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## Membrane-Covered, Rotated Disc Electrode

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**A rotated disc electrode that can be used to evaluate mass transport in membranes is described. In this design, both a disc electrode and reference electrode are mounted in a rotating shaft over which the membrane is placed. A transport model is given for solutes that diffuse through the membrane and undergo simple reaction at the electrode surface. With membrane in place, the diffusion current at low rotation rates approaches the theoretical (Levich) current that would be seen without membrane. At high rotation rates, however, the current is dependent on membrane permeability.**

In recent years, there has been a growing interest in the development of chemical-specific electrodes for monitoring chemical concentrations (1-4). Sensors for a variety of chemicals have been reported, and some have reached a relatively practical state of development for certain monitoring applications (e.g., 3, 4). One type of electrochemical sensor that has received some attention is the amperometric electrode, in which current is produced when electrochemically active species react at the electrode surface. In this type of sensor, selectivity for the chemical of interest is promoted by operating at a particular electrode potential (5) and by placing a semipermeable membrane between the electrode surface and the sample. In addition, immobilized catalysts can be incorporated in the membrane to extend the range of chemicals that can be monitored. In this way, certain chemicals for which selectivity is not readily obtainable on the basis of transport or electrode potential alone can be converted to other measurable species (6). A variety of potentially useful sensors based on these electrode designs is conceivable.

However, the amperometric-type sensor has had relatively limited application in biochemical monitoring situations because of an inadequate understanding of the complex physicochemical phenomena involved in its operation. An example is the lack of information about the electrochemical reaction. The mechanisms of reaction of most organics at solid electrodes have not been thoroughly studied. These processes at the electrode surface may include: formation of multiple intermediates, adsorption and electrode poisoning, electrochemical reactions in which the number of electrons exchanged is variable, or competitive reactions of other biochemicals that are not completely excluded by the membrane (5). Mass transfer is another example. Chemical transport in the external medium to the membrane surface must be quantitatively defined. The membrane must be designed to provide selectivity for the biochemical of interest and an acceptable response time. For diffusants that saturate the electrode

surface, membrane permeability must necessarily be limited in order to promote linearity of the current with concentration. Additionally, for sensors with catalytically active membranes, account must be taken of such properties as kinetics, catalyst loading, and catalyst inactivation.

A rotated disc electrode on which a membrane could be mounted would offer some unique advantages for sensor development. With such an electrode, a well-defined transport regime could be established that would be analogous to that of the classical rotated disc electrode first described by Levich (7), in which the disc surface is uniformly accessible to reactant and the diffusion boundary layer thickness precisely determined. At low rotation rates, the diffusion boundary layer would be relatively large, so that transport through solution would be a major contribution to total diffusional resistance. Under such conditions, the current would be proportional to the square root of rotation rate. At high rotation rates, however, where the diffusion boundary layer would be small, the resistance of the solution would be negligible making the diffusion current independent of rotation rate and determined by the properties of the membrane. Thus, transport in the membrane could be evaluated quantitatively without complications of external mass transfer. This feature would be particularly useful where the membrane is permeable enough to the solute that transport in membrane is comparable to transport in solution, or where carefully defined conditions are essential for distinguishing the effects of diffusion from chemical reaction in catalyst-containing membranes. This electrode could therefore be used for characterization of membranes that are to be used in nonrotating sensors or, as appropriate, employed directly as a sensor in the rotated configuration.

Two previous investigations with a membrane-covered, rotated disc electrode have been reported. Chien, Olson, and Sokoloski (8) employed fixed potential voltammetry and a carbon rotated disc electrode with a Millipore VC membrane to qualitatively study transport of *p*-nitrophenol. The other electrode was a stationary combination reference/counter electrode. These investigators found that, at steady state, the limiting current was approximately linear with the square root of rotation rate at low rotation rates (although unexplainedly lower than the Levich current), and independent of rotation rate at high rotation rates. No theoretical treatment was proposed to describe the effect of rotation rate on current. Determinations of membrane permeability were made at high rotation rate by injecting a known amount of the reactant and measuring the time to establish a new steady state. In a subsequent publication by the same authors (9), this system was used to determine the relative concentrations of bound

