activation energy of 50 KJ mol⁻¹ was calculated.

Glucose Enzyme Electrode Construction. Although all the ferrocene derivatives shown in Table I lead to the effective electrochemical oxidation of glucose via glucose oxidase at physiological pH and over a broad temperature range, other criteria are important in designing a practical enzyme electrode. The solubility of the reduced form of the ferrocene derivative in aqueous solution must be low to aid entrapment

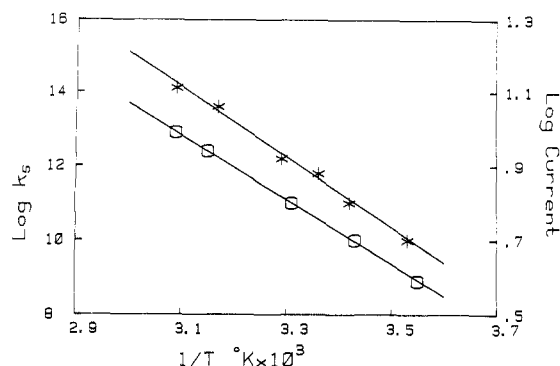


Figure 4. (*) Arrhenius plot showing the effect of temperature on the steady-state current measured at the enzyme electrode using glucose (10 mM). (O) Arrhenius plot for soluble glucose oxidase, conditions as for Figure 1b.

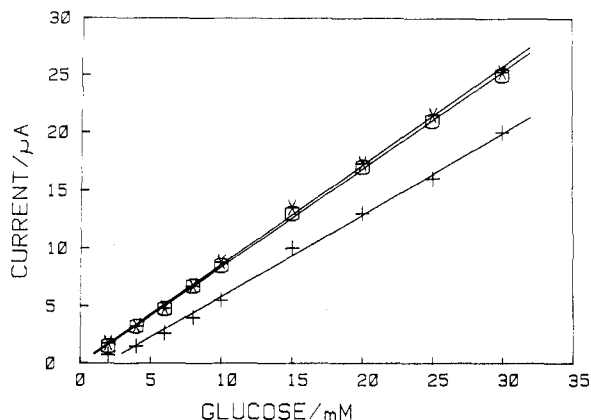


Figure 5. Calibration curve for glucose enzyme electrode in (*) argon, (O) air, and (+) oxygen-saturated buffer. Steady-state current was measured at 160 mV vs. SCE, pH 7.0, and 25 °C.

within the electrode: the oxidized form should be stable at physiological pH; the formal potential should be low to reduce interference from reduced compounds present in physiological samples. 1,1'-Dimethylferrocene proved the best compromise between the constraints imposed by these factors and was chosen for incorporation into the enzyme electrode. The prototype enzyme electrode was constructed by using graphite foil as the base sensor, onto which 1,1'-dimethylferrocene and glucose oxidase were immobilized as described above.

Glucose Enzyme Electrode Calibration. A glucose-dependent current is only realized when the electrode is poised sufficiently positive to generate the ferricinium ion. Figure 5 shows a typical steady-state current, $i_{ss} - i_0$, calibration curve for one of the electrodes, determined on gravimetrically prepared buffered glucose solutions which had been saturated with argon. The electrode was operated potentiostatically at +160 mV vs. SCE (60 mV positive of E° for 1,1'-dimethylferrocene). Figure 5 shows that the electrode gave a linear current response in the range 1–30 mM glucose. The background current in the electrolyte in the absence of glucose, $i_0 = 1.5 \mu\text{A}$, is subtracted from the data shown in Figure 5. Above 30 mM glucose, the current response of the electrode was nonlinear, finally becoming insensitive to additional amounts of glucose at 70 mM glucose.

The limit of the linear range is relevant to the analysis of undiluted plasma samples from diabetics, since changes in the patient's blood glucose concentration (between hypo- and hyper-glycemia) are generally within this range.

The current response of the electrode was investigated in both quiescent and stirred solutions of 8 mM glucose. Typically, the steady-state current increased by ca. 10% when changing from quiescent to rapid stirring. All subsequent experiments were performed in stirred solutions. In the linear

region of the calibration curve, the electrode showed a rapid response time reaching 95% of the steady-state current in 60–90 s.

