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Steady-State and Nonsteady-State Transport through Membranes Using Rotating-Disk Electrode Polarography: Description and Properties of a Rapid Response New Technique

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Abstract □ A new rapid response technique was developed to measure both steady-state and nonsteady-state flux (mass transport rate, moles per second) through membranes. The system utilizes a rotating-disk electrode, which is covered with a porous membrane and connected to a polarographic module. The rotating-disk configuration provides a uniform flux density over the entire surface of the membrane. Since flux is directly measured as current, there is no need to construct a concentration *versus* time plot to obtain steady-state permeability and there is no need to rely on lag time to measure effective diffusivity. The use of lag times for this purpose has recently been shown to introduce significant errors in estimating diffusivity. In this technique, the membrane is placed on the surface of the electrode. In the receiving compartment, response to flux is so rapid that nonsteady-state diffusion may be characterized. Moreover, it is possible to determine steady-state flux in the same experimental trial in short time periods. The technique and its use are described and theoretically explained through basic electrochemical and diffusion principles. The reproducibility achieved in transport measurements is seen to be quite good.

Keyphrases □ Transport through membranes—methodology, equations for measuring steady-state and nonsteady-state flux using rotating-disk polarography □ Membranes, characterization of transport—methodology, equations for measuring steady-state and nonsteady-state flux using rotating-disk polarography □ Polarography, rotating disk—used to measure steady-state and nonsteady-state transport through membranes, theory, equations □ Rotating-disk polarography—used to measure steady-state and nonsteady-state transport through membranes, theory, equations

Transport processes occurring across a membrane barrier separating a solution phase from another solution or solid phase continue to be of great interest to pharmaceutical scientists. Among these interests are: (*a*) a better understanding of the mechanisms of transport in order to develop new membrane systems having controlled drug-release properties, and (*b*) the characterization, through the use of models, of the probable mechanisms and behavior of complex biomembranes in terms of physicochemical principles.

There have been numerous studies dealing with the measurement of mass transport of drug and drug-like molecules across a large variety of membranes or barriers, ranging from model membranes to biological membranes themselves (1–9). In most instances (1–6, 9), the actual measurement of membrane permeation has been conducted along rather classical lines where the barrier separates two compartments of solution, the contents of which are sampled as a function of time. Light absorption is the measurement technique most commonly used. The usual time course of measurement of mass transport ranges from several hours to over a day, primarily because the ratio of compartment volume to barrier surface area is large. Even in recently developed systems (7, 8) where sampling is not required and where the volume to area ratio is low, the detector is positioned away from the immediate surface of the membrane barrier in the receiving compartment, causing a time lag in transport measurement. The technique described in this paper does not suffer from this disadvantage.

It is apparent from the literature that the design of a suitable diffusion measurement system is complex. Variables such as membrane integrity, area, and thickness; compartment volumes and concentrations; reproducibility of sampling; stirring; and temperature must be controlled. Many diffusional cell prototypes may be found (1–6, 9–16) and, considering that most were designed for similar purposes, their diversity is remarkable. Flynn and Smith (7) classified these cell systems and analyzed their advantages and disadvantages.

One prime concern of making permeability measurements is the ability to determine flux (mass transport

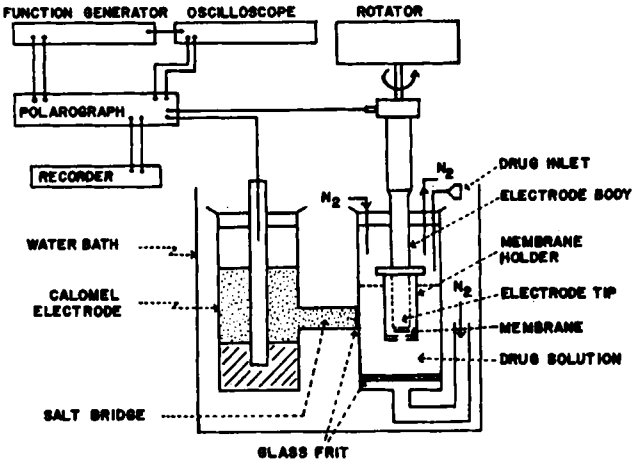

Figure 1—*Schematic representation of the electrochemical system used to measure drug transport.*