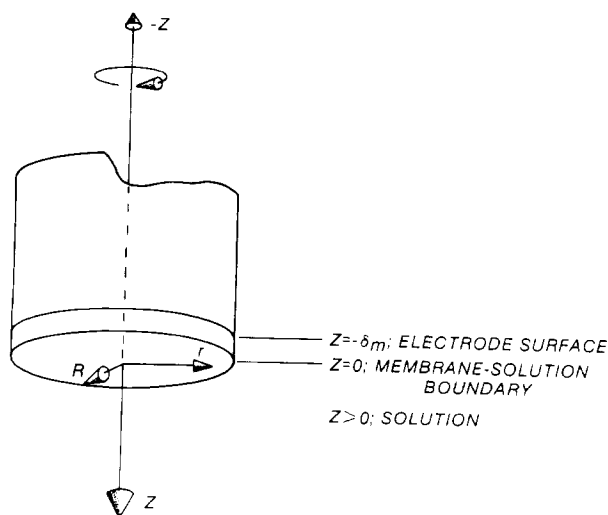


Figure 1. Coordinate system for mass transfer to a rotated, membrane-covered disc

and unbound drug in studies on drug interaction with macromolecules.

We are employing a similar type of rotated disc electrode to characterize transport in new membranes that may eventually be used in biochemical-specific sensors. In this communication, we discuss the construction of this novel electrode system and demonstrate its application to evaluate permeability of certain commercial hydrophilic membranes using solutes that undergo an uncomplicated reaction at the electrode surface. We develop an analytical model for this system and compare its predictions with experimental results. This limited study provides a basis for future work employing solutes of physiological significance and new membranes.

### THEORETICAL

We consider steady-state mass transfer of a solute that is consumed quantitatively at the surface of the disc electrode. As shown in Figure 1, the disc is covered by a homogeneous gel membrane of thickness  $\delta_m$  and rotated at a steady angular frequency  $\omega$ . The radius  $R$  of the disc is large compared to the diffusion boundary layer so that edge effects can be neglected and only variations of chemical concentration in the  $Z$ -direction need be considered. The liquid-filled space between the membrane and the electrode surface is assumed negligible. Employing a coordinate system similar to that of Levich (7), we let  $Z = 0$  at the membrane-solution boundary with  $Z$  positive in the downward direction. Assuming no convective flow within the membrane, the diffusion equation is

$$\frac{d^2 C_m}{dZ^2} = 0 \quad -\delta_m \leq Z \leq 0 \quad (1)$$

where  $C_m$  is the solute concentration in the membrane. In the solution, the convective diffusion equation is

$$v_z(Z) \frac{dC}{dZ} = D \frac{d^2 C}{dZ^2} \quad 0 \leq Z < \infty \quad (2)$$

where  $C$  is the solute concentration,  $D$  is the diffusion coefficient in solution, and  $v_z(Z)$  is the fluid velocity in the  $Z$ -direction. Far from the disc, the concentration of the reacting solute equals the bulk solute concentration  $C_B$

$$C = C_B \quad \text{as } Z \rightarrow \infty \quad (3)$$

and in the limit of complete diffusion control, the concentration at the electrode surface is zero

$$C = 0 \quad \text{at } Z = -\delta_m \quad (4)$$

Conservation of mass at the membrane-solution interface requires

$$D_m \left( \frac{dC_m}{dZ} \right) = D \left( \frac{dC}{dZ} \right) \quad \text{at } Z = 0 \quad (5)$$

where  $D_m$  is the molecular diffusion coefficient within the membrane. A partition coefficient  $\alpha$  is defined as the equilibrium ratio at the membrane-solution interface of solute concentration in the membrane divided by concentration in solution. Thus,

$$\frac{C_m}{C} = \alpha \quad \text{at } Z = 0 \quad (6)$$

We assume both  $D_m$  and  $\alpha$  to be constants. Integration of Equations 1 and 2 in their respective domains analogously to the method of Levich (7), and evaluation of the integration constants by the boundary conditions given in Equations 3 through 6 yield the following concentration profiles

$$C_m(Z) = \frac{DC_B}{D_m \delta_d} \left[ \frac{Z + \delta_m}{1 + \frac{D \delta_m}{\alpha D_m \delta_d}} \right] \quad (7)$$

and

$$C(Z) = C_B \left\{ \frac{1}{\delta_d} \left[ \frac{1}{1 + \frac{D \delta_m}{\alpha D_m \delta_d}} \right] \int_0^Z \exp \left[ \frac{1}{D} \int_0^\xi v_z(u) du \right] d\xi + \frac{1}{1 + \frac{\alpha D_m \delta_d}{\delta_m D}} \right\} \quad (8)$$

