magnetization upon the adsorption of one benzene molecule  $\mu_{\rm B}$  on the bare surface, 2  $\mu_{\rm B}$  on the surface covered with hydrogen, and 2.7  $\mu_{\rm B}$  in the case of cyclohexene.

Finally, the exchange experiments corroborate these findings: in the present case all the molecules present on the nickel surface can be exchanged for <sup>14</sup>C-labeled benzene while, if benzene is directly chemisorbed on bare nickel, only 80–90% of the benzene can be exchanged.<sup>9</sup>

At higher cyclohexene pressure, neutron measurements show that cyclohexane is present in the adsorbed phase with benzene and hydrogen. However, cyclohexane is only slightly perturbed: the characteristic frequencies are very close to their values in the solid state; cyclohexane is thus probably physisorbed at the surface; therefore, it is not surprising that pumping off at 300 K leads to a condensed phase containing cyclohexane, benzene, and cyclohexene.

These results on polycrystalline samples can be interestingly compared with those reported by Tsai, Friend, and Muetterties,<sup>10</sup> on Ni flat or stepped single crystal faces. Operating under ultrahigh-vacuum conditions, i.e., equilibrium pressures not greater than 10<sup>-9</sup> torr, they have found that, for temperatures of 300-400 K, cyclohexene is converted to benzene and hydrogen and they did not detect any cyclohexane. This is guite similar to what we have observed in the second and third parts of our experiment. Indeed, when cyclohexane is formed, its sticking coefficient on an already covered surface is so low that one should increase the pressure to 1 torr to observe a physisorbed layer containing this molecule.

Now the question remains of the existence of cyclohexadiene as an intermediate in the formation of benzene from cyclohexene. No direct proof of the existence of this molecule has been afforded by the above authors. A separate neutron measurement on Raney nickel where cyclohexadiene was directly adsorbed on the bare surface gave a spectrum very similar to that observed upon adsorption of cyclohexene at low pressure. It is thus not conclusive, insofar as cyclohexadiene might be a transient state but with a very short lifetime.

Finally, at low pressure, cyclohexene (or cyclohexadiene) forms directly chemisorbed benzene and hydrogen. If now the cyclohexane pressure is augmented, this molecule reacts with adsorbed hydrogen in a bimolecular process which is probably rate limiting.

Registry No. Nickel, 7440-02-0; cyclohexene, 110-83-8.

# Hydroxide Complex of the Magnesium Octaethylporphyrin Cation: Spectrum and Formation Constant in Alcohol–Water Solvents

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The formation of the complex between photogenerated magnesium octaethylporphyrin cation and hydroxide has been studied in mixed alcohol/water solvents. This complex has been postulated as a participant in the transport of hydroxide and/or hydrogen ion across bilayer lipid membranes. The absorption spectrum of the complex ( $\lambda_{max} = 665 \text{ nm}$ ) is red shifted from that of the cation ( $\lambda_{max} = 680 \text{ nm}$ ) in both methanol/water (M/W) and ethanol/water (E/W) solvents. The formation constants for the complex in the two solvent systems are 16 (in M/W) and 190 M<sup>-1</sup> (in E/W). The difference in the two constants is too great to be explained entirely by the Born equation (with correction for ion screening) in which the dielectric constant of the solvent system is the pertinent variable. Differential solvation effects appear to be involved. The rate constants,  $k_2$ , for the reaction of the cation with the p-dinitrobenzene anion, p-DNB<sup>-</sup> (p-DNB is the electron acceptor for the photogeneration of the magnesium octaethylporphyrin cation), and  $k_4$ , for the reaction of the complex with p-DNB<sup>-</sup>, are  $1.1 \times 10^9$  and  $2.1 \times 10^9$  M<sup>-1</sup> s<sup>-1</sup> in M/W and  $1.3 \times 10^9$  and  $1.2 \times 10^9$  M<sup>-1</sup> s<sup>-1</sup> in E/W. Although the rate constants are within an order of magnitude of being diffusion controlled, the fact that  $k_4 \ge k_2$  is indicative of some degree of activation control.

# Introduction

Recent work<sup>1</sup> has suggested that the photogenerated cation of magnesium octaethylporphyrin in a lipid bilayer membrane can mediate the transport of  $OH^-$  and/or  $H^+$ across the membrane. There is much evidence to support the contention<sup>1</sup> that under these conditions a water molecule is probably tightly bound to the Mg in both the oxidized (cationic) and unoxidized (neutral) magnesium octaethylporphyrin (e.g., electrochemical evidence for anionic complexation of cations of magnesium porphyrins,<sup>2</sup> of magnesium chlorin,<sup>3</sup> and of chlorophyll a;<sup>3</sup> X-ray crystallographic data for  $MgTPP^+ClO_4^-$  (TPP = tetraphenylporphyrin) indicating that the Mg is pentacoordinate ligating  $ClO_4^-$  through the oxygen;<sup>4</sup> and X-ray

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<sup>(10)</sup> M. C. Tsai, C. M. Friend, and E. L. Muetterties, J. Am. Chem. Soc., 104, 2539 (1982).