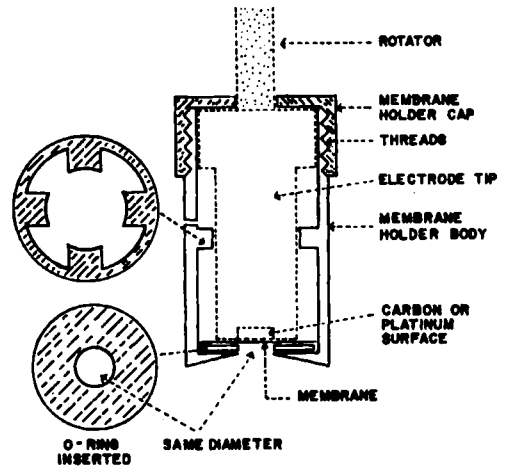

Figure 2—*Schematic representation of the membrane holder assembly showing its relation to the electrode tip. The electrode tip can be removed from the rotator. The cap is placed over the electrode tip with a membrane in place and the body is then attached.*

rate, moles per second) reliably in as short a time as possible. With this in mind, a relatively simple and easily assembled transport cell was constructed. A membrane is positioned smoothly on the immediate surface of a rotating-disk electrode. The flux or transport of material through the membrane is proportional to the current measured polarographically at a constant potential. The response is very rapid, permitting direct measurement of both the initial nonsteady-state and the steady-state membrane transport. The sensitivity of a diffusional cell is proportional to the available diffusional area divided by the solution volume of the receiving compartment (8). The receiving compartment in the present system has a negligible volume because the detector is positioned directly on the membrane surface. Therefore, the detector signal is directly proportional to the flux across the membrane rather than to the receiving compartment concentration.

The high speed rotating-disk configuration has the advantage of uniform, controlled flux to the outside membrane interface. At high rotation speed, the solution diffusion layer thickness may become negligible with respect to membrane thickness. In any case, the thickness of the convective diffusion layer on the surface of the membrane may be controlled and calculated. The concentration at the electrode surface can be set to zero by setting the voltage on the diffusion current plateau. The high sensitivity, rapid response, and nonsampling method utilized permits the observation of transient phenomena which may be significant to the mechanism of membrane transport but which may be overlooked using slower conventional methodology.

The lag time technique of Daynes (17) and Barrer (18) has been popularly applied in conventional mass transport systems to calculate the diffusivity of drug species. Steady-state transport should be calculated from data taken after two lag times; otherwise, significant errors may result in determining the extrapolated lag time and in calculating diffusivity.

Siegel and Coughlin (19) recently made a theoretical analysis of such calculations and they concluded that the relative errors in lag time calculations can be as much as five times larger than the errors obtained from calculations based on the slope of steady-state permeability. Their analysis suggests that the error in diffusivity will always be several times larger than the error in permeability when such techniques are employed. In the method introduced here, diffusivity can be estimated either directly from steady-state permeability or from kinetic analysis of nonsteady-state transport without the use of lag time estimates.

Although extensive experimental and theoretical studies have been made on the permeation of gases through various materials, diffusion in polymer membranes has received relatively limited attention, particularly with regard to the short-time dynamic nature of drug permeation across barriers. In general, two different phenomenological mechanisms are met in the flow of penetrant species through thin membranes. These are molecular transport involving partitioning of the penetrant into the membrane structure, followed by molecular diffusion across it, and passage of penetrant molecules through membrane pore channels filled with solvent. Transport through a nonporous barrier depends on the relative solubility of the penetrant in the barrier as compared to its solubility in the donor and receiving compartments.

The new technique described in this paper provides a direct measurement of flux both at steady-state and nonsteady-state diffusion conditions.

