MASS TRANSFER OF CO₂ ACROSS MEMBRANES: FACILITATION IN THE PRESENCE OF BICARBONATE ION AND THE ENZYME CARBONIC ANHYDRASE

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SUMMARY

A theoretical and experimental analysis of facilitated transport of CO₂ across membranes containing NaHCO₃ and the enzyme carbonic anhydrase (carbonate hydro-lyase EC 4.2.1.1) is presented. The necessary diffusion reaction equations are derived and the system constraints defined. For the CO₂–HCO₃⁻ system, mathematical simplifications based on the magnitude of various reaction and concentration terms are made to make the equations tractable to solution. The resultant equations are solved by a number of analytical and numerical techniques, each having a limited, though useful, range of validity.

The experimental arrangement consists of a liquid membrane (created by soaking a porous filter paper in the test solution), a diffusion chamber, and gas metering and analysis equipment. Conditions were selected to cover the range from diffusion-to reaction-dominated behavior.

The flux of CO₂ across a membrane containing 1 M NaHCO₃ was measured at various partial pressures of CO₂ (2–28 in Hg) and with membrane thicknesses of 0.02, 0.06 and 0.10 cm. The extent of facilitation (defined as the ratio of reaction-related flux to the expected Fick’s Law flux in the absence of reaction) ranged from near zero to nearly 5 in these experiments. The agreement between model calculations and experimental observation was found to be excellent over the entire range of near-diffusion to near-equilibrium behavior.

In the presence of enzyme carbonic anhydrase (0.10 mg/ml, activity approx. 80%) and 1 M NaHCO₃, the CO₂ flux across a 0.02 cm membrane was 3–10-fold higher than the corresponding flux in the absence of enzyme. From experiments at various enzyme concentrations and membrane thicknesses, it appeared that the apparent CO₂ reaction rate was directly proportional to the enzyme concentration. The model calculations for the enzyme-catalyzed reactions agreed with the experimental observations to within ±10%.

INTRODUCTION

Facilitated transport process reported in this work refers to the transfer of permeant(s) across a liquid film containing “entrapped” chemical species with which
the dissolved permeant(s) react in solution. By definition, the flux of the “entrapped” species, also referred to as the non-transferred or carrier species, is zero at the two physical boundaries; this in contrast to other facilitated transport studies where some or all the components may enter or leave the membrane phase [1]. In addition, the physical dimensions of the liquid film (that is, thickness and transfer area) are assumed known and only homogeneous reactions take place in the solution. The theoretical treatment of facilitated transport phenomenon, also referred to as “carrier-mediated” transport, has been based on a model allowing for concurrent processes of diffusion and reversible chemical reaction.

This report concerns itself with the facilitated transport of CO2 across strongly basic aqueous films. Early experiments by Ward [2] had indicated that across an aqueous film containing a weak base, such as pyridine, the observed flux of CO2 increased to a level twice that across a corresponding water film. In the presence of a strong base, such as saturated CsHCO3 solution, Ward and Robb [3] observed that the relative flux ratio of CO2 to O2 increased by a factor of 75. Experiments by Longmuir et al. [4] and by Ward and Robb [3] indicated that the CO2 flux could also be increased by catalyzing the CO2 reactions; among the catalysts used for this purpose were sodium aresenite and enzyme carbonic anhydrase. Later experiments by Enns [5] demonstrated that CO2 flux across buffer solutions was enhanced by orders of magnitude in the presence of enzyme carbonic anhydrase. More recently, Otto and Quinn [6] investigated the CO2–HCO3− system in further detail. In their experiments, the CO2 partial pressures at the two gas–liquid interfaces were always maintained at 1 and 0 atm, and the observed CO2 facilitation in the absence of the enzyme was rather small. In the presence of varying amounts of carbonic anhydrase, Otto and Quinn indicate an apparent abnormality in the enzymatic reactions. Based on a calculated equilibrium flux of CO2, the ratio of observed CO2 flux to the equilibrium CO2 flux went through a maxima at an enzyme concentration of approximately 0.5 mg/ml.

The above investigations were limited in their scope – experimental as well as theoretical. Here, the CO2–HCO3− facilitation process is studied in further detail to encompass a larger range of experimental variables, and to present a more general theoretical analysis. The principal features of the CO2 facilitation process are as follows: (a) in the uncatalyzed reaction scheme, a total of nine chemical species participate in six reactions, (b) only the CO2 reactions are rate limiting, (c) a variable reaction “rate constant” which can be obtained by varying amount of carbonic anhydrase concentration in the film, and (d) transport, solubility, and kinetic constants change as the HCO3−–CO32− concentration is increased.

The mathematical equations governing the transport process are, in general, highly non-linear. To this must be added the complexity arising from the zero-flux condition on the carrier species. The theoretical objective was therefore to obtain a unified overall picture of the phenomenon based on various limiting analytical as well as approximate solutions. Such solutions have been reported in the literature for specific examples; a principal task was therefore to generalize the approach so as to accommodate the overall phenomenon. In addition, the CO2 facilitation system required that a method for handling multiple reactions be obtained. The regimes in which the analytical and approximate solutions are valid may be defined as (a) near-diffusion regime [7], (b) near-equilibrium regime [8], (c) boundary-layer approximation [9, 10], (d) near-stationary state regime [11], (e) large carrier concentration limit [2].
Fig. 1. Calculated facilitations in the flux of CO₂ as a function of Damköhler number \( \alpha \). Curves I through IV are limiting analytical solutions. Curve V represents numerical solution by quasilinearization (○ — ○) and boundary layer approximation solution (□ — □). Model approximations: I, near-diffusion; II, near-stationary state; III, large carrier; IV, near-equilibrium; V, "exact".