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<sup>1979, 76, 4170.</sup> 

<sup>(4)</sup> Barkigia, K. M.; Spaulding, L. D.; Fajer, J. Inorg. Chem. 1983, 22, 349

$$H_{H}^{H}O-MgOEP^{+}$$
 HO-MgOEP  $H_{H}^{H}O-MgOEP$   
 $P^{+}$  POH P

We report here the effect of hydroxide ion on the spectrum of  $P^+$  in alcohol/water systems providing direct evidence for the equilibrium between the two species,  $P^+$  and POH, in waterlike solvents. This particular solvent system was selected in order to mimic the aqueous environment in the membrane experiments<sup>1</sup> and at the same time maintain adequate solubility of the magnesium octaethylporphyrin (P). Also, the addition of water to the alcohol solvents prevents alkoxide formation.

Conventional methods of generating  $P^+$  (e.g., coulometric) fail because the cation is unstable in alkaline media.<sup>1</sup> Photogeneration of  $P^+$  invokes the reaction between the porphyrin triplet (P\*) and an electron acceptor (A)

$$\mathbf{P}^* + \mathbf{A} \xrightarrow{\kappa_1} \mathbf{P}^+ + \mathbf{A}^- \tag{R1}$$

The approach is ideal since the back-reaction

$$P^+ + A^- \xrightarrow{n_2} P + A \qquad (R2)$$

is fast enough to preclude any undesirable degenerative reactions of  $P^+$  with the solvent, and slow enough to permit spectral analysis of the products of reaction R1. We shall show that when  $P^+$  is generated in the presence of hydroxide ion the following equilibrium exists:

$$P^+ + OH^- \stackrel{K_3}{=} POH + H_2O \tag{R3}$$

Thus one must consider the additional back-reaction

$$POH + A^{-} \xrightarrow{\kappa_{4}} P + OH^{-} + A$$
 (R4)

Assuming that the equilibrium of R3 is instantaneous, we are able to determine the reaction rates  $(k_2 \text{ and } k_4)$  for both P<sup>+</sup> and POH with the concomitantly photogenerated *p*-dinitrobenzene anion, A<sup>-</sup>.

# **Experimental Section**

Materials. The solvents were prepared from methanol (Eastman, spectrophotometric grade) or ethanol (Rossville Gold Shield, 200 proof) with 11.1 M deionized water. Aldrich *p*-dinitrobenzene was recrystallized from a mixture of methanol and water. Purum grade tetramethylammonium hydroxide pentahydrate, TMAOH (Fluka), purum grade tetramethylammonium chloride, TMACI (Fluka), and KCl were used without further purification. The magnesium octaethylporphyrin was a gift from Jack Fajer, Brookhaven National Laboratory.

Methods. The laser flash photolysis apparatus used in this work has been described elsewhere.<sup>8</sup> Changes in optical density  $(\Delta A_{\lambda,t})$  were determined as  $-\log (1 + \Delta I_t/I_0)$ where  $I_0$  is the intensity of the interrogating light transmitted by the experimental solution before the flash and  $\Delta I_t$  is the change in this intensity measured at a time (t)subsequent to the flash. All flash photolysis experiments were performed at  $25 \pm 1$  °C. Optical cells had a path length ( $\delta$ ) of 0.7 cm.

Alcohol-water solutions contained magnesium octaethylporphyrin ( $\sim 2 \times 10^{-5}$  M), p-dinitrobenzene ( $\sim 1 \times 10^{-3}$  M), and in most cases TMAOH and TMACl at a constant total ionic strength (0.5 M assuming 100% ionization). Solutions were outgassed on a grease-free and mercury-free vacuum line by six freeze-thaw cycles and then pumped on once at 195 K to remove carbon dioxide.