EXPERIMENTAL

Apparatus—The system used in these studies is shown in Fig. 1. A polarographic H-cell is immersed in a constant-temperature bath[1] at 30 ± 0.1°. The left side of the cell contains a saturated calomel reference electrode (S.C.E.) connected to the working half of the cell (on the right) *via* a salt bridge. The working cell contains 10–20 ml. of test solution. The electrode is immersed in this solution and either rotated or held stationary. The working and reference electrodes are connected to a polarograph[2]. Current is measured either with a strip-chart recorder[3] or an oscilloscope[4]. The recorder is used for time measurements greater than 0.5 sec., and the oscilloscope is used for shorter times. For short-time

[1] Sargent Thermonitor, model ST.
[2] Heath polarographic module EVA-19-2.
[3] Varian model G-14.
[4] Hewlett Packard model 141A.

measurements, the potential from the polarograph is controlled by a function generator[6]. The test solution is deaerated with nitrogen gas, and a nitrogen atmosphere is maintained above the solution during measurements. Drug is introduced into the working side of the electrode by injecting a small volume (0.2–1.0 ml.) of a concentrated predeaerated drug solution *via* the drug inlet from a calibrated microsyringe.

A membrane sheet is positioned on the electrode surface using a cell holder made of Teflon (Fig. 2). A small hole is drilled on the upper edge of the holder to balance pressures across the membrane and to minimize hydrodynamic transport through the covering membrane. The entire assembly is rotated at a constant speed with a rotating electrode assembly[6]. Speeds ranging from 0.6 to 6000 r.p.m. are possible with this assembly. Two types of electrode tips were used: a platinum tip[7], which was coated with mercury according to the method of Ramaley *et al.* (20), and a carbon electrode[8]. The carbon electrode permits the use of a wide potential range, has a low residual or background current, provides good reproducibility, and requires little pretreatment. Both electrodes have a smooth surface. The membrane is placed directly on the electrode surface as shown in Fig. 2.

Materials—The supporting electrolyte used in the studies was 0.2 *M* acetate buffer, pH 4.0. The pH selected assures that the electroactive species, *p*-nitrophenol[9], is totally undissociated (pKa 7.12) and that the buffer is of sufficient concentration to obviate pH changes at the electrode surface due to the consumption of protons. *p*-Nitrophenol was found to be 99% pure by potentiometric titration.

Two membranes were used in the study: (*a*) Millipore membranes[10] of cellulose acetate–cellulose nitrate mixtures and (*b*) dialysis membranes[11] with a pore radius of 24 Å and a dry thickness of 25 μ.

Method—All of the membranes were compatible with the solutions used and were presoaked in the buffer system overnight in a refrigerator before use.

The general procedure used for the measurement of steady-state and nonsteady-state transport of *p*-nitrophenol through the various membranes was as follows. Seventeen milliliters of supporting electrolyte was placed in the working compartment of the polarographic H-cell and deaerated for 5 min. with nitrogen gas. A solution of *p*-nitrophenol was separately prepared in the same supporting electrolyte and deaerated for 3 min. The transport cell containing the membrane was assembled (Fig. 2) and placed on the electrode tip. The electrode assembly was placed in the solution and rotated at a fixed speed until equilibration occurred. The current-potential curves for *p*-nitrophenol were determined, and the results are listed in Table I. A constant potential of −1.0 v. *versus* S.C.E. was applied, and the steady-state residual current was measured. One milliliter of the concentrated *p*-nitrophenol solution was rapidly injected into the cell compartment. The current increased with time until it reached a steady-state value. After steady state was reached, an additional 0.4 ml. of solution was introduced, giving rise to a new steady-state current. The incremental procedure was continued until a suitable calibration curve was obtained.