**TABLE I**

**PHYSICOCHEMICAL CONSTANTS AT 25 °C**

<table>
<thead>
<tr>
<th></th>
<th>In water</th>
<th>In 1 M NaHCO₃-Na₂CO₃ solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>( k_1, \text{s}^{-1} )</td>
<td>0.0375 [17]</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.0358 [18]</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.026 [19]</td>
<td></td>
</tr>
<tr>
<td>( K_1 )</td>
<td>2.58 \cdot 10^{-3} [20]</td>
<td></td>
</tr>
<tr>
<td>( k_2, \text{cm}^3 \cdot \text{g mole}^{-1} \cdot \text{s}^{-1} )</td>
<td>8.5 \cdot 10^6 [19]</td>
<td></td>
</tr>
<tr>
<td>( K_1, K_3, \text{g moles/cm}^3 )</td>
<td>4.56 \cdot 10^{-10} [20]</td>
<td>1.37 \cdot 10^{-9} [16]</td>
</tr>
<tr>
<td>( K_4, \text{g moles/cm}^3 )</td>
<td>4.69 \cdot 10^{-14} [20]</td>
<td>2.70 \cdot 10^{-13} [21]</td>
</tr>
<tr>
<td>( K_6, (\text{g moles/cm}^3)^2 )</td>
<td>1.01 \cdot 10^{-20} [20]</td>
<td>1.56 \cdot 10^{-20} [21]</td>
</tr>
<tr>
<td>( K', \text{g mole} \cdot \text{cm}^{-3} \cdot \text{atm}^{-1} )</td>
<td>0.33</td>
<td>0.105 [22]</td>
</tr>
<tr>
<td>( n )</td>
<td>24.4</td>
<td>11.46 [23]</td>
</tr>
<tr>
<td>( k_p, \text{s}^{-1} )</td>
<td>8 \cdot 10^5 [13]*</td>
<td></td>
</tr>
<tr>
<td>( K_{at}, \text{g moles/cm}^3 )</td>
<td>1.2 \cdot 10^{-5} [13]*</td>
<td></td>
</tr>
<tr>
<td>( K_{io}, \text{g moles/cm}^3 )</td>
<td>4.6 \cdot 10^{-13} [13]*</td>
<td></td>
</tr>
<tr>
<td>( D_1, \text{cm}^2/\text{s} )</td>
<td>1.96 \cdot 10^{-5} [14]</td>
<td></td>
</tr>
<tr>
<td>( H_1, \text{g moles} \cdot \text{cm}^{-3} \cdot \text{atm}^{-1} )</td>
<td>3.39 \cdot 10^{-5} [15]</td>
<td></td>
</tr>
<tr>
<td>( D_1 H_1, \text{g moles} \cdot \text{cm}^{-1} \cdot \text{atm}^{-1} \cdot \text{s}^{-1} )</td>
<td>3.80 \cdot 10^{-10} [7]</td>
<td></td>
</tr>
<tr>
<td>( D_2, D_3, \text{cm}^2/\text{s} )</td>
<td>1.08 \cdot 10^{4**}</td>
<td>0.89 \cdot 10^{-3***}</td>
</tr>
</tbody>
</table>

* Carbonic anhydrase isolated from bovine red blood cells.

** See text: Physiochemical parameters.
Several factors dictate the validity of each approximation; the principal parameter being the Damköhler number, defined as the ratio of diffusion to reaction resistance. Fig. 1 is an example of the extent of facilitation as a function of Damköhler number. Various limiting solutions as well as the numerical solution are indicated. A detailed discussion is presented in a later section.

The experimental objective of this study was to investigate the CO2 facilitation over a wide range of variables. In the near-diffusion regime, information pertaining to diffusivity and solubility of gases in reacting solutions was obtained [7]. In the near-equilibrium regime, the relative importance of parameters such as CO2 partial pressures at the two interfaces and carbonic anhydrase concentration is investigated. The experimental variables covered in this study are: Total HCO3− concentration, 1 gmoles/l; diffusion path length, 0.02–0.10 cm; CO2 partial pressure, 0.001–1 atm; enzyme concentration, 0.1–1 mg/ml.