where  $\delta_d$  is the thickness of the diffusion boundary layer

$$\delta_d = 1.6 D^{1/3} \nu^{1/6} \omega^{-1/2} \quad (9)$$

with kinematic viscosity  $\nu$ , and  $v_z(Z)$  is obtained from the solution of the hydrodynamic problem (7). A first approximation of  $v_z(Z)$  used in the evaluation of the constants from the boundary conditions is

$$v_z(Z) \simeq -0.51 \omega^{3/2} \nu^{-1/6} Z^2 \quad (10)$$

The resulting diffusion current  $i_d$  can then be obtained

$$i_d = n \mathcal{F} \pi R^2 D_m \left( \frac{\partial C}{\partial Z} \right)_{Z = -\delta_m} = i_L \left[ \frac{1}{1 + \frac{P_s}{P_m}} \right] \quad (11)$$

where  $n$  is the number of electrons transferred,  $\mathcal{F}$  is the Faraday constant,  $i_L$  the current in the absence of a membrane as described by Levich (7)

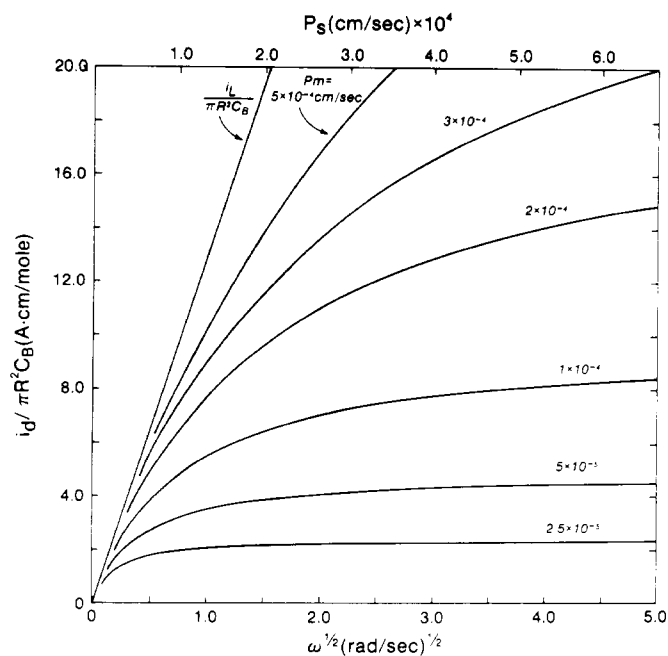
$$i_L = 0.62 n \mathcal{F} \pi R^2 D^{2/3} \nu^{-1/6} \omega^{1/2} C_B \quad (12)$$

and  $P_m$  and  $P_s$  are, respectively, the permeability of the membrane and solution,

$$P_m = \frac{\alpha D_m}{\delta_m} \quad (13)$$

$$P_s = \frac{D}{\delta_d} \quad (14)$$

The solution permeability is here well defined and is directly amenable to experimental manipulation by varying the rotation rate.



**Figure 2.** Predicted effects of mixed membrane and solution transport control with representative values for  $P_s$  and  $P_m$ . Current density normalized by concentration is plotted as a function of rotation rate. The values used in this figure are:  $D = 1 \times 10^{-6} \text{ cm}^2/\text{s}$ ,  $\nu = 1 \times 10^{-2} \text{ cm}^2/\text{s}$ ,  $n = 1$ , and  $P_m$  as shown.  $i_L / \pi R^2 C_B$  is the normalized current density without membrane

The anticipated effects of mixed membrane and solution transport control are shown in Figure 2. Here, the diffusion current density normalized by bulk concentration is given as a function of representative values for membrane permeability, solution permeability, and rotation rate. The normalized Levich current is linear with rotation rate and solution permeability. Membranes with different representative permeabilities are expected to demonstrate characteristic effects. At sufficiently low rotation rates such that  $P_s/P_m \ll 1$ , the diffusion current approaches the Levich current and in the limit the system is in solution transport control

$$\lim_{\omega^{1/2} \rightarrow 0} i_d = i_L \quad (15)$$

However, at high rotation rates where  $P_s/P_m \gg 1$ , the current becomes limited by membrane permeability and