RESULTS AND DISCUSSION

The current–time relation predicted for a plane electrode in a quiet (unstirred) solution is given by the equation:

$$i = \frac{nFAD^{1/2}C}{\sqrt{\pi t}} \qquad \text{(Eq. 1)}$$

where n is the number of electrons transferred (4 for *p*-nitrophenol at pH 4), F is the Faraday constant (96,500 coulombs per equivalent), A is the electrode surface area in square centimeters, D is the diffusion coefficient (9.18×10^{-6} cm.^{2} sec.$^{-1}$ for *p*-nitrophenol), C is the bulk concentration of electroactive species in millimoles per liter, t is the time after start of electrolysis in seconds, and i is the faradaic current in microamperes. A plot of i *versus* $(t)^{-1/2}$ should be a straight line with slope $nFAD^{1/2}C(\pi)^{-1/2}$. When a membrane covers the electrode surface, the effective area of the electrode is reduced to an amount equal to the porosity of the membrane. Figure 3 shows the results for the reduction of *p*-nitrophenol with and without a Millipore membrane on the electrode surface. The experimental slope without a membrane was 2.89×10^3 μa. sec.$^{1/2}$ M^{-1} compared to a theoretical value of 2.87×10^3 (A = 0.232 cm.^{2}), which is excellent agreement. The experimental slope with the Millipore membrane was 2.23×10^3 μa. sec.$^{1/2}$ M^{-1} compared to a theoretical value calculated to be 2.13×10^3, based on the manufacturer's specifications of 74% porosity, which again is in good agreement.

If a disk electrode is placed into solution and rotated at a constant angular velocity, the mass transport of electroactive material to the electrode surface by means of convective diffusion is much greater than obtained by linear diffusion to a stationary electrode of equal area (22–25). A relationship describing the thickness of the convective diffusion layer, δ_D, at a rotating-disk electrode was derived by Levich (26–27):

$$\delta_D = 1.62D^{1/3}\nu^{1/6}\omega^{-1/2} \qquad \text{(Eq. 2)}$$

where D is again the diffusion coefficient, ν is the kinematic viscosity, and ω is the angular rotation in radians per second. The rotating-disk configuration is the only convective diffusion geometry where flux, *i.e.*, δ_D, is a constant over the entire surface. The

Table I—Decomposition and Half-Wave Potential for *p*-Nitrophenol in Acetate Buffer[a]

Electrode	Decomposition Potential, v.	Half-Wave Potential, v.
Mercury-coated platinum disk[b]	−0.48	−0.63
Carbon disk[b]	−0.63	−0.81
Dropping mercury[c]	−0.52	−0.68

[a] The *p*-nitrophenol concentration is 4×10^{-5} *M*. The buffer is 0.2 *M* and pH 4.0. [b] The electrode was rotated at 3000 r.p.m. [c] From *Reference 21.*

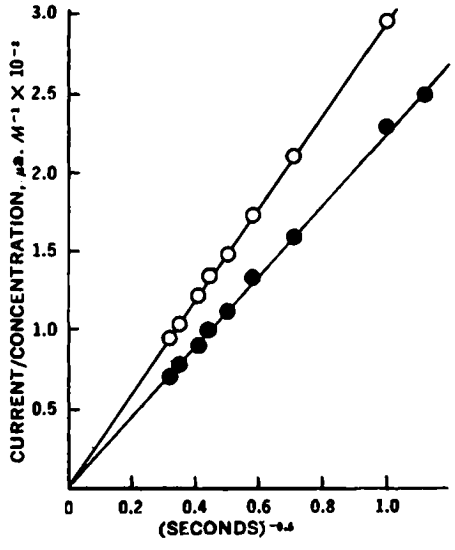

Figure 3—*Relation between current divided by concentration of* p-*nitrophenol and the reciprocal of the square root of time. The carbon disk electrode is not rotating. Key:* ○, *without a covering membrane, concentration of* p-*nitrophenol* = 2×10^{-3} M, *slope* = 2.89×10^3 μa. sec.$^{1/2}$ M^{-1} (*theoretical slope* 2.87×10^3); *and* ●, *with a covering Millipore membrane, type VC (0.1-μ pore, porosity 74%), concentration of* p-*nitrophenol* = 5×10^{-3} M, *slope* = 2.23×10^3 μa. sec.$^{1/2}$ M^{-1} (*theoretical slope* 2.13×10^3).