NOMENCLATURE

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>A_i</td>
<td>Chemical species i</td>
</tr>
<tr>
<td>C_i^0</td>
<td>Dimensionless total concentration of A_i,</td>
</tr>
<tr>
<td>C_i</td>
<td>Concentration of A_i, gmoles/cm^3</td>
</tr>
<tr>
<td>C_i(0), C_i(L)</td>
<td>C_i at y = 0, L, respectively, gmoles/cm^3</td>
</tr>
<tr>
<td>C_i^0</td>
<td>Total concentration of A_i, gmoles/cm^3</td>
</tr>
<tr>
<td>C_i</td>
<td>Dimensionless concentration of A_i at x = 0, 1, respectively</td>
</tr>
<tr>
<td>C_1</td>
<td>Dimensionless CO2 concentration defined in Eqn 26</td>
</tr>
<tr>
<td>D_i</td>
<td>Diffusivity of A_i in the reaction solution, cm^2/s</td>
</tr>
<tr>
<td>D_i^0</td>
<td>Diffusivity of A_i in the solvent, cm^2/s</td>
</tr>
<tr>
<td>E_0</td>
<td>Total enzyme concentration, gmoles/cm^3</td>
</tr>
<tr>
<td>F</td>
<td>Facilitation factor defined in Eqn 18</td>
</tr>
<tr>
<td>F_e</td>
<td>Equilibrium facilitation factor</td>
</tr>
<tr>
<td>f</td>
<td>Activity coefficient</td>
</tr>
<tr>
<td>g_{ij}</td>
<td>Diffusion-stoichiometric ratio of A_i defined in Eqn 15</td>
</tr>
<tr>
<td>H_i, H_i^0</td>
<td>Henry's Law constant, gmoles • cm^{-3} • atm^{-1}</td>
</tr>
<tr>
<td>I_i</td>
<td>Reaction invariant defined in Eqn 11</td>
</tr>
<tr>
<td>K</td>
<td>Overall equilibrium constant defined in Eqn 21</td>
</tr>
<tr>
<td>K_i</td>
<td>Equilibrium constant for i_th reactions</td>
</tr>
<tr>
<td>K_H, K_{ms}, K_{mi}</td>
<td>Equilibrium constants for enzyme-catalyzed reactions, gmoles/cm^3</td>
</tr>
<tr>
<td>k_i</td>
<td>Forward rate constant for i_th reaction</td>
</tr>
<tr>
<td>k_p</td>
<td>Rate constants for enzyme catalyzed reaction, s^{-1}</td>
</tr>
<tr>
<td>L</td>
<td>Diffusion path length, cm</td>
</tr>
<tr>
<td>m</td>
<td>Equilibrium constant defined in Eqn 26</td>
</tr>
<tr>
<td>N_i</td>
<td>Flux of A_i, gmoles • cm^{-2} • s^{-1}</td>
</tr>
<tr>
<td>N_i^0</td>
<td>Fick's Law flux of A_i through the reaction solution, gmoles • cm^{-2} • s^{-1}</td>
</tr>
<tr>
<td>N_i^e</td>
<td>Equilibrium flux of A_i, gmoles • cm^{-2} • s^{-1}</td>
</tr>
<tr>
<td>N_i^w</td>
<td>Fick's Law flux of A_i through solvent, gmoles • cm^{-2} • s^{-1}</td>
</tr>
<tr>
<td>n</td>
<td>Rate constant defined in Eqn 26</td>
</tr>
<tr>
<td>P_0, P_L</td>
<td>Partial pressure of A_i at y = 0, L, respectively, atm</td>
</tr>
</tbody>
</table>
Before proceeding to derive the necessary equations for the CO₂ facilitation model, we will consider the chemistry and kinetics of the reactions. Dissolution of CO₂ in water is accompanied by the formation of the hydrated compound, H₂CO₃, which subsequently dissociates into HCO₃⁻, CO₃²⁻, and H⁺ (see Kern [12] for historical background). The first step in the neutralization reaction involves the reaction of dissolved CO₂ by either one or both of the following mechanisms:

\[(i) \quad \text{CO}_2 + \text{H}_2\text{O} ~\overset{k_1}{\underset{k_{-1}}{\rightleftharpoons}}~ \text{H}_2\text{CO}_3 \quad (\text{pH} \leq 8)\]

\[(ii) \quad \text{CO}_2 + \text{OH}^- ~\overset{k_2}{\underset{k_{-2}}{\rightleftharpoons}}~ \text{HCO}_3^- \quad (\text{pH} \geq 10)\]  

(1)

Whereas Reactions i and ii, Eqn 1, have finite rate constants, the following extremely rapid reactions are also involved in the reaction scheme:

\[(iii) \quad \text{H}_2\text{CO}_3 \rightleftharpoons \text{H}^+ + \text{HCO}_3^-; \quad K_3\]

\[(iv) \quad \text{HCO}_3^- \rightleftharpoons \text{H}^+ + \text{CO}_3^{2-}; \quad K_4\]  

(2)

In the study of the facilitated transport of CO₂, the reactions listed in Eqns 1 and 2 are carried out in the presence of a base, for example NaHCO₃, and the following rapid and complete dissociation reaction is also to be expected:
(v) \[ \text{MHCO}_3 \rightleftharpoons \text{M}^+ + \text{HCO}_3^- ; K_5 \approx \infty \] (3a)  

To the above set of reactions must be added yet another for the rapid dissociation of water:

(vi) \[ \text{H}_2\text{O} \rightleftharpoons \text{H}^+ + \text{OH}^- ; K_6 \] (3b)  

The overall reaction between \( \text{CO}_2 \), \( \text{HCO}_3^- \), and \( \text{CO}_3^{--} \) may be written as

\[ \text{CO}_2 + \text{CO}_3^{--} + \text{H}_2\text{O} \rightleftharpoons 2\text{HCO}_3^- \] (4)

Since reaction mechanisms i and ii, Eqn 1, are the limiting steps in the overall scheme of reactions, carbonic anhydrase, a catalyst for these steps was used to study the effect of an enhanced rate of reaction on facilitation. Indications are that the enzyme-catalyzed hydration and dehydration reaction obeys the following mechanism [13]:

\[ \text{CO}_2 + \text{H}_2\text{O} + \text{E} \rightleftharpoons \text{X} \rightleftharpoons \text{HCO}_3^- + \text{H}^+ + \text{E} \] (5)

where \( \text{E} \) is the enzyme carbonic anhydrase (\( \text{E}^- \), \( \text{EH} \))

and \( \text{X} \) is an intermediate of the reaction (\( \text{ECO}_2^- \), \( \text{EHCO}_2 \))

The hydration and the inhibition reactions of the enzyme are

\[ \text{E}^- + \text{H}^+ \rightleftharpoons \text{EH} \] (6)

\[ \text{E} + \text{HCO}_3^- \rightleftharpoons \text{EHCO}_3^- (\text{E} = \text{E}^-, \text{EH}) \]

**Physicochemical parameters**

In order to calculate the flux of \( \text{CO}_2 \), several parameters must be known or evaluated. These are the rate and equilibrium constants (for the uncatalyzed as well as the enzyme-catalyzed reactions), diffusivities, and the solubility of \( \text{CO}_2 \) in the reactant solution.

Values for the various rate and equilibrium constants given in the literature are summarized in Table I. The salts \( \text{MHCO}_3 \), \( \text{M}_2\text{CO}_3 \) are assumed to be completely dissociated. The value of \( K_m \) remains unknown at this time.