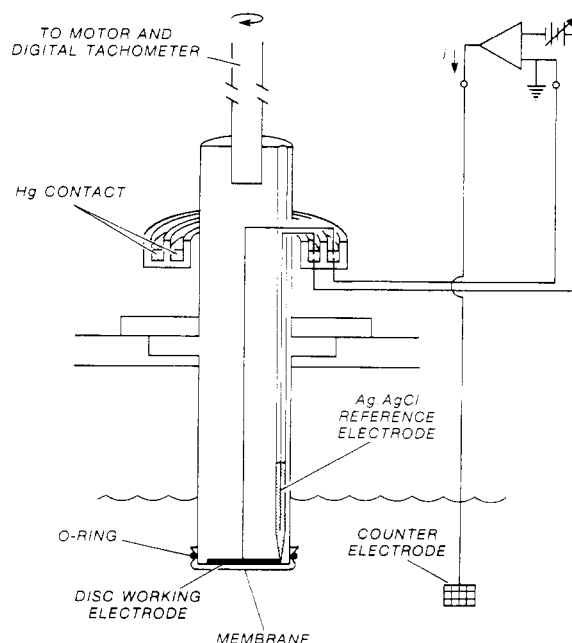
$$\lim_{\omega^{1/2} \rightarrow \infty} i_d = n F \pi R^2 P_m C_B \quad (16)$$

This effect becomes more apparent with decreasing membrane permeability as shown in Figure 2. Thus, a possible explanation might be afforded for the observation previously reported in the literature (8) of the effect of rotation rate on current density: slight inaccuracy in the measurement of diffusion current might lead to the incorrect conclusion that the current density is linear with rotation rate at low rotation rates but proportionally lower than the Levich current.

It should be emphasized that, in this derivation, membrane permeability appears only as a function of the product of the partition coefficient and the diffusion coefficient in the membrane, and that these two latter properties represent different mechanisms of transport control by the membrane. Nevertheless, if the partition coefficient can be determined by an independent measurement, the diffusion coefficient in the membrane can be found.

### ELECTRODE DESIGN

It may be difficult in practice to demonstrate these theoretical predictions because of certain technical considerations. For example: when employing conventional electrochemical



**Figure 3.** Design schematic of a membrane-covered, rotated disc electrode

instrumentation with the standard three-electrode configuration, the potential of the disc working electrode is usually determined by a Luggin capillary, located very close to the electrode surface to minimize  $iR$  drop in solution (10). With a hydrophilic membrane placed over the disc electrode, potential determinations would be of questionable accuracy because of possible  $iR$  drop in the membrane and accurate compensation would be difficult. For membranes of substantial electrical resistivity, reliable measurement by such an arrangement would be impossible.

This objection could be resolved by mounting both the disc and reference electrodes behind the membrane on the rotating shaft. This configuration would eliminate  $iR$  drop through the membrane, and any remaining  $iR$  drop in the thin layer of solution between the membrane and the electrode surface could be reduced by using high conductivity electrolyte or could be compensated by conventional methods. The compliance voltage of the counter electrode would be sufficient to pass current through membranes of low and moderate electrical resistivity. Distribution of the current between the disc working electrode and the external counter electrode could be made symmetrical by using high conductivity background electrolyte and properly positioning the counter electrode far enough away from the membrane. Thus, the membrane-covered disc electrode would be uniformly accessible to both reactant and background electrolyte, thereby ensuring a uniform potential distribution across the disc. This arrangement requires an independent measurement of membrane conductivity and the assurance that membrane resistance is always lower to electrolyte flux than to reactant transport.

We have constructed a new electrode system incorporating these features. As shown schematically in Figure 3, the working disc electrode and an internal silver-silver chloride reference electrode (ISSCE) are mounted in the rotated shaft over which the membrane is placed. The liquid junction of the reference electrode is a small opening in the inactive region of the disc near the edge of the working electrode. A background electrolyte of sufficiently high conductivity is used and the counter electrode can be positioned as appropriate for symmetrical current distribution. In this configuration, noise-free electrical contact established through mercury pools

is essential for maintenance of a stable reference electrode.