P<sup>+</sup>, which is unstable over a period of seconds in solutions containing hydroxide, was produced by flash photolysis of P in the presence of the electron acceptor (pdinitrobenzene). The wavelength of the light pulse produced by the dye laser with rhodamine 6G peaks at 590 nm,<sup>9</sup> nicely overlapping the 578-nm absorption peak of magnesium octaethylporphyrin.<sup>10</sup> The optical density of the solutions at 590 nm is approximately 0.05. The intensity of the flash was kept high to effect virtually complete (>90%) bleaching of the porphyrin. This ensures that the concentration of the photogenerated species is nearly uniform throughout the interrogated region of the cell-this is particularly important for study of secondorder rate processes. Normally the laser was operated at 20 kV with [rhodamine 6G] = 25  $\mu$ M, but in several experiments, in order to ensure complete (>99%) bleaching of the porphyrin, the laser was operated at 25 kV with [rhodamine 6G] = 50  $\mu$ M.

Theory. The experimental objective of the present work is to demonstrate that oxidized magnesium octaethylporphyrin can exist in two forms in the presence of  $OH^-$ :  $P^+$  and POH. The theoretically predicted changes in the optical density are based on two suppositions: (1) that the spectra of  $P^+$  and POH are distinguishable; and (2) that the system chemistry is adequately described by reactions R1-R4. In making the first assumption we take advantage of "prescience" gained by preliminary experimentation. It is important to note at the outset that even if  $P^+$  and POH were spectrally indistinguishable their existence might still be demonstrated by their different rates of reaction (reactions R2 and R4). The development of the theory is prerequisite to discussion of the experimental procedures.

At a particular time, t, the general expression for the change in optical density,  $\Delta A_{\lambda,t}$ , at a given wavelength,  $\lambda$ , is

$$\Delta A_{\lambda,t} = \delta \sum_{j} \epsilon_{j,\lambda} c_{j,t} \tag{1}$$

where  $\epsilon_{j,\lambda}$  is the decadic extinction coefficient of species j at wavelength  $\lambda$ ,  $c_{j,t}$  is the concentration of species j at time t, and  $\delta$  is the optical path length of the cell (0.7 cm). We assume that equilibrium for reaction R3 obtains at all times. Thus

$$[\mathbf{P}^+]_t = \frac{[\mathbf{P}^+]_{\mathbf{T},t}}{1 + K_3[\mathbf{OH}^-]}$$
(2)

$$[POH]_{t} = \frac{K_{3}[OH^{-}][P^{+}]_{T,t}}{1 + K_{3}[OH^{-}]}$$
(3)

where

$$[\mathbf{P}^{+}]_{\mathbf{T},t} = [\mathbf{P}^{+}]_{t} + [\mathbf{POH}]_{t}$$
(4)

and, since  $[POH]_t < [P^+]_{T,t} << [OH^-]$  in all cases,  $[OH^-]$  is the total concentration of hydroxide in the solution. The rate expression for the disappearance of  $[P^+]_{T,t}$  effected

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<sup>(6)</sup> Serbin, R.; Chow, H. C.; Strouse, D. C. J. Am. Chem. Soc. 1975, 97, 7237.

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<sup>(10)</sup> Fuhrhop, J.-H.; Mauzerall, D. J. Am. Chem. Soc. 1968, 90, 3875. Fajer, D. C.; Forman, A.; Dolphin, D.; Felton, R. H. Ibid. 1970, 92, 3451.

by reactions R2 and R4 is (remembering that  $[P^+]_{T,t}$  = [A<sup>-</sup>])

$$-\frac{\mathrm{d}[\mathrm{P}^+]_{\mathrm{T},t}}{\mathrm{d}t} = \frac{k_4 K_3 [\mathrm{OH}^-] + k_2}{1 + K_3 [\mathrm{OH}^-]} [\mathrm{P}^+]_{\mathrm{T},t}^2 \tag{5}$$

Thus

$$[\mathbf{P}^+]_{\mathrm{T},t} = \frac{(1 + K_3[\mathrm{OH}^-])[\mathbf{P}^+]_{\mathrm{T},t=0}}{1 + K_3[\mathrm{OH}^-] + (k_4 K_3[\mathrm{OH}^-] + k_2)[\mathbf{P}]_{\mathrm{T},t=0}t}$$
(6)

If we assume that OH<sup>-</sup> does not absorb in the spectral region of interest and that the spectra of A<sup>-</sup> and A are not affected by OH<sup>-</sup>, then the general expression for  $\Delta A_{\lambda,t}$  is (from eq 1)

$$\Delta A_{\lambda,t} = \delta[\mathbf{P}^+]_{\mathrm{T},t} \frac{\epsilon_{\lambda,a} K_3[\mathrm{OH}^-] + \epsilon_{\lambda,b}}{1 + K_3[\mathrm{OH}^-]}$$
(7)

where

$$\epsilon_{\lambda,a} = \epsilon_{\lambda,\text{POH}} + \epsilon_{\lambda,A^-} - \epsilon_{\lambda,P} - \epsilon_{\lambda,A} \tag{8}$$

$$\epsilon_{\lambda,b} = \epsilon_{\lambda,P^+} + \epsilon_{\lambda,A^-} - \epsilon_{\lambda,P} - \epsilon_{\lambda,A} \tag{9}$$