The widely accepted values quoted in the literature for the diffusivity \( D_1 \) and the solubility \( H_1 \) of \( \text{CO}_2 \) in water are \( 1.96 \cdot 10^{-5} \text{ cm}^2/\text{s} [14] \) and \( 3.39 \cdot 10^{-5} \text{gmoles} \cdot \text{cm}^{-3} \cdot \text{atm}^{-1} [15] \), respectively. In this study, however, it was necessary to know the effect of concentration and ionic strength on these parameters, since \( \text{NaHCO}_3-\text{Na}_2\text{CO}_3 \) solutions were used in the facilitation experiments. In a related publication, we estimated the physical permeability \( (D_1H_1) \) of \( \text{CO}_2 \) through \( \text{HCO}_3^- \) solutions (1 M) to be \( 3.80 \cdot 10^{-10} \text{gmoles} \cdot \text{cm}^{-1} \cdot \text{atm}^{-1} \cdot \text{s}^{-1} [7] \).

The ionic diffusivities of \( \text{HCO}_3^- \) and \( \text{CO}_3^{2-} \) depend on the individual ionic mobilities as well as their concentrations. In dilute solutions, the diffusivities are bounded by the following two limits:

\[ D_2^0, D_3^0 \leq (RTu_2/2, RTu_3) \]

and, at 25 °C, the limits are [16]:
\[ RTu_2 = 0.92 \cdot 10^{-5} \text{ cm}^2/\text{s} \]

\[ RTu_3 = 1.18 \cdot 10^{-5} \text{ cm}^2/\text{s} \]

(Subscripts 2 and 3 refer to \( \text{CO}_3^{2-} \) and \( \text{HCO}_3^- \), respectively.)

In light of the fact that the two bounds for \( D_2^0 \) and \( D_3^0 \) are nearly the same, we assumed that the diffusivities of \( \text{HCO}_3^- \) and \( \text{CO}_3^{2-} \) are equal and a common value was selected arbitrarily at a \( \text{HCO}_3^- \) concentration equal to twice the \( \text{CO}_3^{2-} \) concentration; thus \( D_2^0 \approx D_3^0 \approx 1.08 \cdot 10^{-5} \text{ cm}^2/\text{s} \).

In concentrated solutions, ionic diffusivities \( D_i \) can be obtained from \([16]\)

\[
D_i = (D_i^0 + \mu_i) \left(1 + \frac{d\ln f}{d\ln C}\right) \eta'
\]

(20)

For a 1 M \( \text{NaHCO}_3-\text{Na}_2\text{CO}_3 \) solution, the ‘correction’ terms for the \( \text{HCO}_3^- \) and \( \text{CO}_3^{2-} \) are estimated as

\[
\mu_i \approx 0.04 \cdot 10^{-5} \text{ cm}^2/\text{s}
\]

\[
\frac{d\ln f}{d\ln C} \approx -0.007
\]

and the relative solution viscosity is taken as the average of \( \text{NaHCO}_3 \) and \( \text{Na}_2\text{CO}_3 \) solution viscosities \([24]\) : \( \eta' = 1.27 \). Therefore, for a one normal solution, the ionic diffusivities of the \( \text{HCO}_3^- \) and \( \text{CO}_3^{2-} \) are estimated to be \( D_2 \approx D_3 \approx 0.89 \cdot 10^{-5} \text{ cm}^2/\text{s} \).

**Model formulation**

In presenting the equations for the facilitation of \( \text{CO}_2 \), the derivation for the uncatalyzed reaction scheme, Eqns 1–3, is shown in detail. A model for the enzymatically catalyzed reaction scheme may be similarly derived. The development is based on the premise of diffusion accompanied by homogeneous reversible chemical reaction everywhere within the film. Sufficient boundary conditions for the diffusion–reaction equations are obtained from a knowledge of concentrations (or partial pressures) of the transferred species at the gas–liquid interfaces and the zero-flux condition on entrapped species.

We will adopt the following notation to simplify the presentation of the various diffusion–reaction equations. The various chemical species are denoted as follows:

<table>
<thead>
<tr>
<th>Subscript</th>
<th>Species ( A_i )</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>( \text{CO}_2 )</td>
</tr>
<tr>
<td>2</td>
<td>( \text{CO}_3^{2-} )</td>
</tr>
<tr>
<td>3</td>
<td>( \text{HCO}_3^- )</td>
</tr>
<tr>
<td>4</td>
<td>( \text{M}^+ )</td>
</tr>
<tr>
<td>5</td>
<td>( \text{H}^+ )</td>
</tr>
<tr>
<td>6</td>
<td>( \text{MHCO}_3 )</td>
</tr>
<tr>
<td>7</td>
<td>( \text{OH}^- )</td>
</tr>
<tr>
<td>8</td>
<td>( \text{H}_2\text{O} )</td>
</tr>
<tr>
<td>9</td>
<td>( \text{H}_2\text{CO}_3 )</td>
</tr>
</tbody>
</table>
The overall reaction, Eqn 4, is represented as

\[-A_1 - A_2 - A_8 + 2A_3 = 0\]  

(8)

and the six individual mechanistic steps, Eqns 1-3, are given in matrix form by

\[\mathbf{v}^T(A) = (0)\]  

(9)

where \(v_{ij}\) component of the matrix \(\mathbf{v}\) represents the stoichiometric coefficient of \(i^{\text{th}}\) species participating in the \(j^{\text{th}}\) reaction:

\[
\begin{bmatrix}
-1 & -1 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 1 & 0 & 0 \\
0 & 1 & 1 & -1 & 1 & 0 \\
0 & 0 & 0 & 0 & 1 & 0 \\
0 & 0 & 1 & 1 & 0 & 1 \\
0 & 0 & 0 & 0 & -1 & 0 \\
0 & -1 & 0 & 0 & 0 & 1 \\
-1 & 0 & 0 & 0 & 0 & -1 \\
1 & 0 & -1 & 0 & 0 & 0
\end{bmatrix}
\]

A cursory examination of the stoichiometric coefficients \(\mathbf{v}\) and column operations reveals that only five of the mechanistic steps are stoichiometrically independent. For convenience, the first five reaction rates are chosen to be the linearly independent set. That is, \(v_{16} = v_{11} - v_{12} + v_{13}\).