## EXPERIMENTAL

**Electrode Construction.** The electrode body was precision machined from a 1.9-cm diameter Lucite rod. A disc electrode of 0.80-cm diameter was machined from 0.5-mm thick platinum plate (Engelhard Industries, Los Angeles, Calif.). A length of platinum wire was spot welded to the center of the disc, passed through the central cavity of the electrode body, and attached to the ring fixture described below for electrical contact. The disc and wire were cemented into the electrode body, and the disc surface was polished to a mirror finish. The reference electrode was a chlorided silver wire with saturated KCl as the internal electrolyte. A linen fiber was inserted at the liquid junction of the reference electrode to minimize the rate of convective outflow of electrolyte.

A two-piece fixture was designed for the electrical contact. The lower, immobile piece contained two concentric channels with a precision-fitted stainless steel ring cemented into the bottom of each channel, and a wire from the back of the rings for connection to the potentiostat. A small volume of mercury was placed in each channel. The upper piece, which was fastened to the rotated shaft, contained two protruding stainless steel rings, each connected from above to the respective electrode and mated into the channels below. The shaft was fitted with Teflon bearings and mounted, together with the electrical contact fixture, onto the vessel lid. The electrode could be rotated freely while maintaining uninterrupted electrical contact.

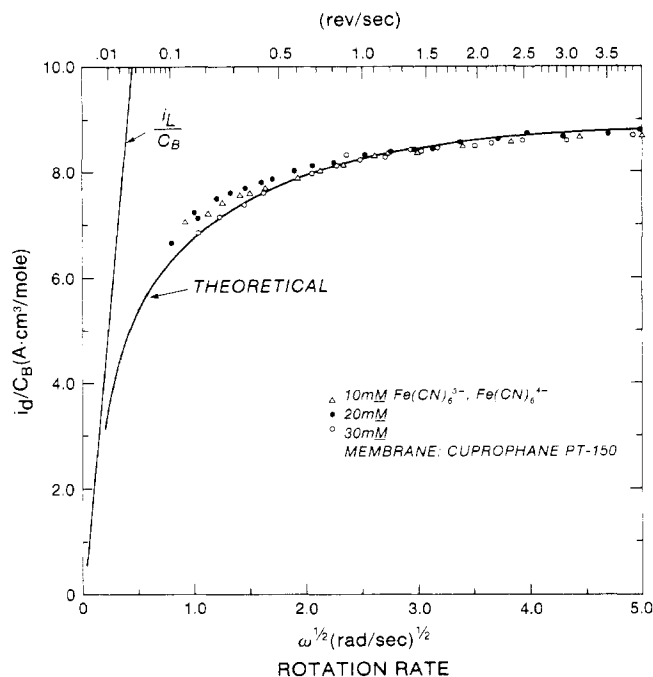
**Apparatus.** The shaft was coupled directly to a model M-062 stepper motor (Minarik Corp., Los Angeles, Calif.), and rotation rate was controlled by a corresponding model TBM regulator of the same manufacturer that had been modified to allow continuously variable rotation rate from 0 to 3000 rev/min as dictated by a voltage input. Rotation rate was monitored by a digital tachometer of our own construction employing a photo transistor sensor, and was found to be precisely maintainable over the entire range.

Voltammetric measurements were made with a model 173/178 potentiostat (Princeton Applied Research Corp., Princeton, N.J.). Other equipment included a model 176 programmer (Princeton Applied Research), a model ABA-26 slow sweep generator (Elsinct Ltd., Haifa, Israel) for producing linear voltage sweep, and an X-Y recorder (Hewlett-Packard, Palo Alto, Calif.). The sealed vessel was thermostated to  $30 \pm 0.5^\circ\text{C}$  and equilibrated with prepurified nitrogen gas. The two different background electrolyte solutions used in this study were 1 N KCl in  $10^{-2}$  M sodium phosphate buffer, pH 7.3, and 1 N NaOH. Solutions were prepared fresh daily from analyzed reagent grade chemicals and double distilled water.

A special conductivity cell was constructed for estimation of ionic resistance in the membrane. Two rectangular platinum plate electrodes, each of  $0.5\text{ cm}^2$  geometric area were spot welded to respective platinum leads, and each lead was sealed into the end of a 1-cm diameter glass tube. Two pieces of such tubing were joined through a ball and socket, ground glass joint in which the membrane could be clamped. The electrodes were platinized and provision made for filling the cell.

**Membrane.** Bemberg Cuprophane PT-150 was used in this study. This is a regenerated cellulosic membrane that contains about 65 vol % water at equilibrium. Other properties have been reported elsewhere (14). Membranes were discarded after 1-h use to minimize any effects of possible membrane degradation in the alkaline electrolyte.