The expression for the time dependence of  $\Delta A_{\lambda,t}$  is obtained by combining eq 6 and 7

$$\Delta A_{\lambda,t} = \delta \frac{[\mathbf{P}^+]_{\mathbf{T},t=0}(\epsilon_{\lambda,\mathbf{a}}K_3[\mathbf{OH}^-] + \epsilon_{\lambda,\mathbf{b}})}{1 + K_3[\mathbf{OH}^-] + (k_4K_3[\mathbf{OH}^-] + k_2)[\mathbf{P}^+]_{\mathbf{T},t=0}t}$$
(10)

Experimental Procedures and Data Analysis. A linear plot of  $1/\Delta A_{\lambda t}$  vs. t (see eq 10) confirms the second-order character of reactions R2 and R4. The slope,  $S_{\lambda,[OH^-]}$ , and intercept,  $I_{\lambda,[OH^{-}]}$ , are

$$S_{\lambda,[\text{OH}^-]} = \frac{(k_4 K_3 [\text{OH}^-] + k_2)}{\delta(\epsilon_{\lambda,a} K_3 [\text{OH}^-] + \epsilon_{\lambda,b})}$$
(11)

$$I_{\lambda,[OH^-]} = \frac{(1 + K_3[OH^-])}{\delta[P^+]_{T,t=0}(\epsilon_{\lambda,a}K_3[OH^-] + \epsilon_{\lambda,b})}$$
(12)

and the slope/intercept ratio, which is independent of  $\lambda$ , is

$$R_{[OH^-]} = \frac{(k_4 K_3 [OH^-] + k_2) [P]_{T,t=0}}{1 + K_3 [OH^-]}$$
(13)

Although a plot of  $1/\Delta A_{\lambda,t}$  vs. t (eq 10) is a useful demonstration of second-order kinetics, parameters  $S_{\lambda,[OH^-]}$  and  $I_{\lambda,[OH^-]}$  (eq 11 and 12) are evaluated from a nonlinear least-squares fit to eq 10.

An additional useful relationship is obtained by comparing the changes in absorption obtained at two different wavelengths,  $\lambda_i$  and  $\lambda_k$ , and at a given time t. Thus (see eq 10)

$$\frac{\Delta A_{\lambda_{j,t}}}{\Delta A_{\lambda_{k,t}}} = \frac{\epsilon_{\lambda_{j,\mathbf{a}}} K_3[\mathrm{OH}^-] + \epsilon_{\lambda_{j,b}}}{\epsilon_{\lambda_{k,\mathbf{a}}} K_3[\mathrm{OH}^-] + \epsilon_{\lambda_{k,b}}}$$
(14)

For notational convenience we define

$$r_{n,x/m,z} = \epsilon_{\lambda_n,x} / \epsilon_{\lambda_m,z}$$
(15)

Equation 14 can now be rewritten

$$\frac{\Delta A_{\lambda_{j,t}}}{\Delta A_{\lambda_{k,t}}} = \frac{r_{j,\mathbf{a}/k,\mathbf{b}}K_{3}[\mathrm{OH}^{-}] + r_{j,\mathbf{b}/k,\mathbf{b}}}{r_{k,\mathbf{a}/k,\mathbf{b}}K_{3}[\mathrm{OH}^{-}] + 1}$$
(16)

A given solution is flashed with the changes in optical density monitored at each of four different wavelengths

 $(\lambda = 660, 670, 680, \text{ and } 690 \text{ nm})$ . This procedure, repeated five times for a total of 20 flashes, permits confirmation that the flash is producing a constant value of  $[P^+]_{t=0}$ . A nonlinear least-squares analysis of the absorbance-ratio data (eq 16) for four wavelengths and solutions of varying  $[OH^-]$  yields the values of the three unknowns in eq 16:  $r_{j,a/k,b}K_3$ ,  $r_{j,b/k,b}$ , and  $r_{k,a/k,b}K_3$ . Using the saturating (>99% bleaching) high-intensity

flash we can presume that

$$[\mathbf{P}^+]_{\mathbf{T},t=0} = [\mathbf{P}]_0 \tag{17}$$

where  $[P]_0$  is the total concentration of P in the solution. Equation 12 can be rewritten to give

$$I_{\lambda_{n},[OH^{-}]}[P]_{0} = \frac{(1 + K_{3}[OH^{-}]) / \epsilon_{\lambda_{n},b}}{\delta(r_{n,a/n,b}K_{3}[OH^{-}] + 1)}$$
(18)