[6] Wavetec model 112.
[7] Beckman Drive Unit 188501W. Electrode Body 188551W.
[8] Beckman No. 39086.
[9] Beckman No. 39084.
[9] Eastman.
[10] Millipore Filter Co. [Various pore sizes are available (10 nm.–8 μ) but all types have the same approximate thickness (150 μ) and porosity (80%).]
[11] Union Carbide.

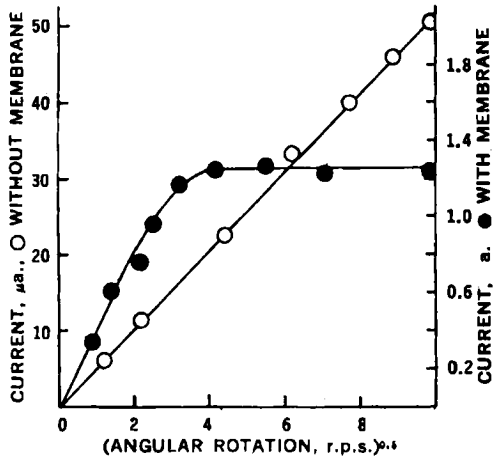

Figure 4—*Effect of the square root of angular rotation (r.p.s.) on the steady-state current of* p-*nitrophenol* (1×10^{-4} M). *Key:* O, *left ordinate, without covering membrane; and* ●, *right ordinate, with a covering dialysis membrane.*

diffusion-limited current at a rotating-disk electrode is calculated as:

$$i = \frac{nFADC}{\delta_D} \qquad \text{(Eq. 3)}$$

or:

$$i = 0.62\, nFAD^{2/3}\, \omega^{1/2}\, \nu^{-1/6}\, C \qquad \text{(Eq. 4)}$$

where the terms are as previously described. The behavior of the rotating-disk electrodes used in these studies was in agreement with theory.

Table II—Determination of the Porosity of Millipore Membranes from Steady-State and Nonsteady-State Transport of *p*-Nitrophenol at pH 4, 30°

Millipore Type	$\pi^2 D/\delta^2$, sec.$^{-1a}$	$P_m \times 10^{-4}$, μa. M^{-1b}	Porosity	
			Measured	Reported[c]
SC	0.333	3.89	0.78	0.74
SM	0.222	3.26	0.80	0.84
SS	0.119	2.52	0.85	0.83
RA	0.0952	2.18	0.82	0.82
GS	0.0054	0.50	0.79	0.75
VC	0.0042	0.44	0.79	0.74
VM	0.0040	0.42	0.77	0.72

[a] Slope of plots of $\ln (i_{ss} - i_t)$ *versus* t. [b] Slope of plots of i *versus* concentration. [c] From Millipore Catalog MF-84.

The current obtained at a membrane-covered rotating-disk electrode is controlled by both the permeability of the membrane and the convective diffusion layer at the outer surface of the membrane. Figure 4 shows a typical plot of current *versus* electrode rotation speed for both a bare and a membrane-covered electrode. When the rotational velocity of the electrode becomes sufficiently large, the transport of material to the electrode surface is controlled primarily by diffusion across the membrane. The measured current, therefore, reaches a plateau, becomes independent of increased rotational velocity, and is directly proportional to the diffusion-controlled transport or flux of material across the membrane.