**Procedure.** The electrode surface was prepared prior to each day's experiment by 10 to 20 repeated cyclic polarizations in 1 N  $\text{H}_2\text{SO}_4$  until a reproducible scan was obtained. The membrane was equilibrated in the background electrolyte and held in place taut across the disc surface with an O-ring. The limiting current was recorded at individual rotation rates, allowing adequate time after adjusting the rotation rate to attain steady state. The partition coefficient for  $\text{Fe}(\text{CN})_6^{3-}/\text{Fe}(\text{CN})_6^{4-}$  in Cuprophane was estimated by equilibrating a 30-inch square piece of membrane in background electrolyte solutions that contained  $10^{-2}$  M of each salt, then superficially drying to remove excess solution from the surface, and transferring into exactly 50 mL of the solute-free electrolyte solution. After reequilibration the concentration was



**Figure 4.** Normalized limiting current at a membrane-covered, rotated disc electrode.  $i_L/C_B$  is the normalized Levich current in the absence of membrane. The theoretical curve was calculated from Equation 11 using permeability data obtained in this study

determined from the limiting current. Thickness of the wet membrane was determined to within  $\pm 0.0005$  cm with a precision micrometer. Conductivity measurements of the background electrolyte were made by the conventional method (11) with and without the membrane in place.

## RESULTS AND DISCUSSION

The  $\text{Fe}(\text{CN})_6^{3-}/\text{Fe}(\text{CN})_6^{4-}$  couple was chosen for this study because it reacts in a simple, reversible electrochemical process and the diffusion properties in solution have been well documented (12, 13). Cuprophane was used because its permeability to a variety of solutes representing a spectrum of molecular weight is known (14). Although the transport of  $\text{Fe}(\text{CN})_6^{3-}/\text{Fe}(\text{CN})_6^{4-}$  in Cuprophane has not been studied, a correlation has been reported between molecular dimensions and membrane permeability that may be applicable to these solutes (14).

The properties of the electrode with no membrane were first studied using a Luggin capillary for the reference electrode. It was observed that at rotation rates greater than 2 rev/s the theoretical Levich current could be obtained to within 1% accuracy, with deviations becoming increasingly larger at lower rotation rates because of natural convection. The current was also recorded at much higher rotation rates and concentrations in order to reveal any possible kinetic limitation. The Levich current was obtained at all rotation rates and concentrations, confirming that the process was under complete diffusion control in the conditions of our experiment. The anticipated difference in magnitude of anodic and cathodic limiting currents due to the difference in diffusivities of ferro- and ferricyanide was also observed.

Results shown in Figure 4 were obtained with the membrane over the electrode. The data points represent the steady-state diffusion current  $i_d$  normalized by concentration for individual values of rotation rate. As predicted, the diffusion current approaches the theoretical normalized Levich current  $i_L/C_B$  at low rotation rates, and is limited by membrane at high rotation rates. It is difficult to demonstrate more definitive correlation with this membrane at low rotation rates because of natural convection. Although certain other

Table I. Mass Transfer Properties of Ferricyanide in Cuprophane ( $\delta_m = 25 \times 10^{-4}$  cm,  $T = 30^\circ\text{C}$ )

electrolyte	$R_m$ min/cm	$P_m$ , cm/s $\times 10^4$	$\alpha$ , cm <sup>3</sup> /cm <sup>3</sup>	$D$ , cm <sup>2</sup> /s $\times$ $10^7$	$D_{\text{eff}}$ , cm <sup>2</sup> /s $\times 10^7$	$D_m$ , cm <sup>2</sup> /s $\times 10^7$	$D_{\text{eff}}/D$
1 N NaOH	86.6	1.92	0.82	65.2 (12)	4.80	5.85	0.07
1 N KCl in 0.01 M sodium phosphate buffer, pH 7.3	99.7	1.67	0.74	63.2 (13)	4.18	5.65	0.07

commercial membranes are expected to be somewhat more permeable than Cuprophane (14), predictions based on Figure 2 suggest that these relatively small differences would still not permit a significant improvement in correlation at low rotation rates. The effect had a linear concentration dependence over the concentration range studied. The theoretical curve was calculated using Equation 11, with membrane permeability obtained as follows. The membrane molecular resistance  $R_m$  was taken from plots of  $C_B/i_L$  vs.  $1/\omega^{1/2}$  as the intercept at  $1/\omega^{1/2} = 0$ . The membrane permeability and  $D_{\text{eff}}$ , the effective diffusion coefficient within the membrane, were calculated from the formula