Since  $r_{n,a/n,b}K_3$  is already known, data from saturating flashes at four wavelengths for each of two different OHconcentrations lead directly to the determination of  $K_3$ . The ratio of the values of  $I_{\lambda_n,[OH^-]}[P]_0$  at a given  $\lambda_n$  for two different [OH<sup>-</sup>] is

$$\beta_{I} = \frac{I_{\lambda_{n},[\text{OH}^{-}]_{1}}([\text{P}]_{0})_{1}}{I_{\lambda_{n},[\text{OH}^{-}]_{2}}([\text{P}]_{0})_{2}} = \frac{(1 + K_{3}[\text{OH}^{-}]_{1})([\text{OH}^{-}]_{2}\sigma + 1)}{(1 + K_{3}[\text{OH}^{-}]_{2})([\text{OH}^{-}]_{1}\sigma + 1)}$$
(19)

where

$$\sigma = r_{n,a/n,b}K_3$$
(20)  
$$K_3 = \frac{[OH^-]_2\sigma + 1 - \beta_I([OH^-]_1\sigma + 1)}{\beta_I[OH^-]_2([OH^-]_1\sigma + 1) - [OH^-]_1([OH^-]_2\sigma + 1)}$$
(21)

One can now deduce the values of  $\epsilon_{\lambda_n,a}$  and  $\epsilon_{\lambda_n,b}$  by substitution back into eq 18 and combining with previously determined terms.

Thus far the analysis has depended entirely upon the spectrophotometric data and not at all upon the kinetic data (except for intercept data which really has no kinetic information). Clearly, if  $k_2 = k_4$ , the kinetics can reveal no information about the distribution of the forms  $P^+$  and POH. Once the value of  $K_3$  has been established, however, it is possible to deduce the values of  $k_2$  and  $k_4$ . Since all the other variables have been determined one can compute the term (see eq 11)

$$\gamma_{[\text{OH}^-]} = \frac{\delta S_{\lambda,[\text{OH}^-]}(\epsilon_{\lambda,a}K_3[\text{OH}^-] + \epsilon_{\lambda,b})}{(1 + K_3[\text{OH}^-])} = \frac{k_4K_3[\text{OH}^-] + k_2}{(1 + K_3[\text{OH}^-])}$$
(22)

Defining

$$\alpha_{[OH^-]} = \frac{K_3[OH^-]}{1 + K_3[OH^-]}$$
(23)

eq 22 can be rewritten

$$\gamma_{[OH^{-}]} = \alpha_{[OH^{-}]}(k_4 - k_2) + k_2 \tag{24}$$

A plot of  $\gamma_{[OH^-]}$  vs. the corresponding value of  $\alpha_{[OH^-]}$  should be linear with a slope,  $k_4 - k_2$ , and intercept,  $k_2$ .

#### Results

Confirmation of Second-Order Kinetics. Several examples of the experimental plot  $1/\Delta A_{\lambda,t}$  vs. t are shown in Figure 1. Data for methanol-water solvent systems exhibit excellent conformation to the anticipated secondorder kinetics. Data obtained at hydroxide concentrations between  $10^{-2}$  and  $10^{-1}$  M for the ethanol-water systems (see



**Figure 1.** Plot of  $1/\Delta A_{\lambda,t}$  (1/DA) vs. *t* demonstrating second-order kinetics for reactions R2 and R4. Conditions were as follows: (a) CH<sub>3</sub>OH/H<sub>2</sub>O, [OH<sup>-</sup>] = 3.01 × 10<sup>-2</sup> M,  $\lambda = 680$  nm; (b) CH<sub>3</sub>OH/H<sub>2</sub>O, [OH<sup>-</sup>] = 3.01 × 10<sup>-1</sup> M,  $\lambda = 670$  nm; (c) C<sub>2</sub>H<sub>5</sub>OH/H<sub>2</sub>O, [OH<sup>-</sup>] = 1.00 × 10<sup>-2</sup> M,  $\lambda = 660$  nm; (d) C<sub>2</sub>H<sub>5</sub>OH/H<sub>2</sub>O, [OH<sup>-</sup>] = 5.01 × 10<sup>-1</sup> M,  $\lambda = 665$  nm.

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**Figure 2.** Plot of  $\Delta A_{\lambda,t=6\mu s}/\Delta A_{\lambda_{max},t=6\mu s}$  vs.  $\lambda$ . Data obtained with a nearly saturating flash. Curves approximate the spectra of P<sup>+</sup> under various conditions.