A typical result obtained in measuring transport through a membrane covering a rapidly rotating disk electrode (3000 r.p.m.) is presented in Fig. 5. *p*-Nitrophenol is rapidly injected into the cell at time zero after allowing the system to come to equilibrium. After a short lag time, the current increases with time, eventually reaching a steady-state flux as defined by Eq. 3, where δ_D becomes the sum of the thickness of the stagnant layer and the effective membrane path. At the steady-state condition, a subsequent addition of *p*-nitrophenol from a concentrated deaerated solution leads to a new

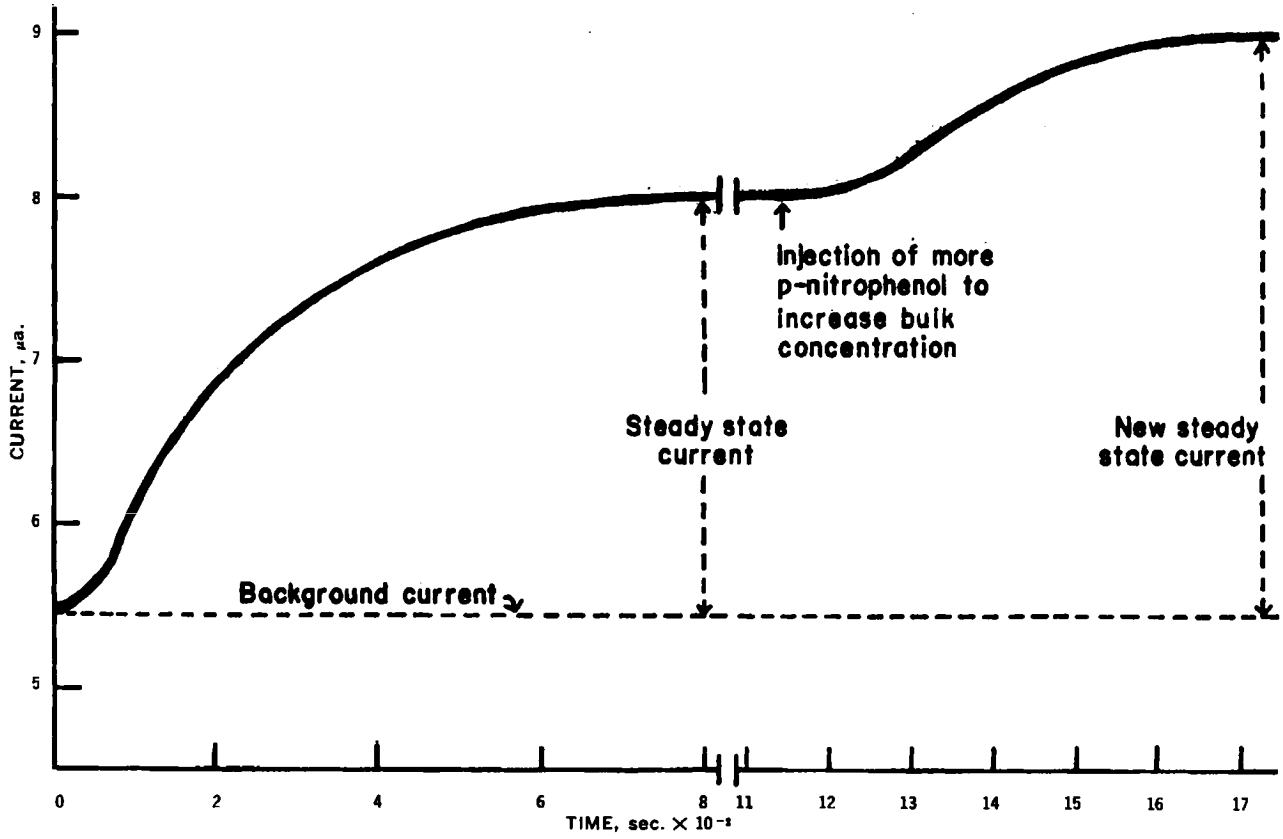

Figure 5—*Relation between current and time in the transport of* p-*nitrophenol through a Millipore membrane, type GS, where successive amounts of compound are added.*

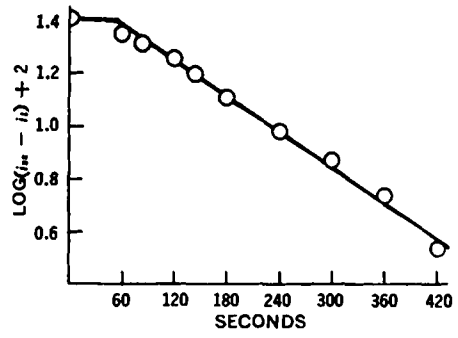

Figure 6—*Relation between the log (steady-state current − current at time t) and the time for p-nitrophenol diffusing through a Millipore membrane, type GS.*