Denoting \(\omega_j\) as the net rate (forward) of reaction \(j\), the rate of consumption of \(A_i\) is given by

\[-(r) = \mathbf{v}(\omega)\]  

(10)

Now, by row operations on the matrix \(\mathbf{v}\), one finds that certain combinations of reaction rates for various species cancel each other out. The reaction rates of \(A_6\) through \(A_9\) are then given by the following linear equations:

\[
\begin{align*}
r_6 &= -r_4 \\
r_7 &= -2r_2 - r_3 + r_4 + r_5 \\
2r_8 &= -r_3 - r_5 - r_6 - r_7 + 2r_9 \\
r_9 &= -r_1 - r_2 - r_3 - r_6
\end{align*}
\]

These relations determine some invariant properties of the system, which in this case are four in number

\[I_k \equiv \sum_{i=1}^{9} \xi_{ki} r_i = 0, \quad k = 1, 4, \text{ or} \]

\[
\begin{align*}
r_4 + r_6 &= 0 \\
2r_2 + r_3 - r_4 - r_5 + r_7 &= 0 \\
r_3 + r_5 + r_6 + r_7 + 2r_8 + 2r_9 &= 0 \\
\text{and} \\
r_1 + r_2 + r_3 + r_6 + r_9 &= 0
\end{align*}
\]
Of these four relationships, first three are seen to be independent of \( r_1 \), the reaction rate of the transferred species.

Up to this point, we have only considered stoichiometric relationships which result from the kinetic steps in the \( \text{CO}_2 \) reaction mechanism. To complete the analysis one must consider the diffusion process; in general the unsteady-state conservation diffusion equations for each of the species involved are of the form

\[
\frac{\partial C_i}{\partial t} = \frac{\partial N_i}{\partial y} + r_i, \quad i = 1, 9
\]  

(12)

Of these, the first five form, once again, a linearly independent set. For \( i = 6, 8 \), a set of three integral relationships is obtained corresponding to a subset of the reaction invariants given in Eqn 11, i.e. those not involving the permeable species, \( \text{CO}_2 \) [25] or

\[
\frac{1}{L} \int_0^L \sum_{i=2}^9 \xi_{ki} C_i \, dy = C_k^0, \quad \text{or}
\]

\[
\frac{1}{L} \int_0^L (\bar{C}_4 + \bar{C}_6) \, dy = \bar{C}_6^0
\]

\[
\frac{1}{L} \int_0^L (2\bar{C}_2 + \bar{C}_3 + \bar{C}_7 - \bar{C}_4 - \bar{C}_5) \, dy = 0
\]

and

\[
\frac{1}{L} \int_0^L (\bar{C}_3 + \bar{C}_5 + \bar{C}_6 + \bar{C}_7 + 2\bar{C}_8 + 2\bar{C}_9) \, dy = 2\bar{C}_8^0
\]

On inspection, the three integral constraints are seen to define the time invariant "species" of the system, namely, total metal \( M \), total charge density, and total hydrogen \( H \).

Defining the flux \( N_i \) for any species (ionic or molecular) via a binary diffusivity, that is, neglecting any diffusive coupling effect,

\[
N_i = -D_i \frac{dC_i}{dy}
\]

and, further treating the diffusivity as being independent of concentration (dilute solution), Eqn 12 under steady state becomes

\[
D_i \frac{d^2C_i}{dx^2} = r_i
\]  

(14)

The linearly independent diffusion–reaction equations, Eqn 14, are now dimensionalized to give

\[
\frac{d^2C_i}{dx^2} = \sum_{j=1}^6 g_{ij} x_j^2 \phi_j, \quad i = 1, 5
\]  

(15)

where: \( g_{ij} = -D_i v_{ij}/D_i \), \( x_j = k_j^* L^2/D_i \), \( \phi_j = r_j/(k_j^* C_6^0) \), \( C_i = \bar{C}_i/\bar{C}_6^0 \) and \( x = y/L \). \( k_j^* \) is a typical first-order rate constant for the \( j \)th reaction. The appropriate boundary conditions for Eqn 15 are
\[
x = 0 \quad C_1 = \overline{C}_1 \\
x = 1 \quad C_1 = \overline{C}_1
\]

\[
\frac{dC_i}{dx} = 0 \quad \text{at} \quad x = 0, 1, \quad i = 2, 5
\]

where \( \overline{C}_1 \) and \( C_1 \) are dimensionless concentrations of \( C_1 \) at \( x = 0, 1 \), respectively.

The flux of CO₂ across the film is given by

\[
N_1 = \frac{-D_1 \overline{C}_0}{L} \frac{dC_1}{dx} \bigg|_{x=0}
\]

and the facilitation in the flux of CO₂ is defined as

\[
F = \frac{N_1}{N_0^0} - 1
\]

At this stage of the development no general analytical solution exists for this set of equations. However, we have established the minimum equations and boundary conditions that are required for a solution. A method for determining which of these are relatively slow and fast reactions to reduce these equations further, as well as the situation of multiple reactions that are rate limiting, is given elsewhere [25].