**Figure 3.** The variation of the ratio  $R = \Delta A_{\lambda=690,t=6\mu s}/\Delta A_{\lambda=670,t=6\mu s}$  as a function of [OH<sup>-</sup>]. Ionic strength of all solutions is 0.5 M. Values of parameters in eq 16 used to fit data (for C<sub>2</sub>H<sub>5</sub>OH/H<sub>2</sub>O and CH<sub>3</sub>OH/H<sub>2</sub>O, respectively):  $r_{690,a/670,b}K_3 = 3.1 \times 10^1$  and 4.9 M<sup>-1</sup>;  $r_{670,a/670,b}K_3 = 1.1 \times 10^2$  and 1.4  $\times 10^1$  M<sup>-1</sup>;  $r_{690,b/670,b} = 1.1$  and 1.1.

Figure 1c), however, indicate the possibility of some other kinetic phenomena at longer times. These aberrations from a "perfect" second-order plot are not dependent upon  $\lambda$ . Since these data are analyzed by a nonlinear least-squares fit of eq 10 the values of the reported rate constants are strongly dependent upon the data at the shorter times.

Spectrophotometric Evidence for P<sup>+</sup> and POH. A plot of  $\Delta A_{\lambda,t=6\mu s} \Delta A_{\lambda_{max},t=6\mu s}$  clearly indicates (Figure 2) that the solution spectra are dependent upon the concentration of OH<sup>-</sup>. Since P,<sup>10</sup> A, and A<sup>-11</sup> do not absorb significantly at  $\lambda > 600$  nm, the spectral changes are evidence that P<sup>+</sup> is being complexed. The data conform to reactions R1–R4 as shown in Figure 3 by a plot of  $\Delta A_{\lambda=690,t=6\mu s} / \Delta A_{\lambda=670,t=6\mu s}$ as a function of [OH<sup>-</sup>] (see eq 16). Similar curves are obtained by using data from any pair of the four wave-

<sup>(11)</sup> Shida, T.; Swata, S. J. Phys. Chem. 1971, 75, 2591.

**TABLE I: Experimental Results** 

solvent	methanol/ water	ethanol/ water
dielectric constant at 298 K <sup>a</sup>	41.8	34.6
viscosity at 298 K, <sup>b</sup> cP	1.131	1.736
$\lambda_{max,P^+}, nm$	680	680
$\lambda_{max,POH}$ , nm	665	665
$\epsilon_{665 \text{nm},a}, \text{M}^{-1} \text{ cm}^{-1}$	$1.5 imes 10^4$	$1.6 imes 10^4$
$\epsilon_{690}$ nm.a, M <sup>-1</sup> cm <sup>-1</sup>	$0.5 imes 10^4$	$0.5 \times 10^{4}$
$\epsilon_{690}$ nm,b, M <sup>-1</sup> cm <sup>-1</sup>	$1.9 imes 10^4$	$2.8 imes 10^4$
$\epsilon_{680nm,a}, M^{-1} cm^{-1}$	$0.9 \times 10^4$	$1.0 \times 10^{4}$
$\epsilon_{680}$ nm,b, $M^{-1}$ cm <sup>-1</sup>	$2.3 imes 10^4$	$2.8 imes~10^4$
$\epsilon_{670}$ nm, a, $M^{-1}$ cm <sup>-1</sup>	$1.4 imes 10^4$	$1.5 imes 10^4$
$\epsilon_{670}$ nm,b, M <sup>-1</sup> cm <sup>-1</sup>	$1.8 imes 10^4$	$2.4 imes 10^4$
$\epsilon_{660}$ nm.a, M <sup>-1</sup> cm <sup>-1</sup>	$1.5 imes 10^4$	$1.5 imes 10^4$
$\epsilon_{660}$ nm,b, M <sup>-1</sup> cm <sup>-1</sup>	$1.4  imes 10^4$	$2.0 imes 10^4$
$K_3, \overline{\mathrm{M}}^{-1}$	$16 \pm 4$	$190 \pm 40$
$10^{-9}k_2, M^{-1}s^{-1}$	$1.1 \pm 0.1$	$1.3 \pm 0.1$
$10^{-9}k_4, M^{-1} s^{-1}$	$2.1 \pm 0.2$	$1.2 \pm 0.3$

<sup>a</sup> Akerlof, K. J. Am. Chem. Soc. 1932, 54, 4125.

<sup>b</sup> West, R. C., Ed. "Handbook of Chemistry and Physics", CRC Press: Cleveland, OH, 1976.