$$1/R_m = P_m = D_{\text{eff}}/\delta_m \quad (17)$$

With a value for the partition coefficient obtained from an independent measurement, the true diffusion coefficient within the membrane was estimated from the relation

$$D_{\text{eff}} = \alpha D_m \quad (18)$$

Experimentally obtained values for these parameters are summarized in Table I. The method used here for determination of  $\alpha$  may, in principle, be subject to relatively large experimental error. Thus, the values reported for  $\alpha$  and  $D_m$ , while reasonable, must be considered as only approximate. Previous studies (14) based on solutes of comparable molecular dimensions suggest an anticipated value of 0.07 to 0.09 for the ratio  $D_{\text{eff}}/D$ . The agreement of our data suggests that no significant artifacts were introduced by either the membrane mounting procedure or possible membrane degradation in the alkaline solution.

Although we have studied a number of commercial hydrophilic membranes by this technique, the permeability of most has not been adequately characterized by conventional methods to allow confident comparison of results. Nevertheless, in every case, the limiting current showed the characteristic response with rotation rate, and membrane permeability for these solutes was readily obtainable. It should be noted that relative membrane permeability as judged by one solute may not reflect permeability properties in general. The latter should be estimated by employing a variety of solutes representing a range of molecular sizes.

Some important technical points must be remembered when working with the membrane-electrode system. First, there may be a lag time due to diffusion in the membrane. Time must be allowed for the current to reach steady state after a change in rotation rate, concentration, or electrode potential. As an illustration, Figure 5 shows the effect of electrode scan rate on current density in the presence of membrane. In this experiment rotation rate was held constant at 3 rads/s and the electrode potential scanned linearly in one direction at successive scan rates. At the lowest scan rate, current density proceeds monotonically from the anodic ferrocyanide oxidation current to the cathodic ferricyanide reduction current. This curve closely approximates the steady-state current density that would be obtained upon application of fixed, discrete electrode potentials in this potential range. With increasing scan rates, however, the current density passes through a

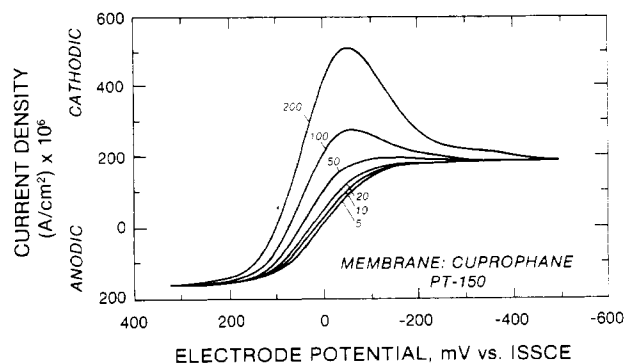


Figure 5. Effect of electrode potential scan rate on the reduction of ferricyanide at the membrane-covered, rotated disc electrode. Curves are shown for a rotation rate of 3 rads/s and scan rates of 5, 10, 20, 50, 100, and 200 mV/min. The arrow indicates the direction of scan. The solution was 10 mM  $\text{Fe}(\text{CN})_6^{3-}/\text{Fe}(\text{CN})_6^{4-}$  in 1 N NaOH.

prominent maximum before returning to the cathodic plateau. Equivalent anodic peaks can be demonstrated with linear potential scans in the opposite direction. This transient phenomenon is analogous to the familiar peaks formed with linear scan voltammetry using conventional, nonrotated electrodes. Here, the solute immediately adjacent to the electrode is vigorously consumed when the electrode potential becomes sufficiently cathodic, thus momentarily producing a high current density. As the solute in this region is depleted, the diffusion distance for subsequent reactant becomes larger and the current density falls off to the cathodic plateau value. This process occurs more rapidly with increasing scan rates resulting in greater peak current densities and, for a given scan rate, peak formation is promoted by greater diffusional resistance such as that provided by the membrane. Thus, studies done under the same conditions as in Figure 5, but without membrane, demonstrated no peaks or effect of scan rate, even at the highest scan rates. Thus, the membrane can cause dramatic time-dependent effects.