The reproducibility of the construction protocol was investigated by measuring the steady-state current of 24 electrodes, in 8 mM glucose. The batch of prototype electrodes gave a mean current response, $i_{ss} - i_0$, of 7.9 μA with a standard deviation of 2.8 μA .

Effect of Oxygen on Enzyme Electrode. Figure 5 shows glucose calibration curves for the enzyme electrode in air-saturated and oxygen-saturated buffer. While some interference from oxygen is expected, the current decrease occurs as the base electrode was not poised sufficiently positive to reoxidize any hydrogen peroxide generated by the enzymatic reaction, eq 1. Operation at a potential sufficiently positive to reoxidize the hydrogen peroxide leads to increased interference from metabolites found in blood samples, particularly ascorbate. At normal blood glucose concentrations (4–8 mM), the decrease in current upon air saturation (equivalent to an oxygen concentration of ca. 260 μM), of previously argon-saturated buffer is ca. 4.0%. The concentration of oxygen in whole venous blood and plasma is usually less than 200 μM , suggesting that interference from oxygen when assaying authentic undiluted clinical samples may be no greater than that observed in air-saturated buffer. All subsequent experiments were performed in air-saturated buffer.

Effect of pH on Enzyme Electrode. The effect of pH on the response of the glucose electrode was investigated over the clinically relevant range. Figure 3 shows that the steady-state current of a typical glucose enzyme electrode is essentially independent of pH. This desirable feature of a non-pH dependent response contrasts with data obtained (14) for an oxygen-mediated glucose enzyme electrode which showed a marked pH dependence in the current response, a maximum occurring at pH 6.5. The difference in behavior may be due to the fact that no proton transfer is involved in the reduction of ferricinium ion.

Effect of Temperature on Enzyme Electrode. The effect of temperature on the enzyme electrode response was studied in the range 10–50 °C. Figure 4, shows the increase in steady-state current with increasing temperature, ca. 4.0% / °C.

Effect of Interfering Substances. The effect of substances which might interfere with the response of the electrode, either through direct electrode oxidation, reaction with the mediator, or inhibition of the enzyme, was examined. Analyses of buffered solutions containing 7 mM glucose, to which metabolites were added to give their normal blood concentrations, were carried out. Though L-ascorbate at a final concentration of 0.13 mM gave a mean increase in current of ca. 4.0%, addition of uric acid (0.20 mM), L-cysteine hydrochloride (0.08 mM), reduced glutathione (0.49 mM), sodium formate (7.35 mM), D-xylose (8.00 mM), α -galactose (7.77 mM), and α -mannose (7.77 mM) did not cause any observable interference to the electrode response to glucose.

Assay of Diabetic Plasma Samples. Experiments were carried out in order to test the performance of the ferrocene-based enzyme electrode against a standard clinical method of glucose analysis. Fresh plasma samples, taken from human diabetics, were first analyzed with a Yellow Springs Instruments glucose analyzer. This device incorporates glucose oxidase and is based on the detection of hydrogen peroxide in prediluted plasma. By this method, the respective concentrations of glucose in the batch of samples were found to encompass the range 2–20 mM. The samples, in an undiluted form, were then reanalyzed with the ferrocene-based glucose enzyme electrode using the three-compartment cell with a working volume of 1 mL. A correlation coefficient of

0.98 was calculated for the two methods, $n = 23$, $y = 0.951 \pm 0.18$. The background current measured by the ferrocene-based enzyme electrode in plasma, from which glucose was removed enzymatically (by the addition of hexokinase and ATP) was $i_0 = 1.2 \mu\text{A}$.

Assay of Diabetic Whole Blood Samples. The response of the enzyme electrode to whole blood vs. plasma was investigated. Samples were taken from diabetics and after addition of heparin were assayed as whole blood. The samples were then centrifuged and reassayed as plasma with the same electrode. A correlation coefficient for the two assays of 0.99 was calculated, $n = 10$, $y = 1.079 \pm 0.86$. In all assays the electrodes were calibrated with 0 mM and 10 mM glucose solutions.