**Figure 4.** A plot of  $\gamma_{[OH^-]}$  vs.  $\alpha_{[OH^-]}$  for CH<sub>3</sub>OH/H<sub>2</sub>O (O) and C<sub>2</sub>H<sub>5</sub>OH/H<sub>2</sub>O (D). Ionic strength of all solutions is 0.5 M.

lengths used. The reproducibility of the variables of eq 16 computed from these data is  $\pm 10\%$ .

Data obtained from saturating flashes at  $[OH^-] = 0.0$ and 0.5 M in methanol and at  $1.0 \times 10^{-3}$ ,  $2.0 \times 10^{-3}$ , and  $0.5~{\rm M}$  in ethanol lead directly (eq 17–21) to an evaluation of  $K_3$  in the two solvent systems (see Table I). When one of the concentrations of OH<sup>-</sup> is zero (as was the case for methanol) or infinity (which was effectively the case for 0.5 M [OH<sup>-</sup>] in ethanol) eq 21 assumes a somewhat simplified form.

Determination of  $k_2$  and  $k_4$ . A plot of the kinetic term,  $\gamma_{\rm [OH^{-}]}$  (eq 24) vs.  $\alpha$  (Figure 4) clearly indicates that the values of  $k_2$  and  $k_4$  are significantly different in methanol and virtually identical (within experimental error) in ethanol. Data are summarized in Table I.

# Discussion

The solvent effects upon the rate constants  $k_2$  and  $k_4$ (Table I) are qualitatively predicted when one considers effects of differing dielectric constants and viscosities. Since the rate constants for these reactions are within an order of magnitude of being purely diffusion controlled it is not surprising that they are similar. The decrease in the value of  $k_4$  from 2.1 × 10<sup>9</sup> M<sup>-1</sup> s<sup>-1</sup> in methanol/water to  $1.2 \times 10^9$  M<sup>-1</sup> s<sup>-1</sup> in ethanol/water approximates the change predicted by the difference in viscosities. The value of  $k_2$ is virtually the same in both solvents, perhaps reflecting the compensating effect of the decrease in the dielectric constant on a reaction between two oppositely charged species. The same reasoning predicts, however, that  $k_2 > k_2 >$ 

 $k_4$  in either solvent. The fact that  $k_4 \ge k_2$  may indicate some activation control.

The  $\sim$ 12-fold difference in the values of  $K_3$  in the two solvents (Table I) is more than a factor of four larger than the value estimated by using the Born equation with corrections for ion screening.<sup>12</sup> It is not unreasonable to assume that chemical (e.g., solvation) effects as well as electrostatic effects are responsible for the change in  $K_3$ .

The work which initiated this study<sup>1</sup> presented evidence for the formation of the POH complex in a lipid bilayer at pH 5: P<sup>+</sup>, photogenerated within the bilayer ( $\epsilon < 5$ , with no other screening ions), reacts with OH<sup>-</sup> in the aqueous phase to form POH in the membrane phase. Both  $P^+$  and POH are insoluble in the aqueous phase. The steady-state conductance at pH 5 ( $g_{ss}$ , attributed to the translocation of the neutral complex POH) is about 20% of the initial conductance  $(g_0, \text{ attributed to translocation of } P^+)$ . With some simple assumptions it is possible to estimate the value of  $K_3$  in a bilayer lipid membrane:

If we assume that the simple carrier transport mechanism is as described by Stark et al.<sup>13</sup> and that the equilibrium for R3 is instantaneously achieved for small values of the transmembrane potential,  $\Delta u$  (i.e.,  $\exp[\Delta u/2] \sim 1$ +  $\Delta u/2$ ), the following relationship is deduced

$$\frac{g_{ss}}{g_0} = \frac{1}{1 + \frac{k_v}{k_s K_s [OH^-]}}$$
(25)

where  $k_{\rm v}$  and  $k_{\rm s}$  are the rate constants for the translocation of P<sup>+</sup> and POH, respectively. Since a charged species will encounter an electrostatic barrier<sup>14</sup> and the neutral species will not, one can qualitatively predict that  $k_s >> k_v$ . Data for carriers such as valinomycin and the actins<sup>13,15,16</sup> support this conclusion.