We are principally interested in solving the CO₂ diffusion-reaction equation, which is

\[
\frac{d^2C_1}{dx^2} = \alpha_1^2 \phi_1 + \alpha_2^2 \phi_2
\]

where

\[
\alpha_1^2 = k_1 \frac{L^2}{D_1} \\
\alpha_2^2 = k_2 \overline{C}_0 \frac{L^2}{D_1}
\]

and

\[
\phi_1 = C_1 - \frac{1}{K_1}C_9 \\
\phi_2 = C_1 C_7 - \frac{1}{K_2} \overline{C}_0 C_3 \\
\phi_3 = 0 = C_9 - \left( \overline{C}_0^0 / K_3 \right) C_3 C_5 \\
\phi_4 = 0 = C_3 - \left( \overline{C}_0^0 / K_4 \right) C_2 C_5 \\
\phi_6 = 0 = 1 - \left( \overline{C}_0^0 / K_2 \right) C_5 C_7 \\
\phi_5 = 0 = C_6 - \left( \overline{C}_0^0 / K_5 \right) C_3 C_4
\]

Up to this point, the procedure for obtaining the minimal set of equations has been general; to reduce the complexity of the problem further one needs to employ constraints which are particular to a given physical problem. For CO₂ transport, one can use the fact that only Reactions i and ii, Eqn 1, are rate limiting; Eqn 15 may thus be simplified, assuming Reactions iii–vi are at equilibrium everywhere. The equilibrium reactions, Eqns 2 and 3, give:

\[
\phi_3 = 0 = C_9 - \left( \overline{C}_0^0 / K_3 \right) C_3 C_5 \\
\phi_4 = 0 = C_3 - \left( \overline{C}_0^0 / K_4 \right) C_2 C_5 \\
\phi_6 = 0 = 1 - \left( \overline{C}_0^0 / K_2 \right) C_5 C_7 \\
\phi_5 = 0 = C_6 - \left( \overline{C}_0^0 / K_5 \right) C_3 C_4
\]
(The water activity coefficient is assumed equal to one.)

Substituting Eqn 20 in Eqn 19 gives

\[
\frac{d^2 C_1}{dx^2} = \frac{k_1 L^2}{D_1} \left( 1 + \frac{k_2 K_b}{k_1 K_4} \right) \left( \frac{C_1 - 1}{C_2} \right)
\]

where \( K = K_4/K_1 K_3 \) and \( C_1(x = 0) = \tilde{C}_1, \ C_1(x = 1) = \tilde{C}_1 \).

The integral relationships, Eqn 13, in de-dimensionalized concentrations are

\[
\int_0^1 (C_4 + C_6)\,dx = 1
\]

\[
\int_0^1 (2C_2 + C_3 + C_7 - C_4 - C_5)\,dx = 0
\]

and

\[
\int_0^1 (C_3 + C_5 + C_6 + C_7 + 2C_8 + 2C_9)\,dx = 2C^0_8
\]

The reaction invariance equation, Eqn 11, when substituted into Eqn 14 upon integration gives

\[
D_1 C_1 + D_2 C_2 + D_3 C_3 + D_4 C_4 + D_5 C_5 + D_6 C_6 + D_7 C_7 + D_8 C_8 + D_9 C_9 = p_{13} x + q_{13}
\]

Before proceeding to solve the above set of equations, some further simplifications can be made, again for the CO₂ problem, by consideration of the magnitude for various terms as shown in Appendix I. In light of all these simplifications, the resultant CO₂ diffusion-reaction equations become

\[
\frac{d^2 \tilde{C}_1}{dx^2} = \alpha^2 \phi
\]

and

\[
\tilde{C}_1 + \phi C_3 = p_{13} x + q_{13}
\]

where

\[
\tilde{C}_1 = \tilde{C}_1/\tilde{C}_1(0)
\]

\[
\phi = \left( \tilde{C}_1 - m \frac{C_3^2}{1-C_3} \right) \left( 1 + n \frac{1-C_3}{C_3} \right)
\]

\[
m = \frac{2C^0_6}{(KH_1)P_0} \quad n = \frac{k_2 k_6}{2k_1 k_4}
\]

with \( \tilde{C}_1(0) = 1 \) and \( \tilde{C}_1(1) = \rho \).

The corresponding kinetic function, \( \phi_e \), and the diffusion reaction resistance, \( \alpha_e \), for the enzyme-catalyzed portion of the total reaction are

\[
\phi_e = \left( \tilde{C}_1 - m \frac{C_3^2}{1-C_3} \right) |Q|
\]
and

\[ \alpha_e^2 = \frac{k_p E_0 L^2}{K_{ms} D_1} \]

where \( Q \) is either

\[ Q_1 = \left( 1 + C_1 \frac{C_1(0)}{K_{ms}} \right) \left( 1 + C_3 \frac{C_6^0}{K_{mi}} \right) \left( 1 + \frac{C_3}{1 - C_3} \frac{2K_4}{K_H} \right) \]

or

\[ Q_2 = \left( 1 + C_1 \frac{C_1(0)}{K_{ms}} + C_3 \frac{C_6^0}{K_{mi}} \right) \left( 1 + \frac{C_3}{1 - C_3} \frac{2K_4}{K_H} \right) \]

\( Q_1 \) refers to the kinetic mechanism proposed by Kernohan [13], discussed earlier in physicochemical parameters. \( Q_2 \), on the other hand, refers to a situation wherein the substrate-enzyme binding step has a finite rate (Haldane [26] scheme).

Solution methods

The model equations, Eqn. 26, are now solved to obtain the \( CO_2 \) facilitation as a function of several parameters: for example \( \alpha_1, p_0, p_1, \) and \( D_3 \).

a. Near-diffusion regime. As indicated earlier, various limiting solutions to the model equations, Eqn 26, can be obtained. The solution in the near-diffusion regime, that is, for \( \alpha_1 \to 0 \), is developed elsewhere [7]. The facilitation in the flux of \( CO_2 \) was shown to be given by

\[ F = \frac{1}{\alpha_1} \frac{1}{\alpha_1} Y_0 \frac{1}{\gamma_2 Y_0} \left[ Y_0 - \frac{6 - \zeta}{g} \right] \alpha_1^4 + \ldots \quad (28) \]

where

\[ Y_0 = 1 + n \frac{1 - \beta_3}{\beta_3} \]

\[ Y_1 = mY_0 \left[ 1 - \frac{1}{(1 - \beta_3)^2} \right] \]

\[ Y_2 = n/\beta_3^2 \]

and

\[ \zeta = \frac{1}{2}(1 - \rho)Y_2^2/Y_1^2 \]