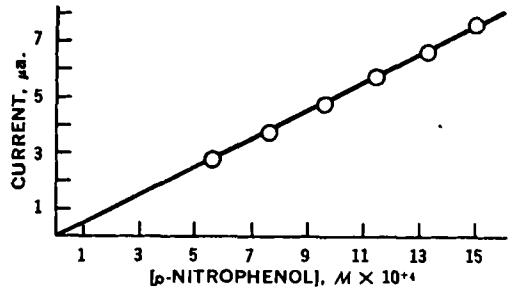

Figure 7—*Relation between steady-state current and concentration of p-nitrophenol at a rotating carbon-disk electrode (3000 r.p.m.) after successive additions of compound. The electrode is covered with a Millipore membrane, type GS.*

steady-state flux. This procedure may be repeated several times to give additional steady-state currents.

Membrane permeability, P_m, calculated from the steady-state current:

$$P_m = \frac{i_{ss}}{nFC} \qquad \text{(Eq. 5)}$$

is determined by two parameters, porosity and effective membrane thickness, which includes tortuosity effects and the stagnant solution layer on the surface of the membrane. To evaluate porosity and effective membrane thickness, additional experimental data are needed. In the present technique the additional data are derived from the time-dependent (nonsteady-state) flux as presented in Fig. 5. Several investigators have derived expressions describing the flux through a membrane as a function of time following a step or rapid change in concentration on one side of the membrane (28–30). In particular, Bowers and Wilson (31) derived an expression relating current to time for a membrane-covered electrode following a step jump in concentration of electroactive species in the stirred solution outside the membrane:

$$i_t = \frac{nFADC}{\delta}\left[1 + 2\sum_{m=1}^{\infty}(-1)^m \exp(-m^2\pi^2)t/\delta^2\right] \qquad \text{(Eq. 6)}$$

where i_t is the current at time t, δ is the effective diffusional thickness, and the other terms are as previously described. Although the electrode type and solution hydrodynamics are different in the Bowers–Wilson experiment, the boundary conditions for transport are the same and the equation is thus valid for a membrane-covered disk electrode when rotational velocity is rapid and constant. Typical experimental values indicate that the summation terms of Eq. 6 converge very rapidly. For example, if it is assumed that $\pi D/\delta^2 = 1$ sec.$^{-1}$, at $t = 1$ sec. an error of 6% would occur in neglecting terms where $m > 1$, and at $t = 2$ sec. the error is less than 0.1%. Neglecting summation terms of $m > 1$ and substituting Eq. 3 into Eq. 6 give:

$$i_t = i_{ss}[1 - 2\exp(-\pi^2 Dt/\delta^2)] \qquad \text{(Eq. 7)}$$

Thus, the value for the effective diffusional path, δ, can be obtained from the slope of a plot of ln $(i_{ss} - i_t)$ *versus* t where the slope is $\pi^2 D/\delta^2$. A typical semilogarithmic plot is shown in Fig. 6. By using the kinetically determined value of δ, it is possible to calculate the porosity, ϵ, of the membrane through the use of the experimentally determined permeability, P_m:

$$P_m = \frac{i_{ss}}{nFC} = \frac{DA}{\delta}\epsilon \qquad \text{(Eq. 8)}$$

Figure 7 shows a typical experimental result demonstrating the applicability of Eq. 8. The slopes of plots of current *versus* concentration give P_m. Thus, by knowing n, F, D, and A (the area of the bare electrode, 0.232 cm.^{2}), the porosity of the membrane, ϵ, may be calculated.