If we make the conservative assumption that  $k_{\rm v} \sim k_{\rm s},$ we can now deduce from the observation<sup>1</sup> that  $g_{\rm ss}/g_0 \sim$ 0.2 at pH 5, that  $K_3[OH^-] \sim 0.25$ . The small radius of the hydroxide ion renders it very hydrophilic and its concentration in the lipid bilayer will be orders of magnitude smaller than its aqueous concentration. Thus we conclude that  $K_3$  is probably orders of magnitude greater than  $10^9$  $M^{-1}$  in order to effect the carrier mechanism suggested in ref 1 for the transport of  $H^+$  and/or  $OH^-$  across lipid bilayer membranes. Extrapolation from the limited data we have would be fanciful to say the least. It is not unreasonable, however, to suggest that in a relatively noncomplexing solvent of low dielectric ( $\epsilon < 5$ ) the value of  $K_3$ could be large enough to effect the observed behavior.<sup>1</sup>

#### Conclusions

The experimental data support the conclusion that a porphyrin cation can form a hydroxide complex in alcohol/water mixtures. The reaction is probably an acid-base reaction in which the aquated  $P^+$  looses a proton:

$$H_{\rm H}^{\rm H}O-MgOEP^+ + OH^- = HO-MgOEP + H_2O$$

As was suggested previously,<sup>1</sup> it is also possible that  $H_2O$ ,

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<sup>(16)</sup> Stark, G., Reiter, D., Bolz, R., Edgel, T. Barger, J. Barger, J New York, 1974; Vol. 3, p 127.

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which will be present in a lipid bilayer at much higher concentrations than  $OH^-$ , may be the active agent:

$$H^{\Pi}O-MgOEP^{+} + H_{2}O = HO-MgOEP + H_{3}O^{+}$$

Although the equilibrium constants which we have determined for these reactions (see Table I) seem, at first glance, too small to effect the phenomenon observed in ref 1 at pH's between 5 and 7, it must be remembered that the low value of the dielectric constant within a bilayer lipid membrane will highly favor deprotonation.

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# Theory of the Biocatalytic Membrane Electrode

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We present a theoretical interpretation of the mechanism of a biocatalytic membrane electrode with particular emphasis on the time dependence of the approach to steady state. We make use of a model based on diffusion from a bulk solution with diffusion constant  $D_{\rm II}$  into an enzyme layer of thickness L with diffusion constant  $D_{\rm I}$ , combined with a chemical reaction obeying Michaelis-Menten kinetics within the enzyme layer. We report an exact calculation of the time dependence of the product concentration within the enzyme layer for the high-concentration case and an approximate treatment for the low-concentration case. We find that in all cases the difference between the product concentration and its steady-state value is proportional to the inverse square root of the time. The approach to steady state is strongly affected by stirring and by the thickness L of the enzyme layer; it is much less dependent on the value of the diffusion constant  $D_{\rm I}$ .

# I. Introduction

An important new device for medicoanalytical and diagnostic purposes is the immobilized enzyme electrode or the biocatalytic membrane electrode. Many enzymes react specifically with one and only one organic or biological substance. If such an enzyme is trapped in a matrix layer of a thickness of the order of 0.01 cm and if the layer is in contact with a substrate solution of the substance that the enzyme reacts with, then the immobilized enzyme electrode can be used for rapid quantitative analysis of the substrate solution. Typical examples of such enzyme electrodes are the glucose electrode, described by Clark and Lyons<sup>1</sup> and constructed by Updike and Hicks,<sup>2</sup> and the urea electrode, developed by Guilbault and Montalvo.<sup>3</sup> In recently proposed electrodes the immobilized enzyme layer has been replaced by bacterial, mitochondrial, or even tissue layers.4

The general theoretical description of the various types of biocatalytic membrane electrodes is based on the onedimensional model that we have sketched in Figure 1. Here it is assumed that the biocatalyst is distributed homogeneously in the layer  $0 \le X \le L$ ; we denote the enzyme layer by the Roman numeral I. The region X > L, which we denote by II, contains the substrate solution. Recently we derived the stationary-state solutions of the substrate and product concentration profiles for the above theoretical model.<sup>5</sup> We obtained analytical expressions for the concentration profiles and we also derived power-series expansions that could be used for convenient numerical evaluation. We should also mention the work by Morf,<sup>6</sup> who approached the same problem from a different viewpoint.

In the present paper we want to derive the time dependence of the approach to steady state; that is, we attempt to calculate the time-dependent solutions of the concentration profiles corresponding to specific initial conditions. We shall see that we are able to derive these time-dependent solutions only for certain limiting situations corresponding to either large or small concentrations. Nevertheless, we hope that our theoretical results contribute toward a general understanding of the time dependence of the approach to steady state.

In most biocatalytic membrane electrodes the substrate solution is stirred and this aspect of the electrode must be incorporated in the theoretical description. Fortunately, the effect of stirring has been studied experimentally by Morf, Lindner, and Simon.<sup>7</sup> Even though this study deals with neutral carriers rather than with biocatalytic mem-

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