The constant \( \beta_3 \) is obtained by solving the quadratic equation:

\[ m \frac{\beta_3^2}{(1 - \beta_3)} = \frac{1}{2}(1 + \rho) \quad (29) \]

b. Near-equilibrium regime. The limiting analytical solution for \( \varepsilon_1 \to 0 \ (\varepsilon_1 = 1/\alpha_1) \), based on the development of Goddard et al. [8] and applied to Eqn 26 is
\[
\frac{N_1}{N_0} = 1 - e_1(\bar{x} + \chi) \tag{30}
\]

where

\[
\chi = \phi^1/\eta(\eta^2 - \phi^1) \\
\eta^2 = \phi^1 + g\phi^3 \\
\phi^i = \frac{\partial \phi}{\partial C_i} \text{ at } C_i = \psi_i
\]

where overbars and underbars refer to evaluation at the boundaries \(x = 0, 1\), respectively, and the "equilibrium" bicarbonate concentration \(\psi_3\) is obtained by solving \(\Phi = (\phi)_{\psi_i} = 0\) or:

\[
m \frac{\psi_3^2}{1 - \psi_3} = \psi_1
\]

\(m\) with \(\psi_1(0) = 1, \psi_1(1) = \rho\).

The convergence of this analytical solution cannot be determined explicitly: the range of \(\alpha_1\) for which Eqn 30 is valid, must therefore be estimated by some alternative method, such as the boundary approximations of Kreuzer and Hoofd [9] and Smith et al. [10].

c. Boundary layer approximation. Based on the generalized equations derived in Appendix II, the boundary layer approximation for the \(\text{CO}_2-\text{HCO}_3^-\) equations, Eqn 26, is as follows:

\[
\psi_1(0) = 1 - \frac{\bar{\phi}^1}{\alpha_1 \bar{\eta}^3} (1 - \rho) \frac{N_1}{N_0} \\
\psi_1(1) = \rho + \frac{\bar{\phi}^1}{\alpha_1 \bar{\eta}^3} (1 - \rho) \frac{N_1}{N_0}
\]

The solution to Eqn 32 is necessarily iterative. For initiating the iterative procedure, it is assumed that the solution corresponds to the equilibrium chemical reaction limit, that is, \(\psi_1(0) \approx 1\) and \(\psi_1(1) \approx \rho\).


\[
\frac{N_1}{N_0} = \frac{1}{1 - \frac{Y_0}{\langle \eta^2 \rangle} \mathcal{J}}
\]

where

\[
\mathcal{J} = 1 - \frac{2e_1}{\langle \eta \rangle} \tanh \frac{\langle \eta \rangle}{2e_1}
\]
and
\[ <\eta^2> = \eta^2 \text{ evaluated at } C_i = \beta_i \]

**e. High carrier concentration.** The Ward analysis [2] for the limit of high carrier concentration gives

\[ \frac{N_i}{N_0} = \frac{\sqrt{Y_0}}{2\epsilon_1} \text{ Coth} \frac{\sqrt{Y_0}}{2\epsilon_1} \]

In the limit \( \zeta \to 0 \), Eqns 33 and 34 reduce, up to a first approximation, to Eqn 28.

**f. Quasilinearization.** The numerical solution to the \( \text{CO}_2-\text{HCO}_3^- \) equation, Eqn 26 is obtained by the quasilinearization technique [27, 28]. The quasilinearization procedure was initiated at small diffusion path lengths and a cubic profile for the concentrations assumed:

\[ C_i \approx \sum_{j=0}^{3} a_{ij} x^j \quad i = 1, 3 \]

The constants \( a_{ij} \) are evaluated by applying the boundary conditions on \( C_i \): \( a_{10} = 1; \Sigma_{j=0}^{3} a_{1j} = \rho; 2a_{12}+3a_{13} = 0 \) and \( a_{21} = 0; 2a_{32}+3a_{33} = 0 \).

An additional relationship results by virtue of reaction invariant equation, Eqn 27: \( a_{32} = -a_{12}/g \).

The two unknowns \( a_{11} \) (related to the flux of \( \text{CO}_2 \) at the two boundaries) and \( a_{20} \) (the \( \text{HCO}_3^- \) concentration at \( x = 0 \)) are obtained from the first order near-diffusion regime solution, Eqn 28.

**Typical solutions**

The various limiting analytical solutions, the boundary layer approximation and the numerical solution outlined above are examined here in order to form a complete picture of the \( \text{CO}_2 \) facilitation system.

Fig. 1 shows facilitation factor \( F \) as a function of the diffusion-reaction parameter \( \alpha_1 \) (Damköhler number). Although the analytical solutions in the near-diffusion regime, Curve I, and the near-equilibrium regime, Curve IV, have limited convergence, such solutions nevertheless provide one with an approximate shape of the facilitation curve. The range of validity of the boundary layer approximation, Curve V, far exceeds the range in which either of the other asymptotic solutions are valid. At higher values of the diffusion-reaction resistance, \( \alpha_1 \), the numerical solution begins to break down, due principally to extreme concentration gradients near the boundary \( x = 1 \) [28]. It is in this region of Damköhler number \( \alpha_1 \), where the quasilinearization numerical analysis nor the asymptotic solutions are valid, that the boundary layer approximation has maximum utility. Even in regions where the numerical method gives a correct solution, the boundary layer approximation is preferred due to its low computational cost. The latter method tends to break down at exceedingly low Damköhler numbers; failure was evident as the computations did not converge (oscillated between two limits) or negative facilitations were calculated*.

The calculated \( \text{CO}_2, N_1 \), flux at various values of diffusion path lengths \( L \) is shown in Fig. 2. Also included in Fig. 2 are fluxes of \( \text{CO}_2 \) across a (i) water film, \( N_1^{\infty} \); (ii)

* See Notes added in Proof (A), p. 439
Fig. 2. Expected total CO₂ flux across a membrane (of thickness L) containing 1 M NaHCO₃. Calculations are for CO₂ partial pressures at the two boundaries of 1.0 and 0.01 atmos.