A second practical consideration that can cause considerable frustration of the investigator if ignored, is the likelihood of forming bubbles under the membrane by gas evolution. While this problem can be ameliorated by operating only at intermediate potentials, it is nevertheless significant because it may limit the use of certain electrochemical pulsing pretreatments for activation of the electrode surface (5).

The nature of the electrochemical reaction must also be considered. In this basic model, we have limited our treatment to simple, diffusion-limited processes, involving no intermediates or side reactions. Few solutes of analytical interest are expected to be similarly uncomplicated. For most gases and low molecular weight organics, the electrochemical processes have not yet been well defined or, where information is available, the reactions are often complex. The membrane may alter these processes in many ways, in addition to the effect on solute transport. For example, the rate of transport of intermediates or products from the electrode may be reduced. This could result in a variable number of electrons exchanged, enhanced side reactions, or electrode poisoning.

Thus, the effect of membrane on the electrochemical process must be carefully evaluated for each solute.

This study prepares the way for characterization of transport of physiologic solutes in membranes and for evaluation of the potential usefulness of new membranes for chemical-specific sensors.

#### ACKNOWLEDGMENT

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## Amperometric Enzymatic Determination of Total Cholesterol in Human Serum with Tubular Carbon Electrodes

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**An amperometric enzymatic assay for total cholesterol in human serum has been developed. The assay utilizes cholesterol esterase, cholesterol oxidase, and peroxidase. H<sub>2</sub>O<sub>2</sub> produced by the oxidase is coupled via peroxidase to produce K<sub>3</sub>Fe(CN)<sub>6</sub>, which is measured at a tubular carbon electrode. The reactions go all the way to completion and the K<sub>3</sub>Fe(CN)<sub>6</sub> formed can be stoichiometrically related to total cholesterol concentration. Therefore, the assay can be calibrated with standard K<sub>3</sub>Fe(CN)<sub>6</sub>. (The reaction time is minimized by running the esterase reaction at 60 °C.) The method shows very good reproducibility, accuracy, and sensitivity. Bilirubin and hemoglobin show no interference and ascorbic acid up to 4 mg/dL did not interfere with the assay. Results obtained by the amperometric method show excellent agreement (correlation coefficient = 0.995) with those obtained by the Leffler method.**

The analysis of total cholesterol (free cholesterol plus cholesterol esters) in human serum is a routine clinical diagnostic test that has been somewhat of a problem for clinical chemists (1, 2). In traditional methods, cholesterol is treated to produce colored reaction products which are measured spectrophotometrically. The most common reactions are the Liebermann-Burchard reaction, the iron salt-sulfuric acid reaction (Kiliani or Zak), and the *p*-toluene sulfonic acid reactions. Color development is usually preceded by extraction and saponification steps. These methods have the disadvantages that the color reactions are very dependent on experimental conditions, other substances interfere with cholesterol, and a fairly large sample volume is required (1).

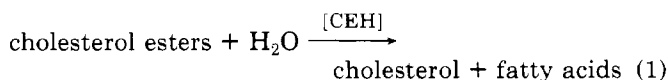
More recently, Flegg (3) and Richmond (4) developed enzymatic methods for analyzing cholesterol utilizing cholesterol oxidase isolated from soil bacteria. Since that time, many enzymatic methods have appeared in the literature (5-17). All these enzymatic cholesterol assays were claimed to be more specific, sensitive, precise, and simple than the direct chemical ones.

A variety of analytical detection methods have been used for the enzymatic methods. These include spectrophotometry (5-12), fluorometry (13), potentiometry (17), and amperometry (14-16). The enzymatic spectrophotometric methods seemed to have some problems with bilirubin and ascorbic acid interferences, and most of the enzymatic procedures required calibration with cholesterol standards which sometimes have questionable reliability.

This paper describes a new amperometric enzymatic method for the analysis of total serum cholesterol that is highly sensitive, relatively free from interferences, and which does not require a cholesterol standard for calibration purposes.

#### THEORY

The assay for total cholesterol utilizes the following reaction scheme. When total serum cholesterol is measured, the cholesterol present in the form of esters must be hydrolyzed since cholesterol esters are not suitable substrates for the enzyme cholesterol oxidase. The two initial reactions are written as follows:



where CEH is the enzyme cholesterol ester hydrolase (CEH: EC 3.1.1.13) and CO is the enzyme cholesterol oxidase (CO:

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