The experimental results obtained for a series of Millipore membranes are given in Table II. The calculated values for porosity are in excellent agreement with the reported values for the Millipore membranes. It is thought that the methodology described is well suited for studying both the steady-state and transient (nonsteady-state) characteristics of membrane transport. From Eqs. 3 and 4, it can be seen that the steady-state current is directly proportional to bulk solution concentration. If a dialysis membrane is placed on the surface of an electrode, macromolecules and molecules bound to macromolecules are excluded from reaching the electrode surface. The use of the methodology as a rapid means to study protein binding is the subject of another paper (32).

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Use of Membrane-Covered Rotating-Disk Electrode to Study Binding of Drugs to Macromolecules: Competitive Binding of 2-(4′-Hydroxybenzeneazo)benzoic Acid and α-(4-Chlorophenoxy)-α-methylpropionic Acid to Serum Albumins

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Abstract □ A new technique is introduced to study the binding of drugs to macromolecules. The method utilizes rotating-disk polarography in which a semipermeable membrane covers the surface of the electrode. The membrane permits passage of an electroactive drug but restricts permeability of the macromolecule and of bound drug. The current generated at the electrode at steady state is directly proportional to the concentration of free drug in bulk solution, from which a calculation of the amount of drug bound may be made. The methodology used provides a complete characterization of binding within about 1 hr. and is, therefore, faster than conventional techniques presently used. Another advantage of this technique is that relatively small amounts of the compounds being studied are required. The method is compared with equilibrium dialysis and ultrafiltration techniques by studying the binding of 2-(4′-hydroxybenzeneazo)benzoic acid (I) to serum albumin and is found to give precisely the same results. α-(4-Chlorophenoxy)-α-methylpropionic acid (II), in previous spectrophotometric studies, showed an anomalous effect on the binding of I to rat serum albumin. At low concentrations, there was an indication that II may increase the binding of I to rat serum albumin. This phenomenon was reinvestigated using the new electrochemical method; it was found that II competitively inhibits the binding of I at all concentration levels of the inhibitor. The conclusion is reached that II affects the nature of the I binding site rather than the number of molecules bound.

Keyphrases □ 2-(4′-Hydroxybenzeneazo)benzoic acid binding to serum albumins—presence, absence of α-(4-chlorophenoxy)-α-methylpropionic acid, rotating-disk polarography method, compared to conventional techniques □ α-(4-Chlorophenoxy)-α-methylpropionic acid—competitive binding with 2-(4′-hydroxybenzeneazo)benzoic acid to serum albumin, rotating-disk polarography method □ Polarography, rotating disk—used in binding studies, compared to equilibrium dialysis and ultrafiltration techniques □ Rotating-disk polarography—used in binding studies, compared to equilibrium dialysis and ultrafiltration techniques □ Serum protein binding, competitive—2-(4′-hydroxybenzeneazo)benzoic acid and α-(4-chlorophenoxy)-α-methylpropionic acid, rotating-disk polarography method □ Binding of drugs to macromolecules—studied using rotating-disk polarography, compared to equilibrium dialysis and ultrafiltration techniques

Classical methods employed to detect and determine the binding characteristics of small molecules to serum albumin have been discussed by several authors (1–3). Techniques such as equilibrium dialysis, ultracentrifugation, light absorption and rotation, and ultrafiltration have been used. The methods conventionally used require long periods of time to collect analyzable data and/or relatively large amounts of reactants, some of which can be costly. Nonequilibrium dialysis techniques (4–7) are generally faster and use less materials than conventional methods but can require cumbersome data analysis. The method of Meyer and Guttman (5), for example, takes about 6 hr. to collect data and requires the use of computer curve-fitting techniques to estimate the number of molecules bound to albumin.

To overcome some of these disadvantages, we applied a technology, developed in these laboratories (8), that was previously used to study mass transport. The technique employed to study mass transport across membranes utilized rotating-disk polarography where a membrane sheet is placed on the surface of a carbon or mercury-coated platinum tip electrode. If the membrane used is semipermeable, allowing only small electrochemically active molecules to penetrate, a system is obtained that is analogous to the thermodynamically well-defined equilibrium dialysis technique. There is,