CONCLUSIONS

We have shown that the ferrocene/ferricinium ion couple acts as an effective mediator between reduced glucose oxidase and a graphite electrode. The incorporation of 1,1'-dimethylferrocene into a graphite electrode upon which glucose oxidase has been immobilized provides a glucose sensor which has many of the features required for the analysis of clinical glucose samples. Its performance rests on a number of features: the rapid rate of electron transfer between reduced enzyme and the ferricinium ion; the good electrochemical properties of ferrocene; the low solubility of the ferrocene resulting in the effective confinement of the mediator to the electrode surface. The greater solubility of the ferricinium ion allows diffusion between the immobilized enzyme and the electrode surface. These features combine to give an electrode which permits rapid reproducible analysis of glucose in solution, whether plasma or whole blood, albeit after the treatment with an anticoagulating agent. Its minimal sensitivity to relevant oxygen concentrations and to change in pH mean that it may be possible to incorporate it in a device

with a wide range of applications.

ACKNOWLEDGMENT

We thank J. C. Pickup, Guy's Hospital, London, for helpful advice and the provision of human diabetic blood samples and G. S. Wilson for helpful discussions.

Registry No. Glucose, 50-99-7; glucose oxidase, 9001-37-0; oxygen, 7782-44-7; ferrocene, 102-54-5; 1,1'-dimethylferrocene, 1291-47-0; L-ascorbic acid, 50-81-7; vinylferrocene, 1271-51-8; carboxyferrocene, 1271-42-7; 1,1'-dicarboxyferrocene, 1293-87-4; [(dimethylamino)methyl]ferrocene, 1271-86-9.

LITERATURE CITED

- (1) Wingard, L. B. *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **1983**, *42*, 288.
- (2) Carr, P. W.; Bowers, L. D. "Immobilized enzymes in Analytical and Clinical Chemistry"; Wiley: New York, 1980; p 460.
- (3) Schlapfer, P.; Mindt, W.; Racine, P. *Clin. Chim. Acta* **1974**, *57*, 283.
- (4) Aleksandrovskii, Y. A.; Bezhikina, L. V.; Rodinov, Y. V. *Biokhimiya* **1981**, *46*, 708.
- (5) Marr, G.; Rockett, B. W. *J. Organomet. Chem.* **1982**, *227*, 373.
- (6) Szentrilmay, R.; Yeh, P.; Kuwana, T. *ACS Symp. Ser.* **1977**, *No. 38*, 143.
- (7) Higgins, I. J.; Hill, H. A. O.; Plotkin, E. V. British Patent Appl. 8 132 034, 1981.
- (8) Duke, R. F.; Weibel, M. K.; Page, D. S.; Bulgrin, V. G.; Luthy, J. J. *Am. Chem. Soc.* **1969**, *91*, 3904.
- (9) Bourdillon, C.; Borgeois, J.-P.; Thomas, D. *J. Am. Chem. Soc.* **1980**, *102*, 4231.
- (10) Nicholson, R. S.; Shain, I. *Anal. Chem.* **1964**, *36*, 706.
- (11) Hill, H. A. O.; Walton, N. J. *J. Am. Chem. Soc.* **1982**, *104*, 6515.
- (12) Weibel, M. K.; Bright, H. J. *J. Biol. Chem.* **1971**, *246*, 2734.
- (13) Lingane, J. J. *Anal. Chim. Acta* **1969**, *44*, 411.
- (14) Ianniello, R. M.; Yacynych, A. M. *Anal. Chem.* **1981**, *53*, 2090.

RECEIVED for review May 16, 1983. Accepted December 7, 1983. The authors thank Genetics International, Inc., for financial support. A.E.G.C thanks St. Hugh's College, Oxford, for the award of a British Petroleum Junior Research fellowship. A.P.F.T is a British Diabetic Association Senior Research Fellow. H.A.O.H is a member of the Oxford Enzyme Group.