1 M NaHCO₃–Na₂CO₃ film in absence of any reaction, \( N_{1}^{0} \); (iii) 1 M NaHCO₃–Na₂CO₃ film at chemical reaction equilibrium, \( N_{1}^{e} \).

EXPERIMENTAL METHODS

The experimental equipment used in this study is essentially the same as the one described by Wittenberg [29] and used by Bassett and Schultz [30]. Only a brief description of the equipment is presented here. Relevant membrane properties and the enzyme activity estimates are also included in this section.

**Experimental equipment**

The experimental equipment consists of the following components: (a) Gas cylinders containing CO₂, N₂, and He. N₂ was used to obtain the desired partial pressure of CO₂ in the upstream chamber of the diffusion cell. He was used as the carrier gas in the gas chromatograph as well as to sweep away the CO₂ (and N₂) that diffused across the test membrane. (b) A gas saturator made of spargers placed in water-filled flasks to humidify all gases prior to their entry into the diffusion chambers. This step was necessary in order to avoid loss of water from the test membrane due to evaporation. (c) A lucite diffusion cell made up of two chambers, which will be referred to hereafter as the upstream and downstream chambers. A membrane consisting of a microporous filter saturated with the test solutions was interposed between the two chambers, and gas mixtures of known composition were circulated through the upstream and downstream sides. The circular area of the membrane exposed in the diffusion cell was 15.5 cm². The diffusion cell and gas humidifier flasks were placed in a water bath maintained at 25±0.5 °C. (d) Soap bubble flow meter (volume: 10 cm³) placed at the exit of downstream chamber of the diffusion cell to measure accurately the flow rate of the downstream gas mixture. The CO₂ flux was calculated from the total flow rate and composition of the downstream gas mixture. (e) A water vapor trap, consisting of a cooling finger placed in an ice–H₂SO₄ flask for removing

** See Notes added in proof (B), p. 439
water to protect the silica gel column packing in the gas chromatograph. (f) A gas chromatograph and accessories (thermal conductivity cell, recorder, and integrator) for analysis of dried influent and effluent gas samples. The gas chromatograph was equipped with a silica gel column (diameter: 1/8 inch, length: 6 feet) to facilitate separation of CO\textsubscript{2} and N\textsubscript{2}. The pressure drop between the diffusion chamber and the gas chromatograph was approximately two inches of water. For all purposes, the partial pressure indicated by the gas chromatograph is assumed to be that at the exit of the diffusion chamber. In each of the diffusion chambers, the gas is assumed to be well mixed*.

Appropriate downstream partial pressure of CO\textsubscript{2} was obtained by adjusting the flow rate of He through the downstream chamber of the diffusion cell**. Various diffusion path lengths were obtained as a "sandwich" formed by placing two or more Gelman membranes side-by-side.

**Membrane properties**

A highly porous Gelman GA-6 membrane*** with the following nominal specifications was used to support the liquid film used in this study: thickness, 150 μm; mean pore size, 0.45 μm; porosity, 85%.

The properties of the Gelman membrane that are of interest to this study are the membrane thickness, porosity, and tortuosity. The thickness and porosity of the membrane were calculated from volume estimates. The "dry" volume of the membrane was estimated from the weight of a sample (membrane polymer density: 1.66 gm/cm\textsuperscript{3}). Subsequently, the sample was soaked in distilled water, and the volume of water estimated. The thickness was calculated from total volume per unit area of the sample, and the porosity calculated as the void fraction volume (that is, volume of water/total volume). For a single membrane, the volume measurements gave the following average results: thickness, 162.3±10.8 μm; porosity, 0.883±0.013.

Measurements on a sandwich of 3–5 membranes revealed that negligible inter-membrane liquid was present.

The diffusion thickness of each membrane is greater than the geometrical thickness because of tortuosity. Based on measurements of CO\textsubscript{2} diffusion through membranes containing only water and no carbonates, a membrane tortuosity of 1.21 was estimated [7] and therefore a diffusion thickness of 200 μm per membrane was used.

**Enzyme assay**

The activity of enzyme carbonic anhydrase was studied by following the enzyme hydrolysis of p-nitrophenyl acetate. Acetazolamide, an inhibitor that binds 1:1 with the enzyme, was used at a number of different levels to titrate the amount of active enzyme available for hydrolysis [31]. From these kinetic studies, the activity of a 1 mg/ml enzyme solution was estimated to be 88 % and it decreased to roughly 77 % at the end of one year. For purposes of subsequent calculations, an enzyme activity of 80 % will be assumed.

* The volumetric flow rate of gases through the diffusion chambers ranged from 20 to 80 cm\textsuperscript{3}/min.


*** It was not possible to obtain the same values of PL in all experiments; in most cases PL/Po was less than 0.04.
Certain precautions were necessary in handling of enzyme carbonic anhydrase. The enzyme solution was prepared at a concentration of 1 mg/ml and stored in 2-ml portions at $-40^\circ$C. The solid enzyme sample was stored at $4^\circ$C. 1 day prior to the experiment, the enzyme solution was removed and placed in water maintained at $4^\circ$C.

RESULTS

The presence of dissolved Na$_2$CO$_3$ and NaHCO$_3$ within the film has two competing effects: namely, a decrease in the CO$_2$ flux due to decreased solubility and diffusivity of CO$_2$, and an increase in the CO$_2$ flux due to the reaction-related facilitation effect. The aim of this section is to investigate the latter effect in detail.

Facilitation in the flux of CO$_2$-uncatalyzed reaction

The results of experiments at three different path lengths, and at various partial pressure driving forces, are shown in Fig. 3. The total sodium base concentration $(C_0^\infty)$ in all these experiments was 1 gmole/l. Also shown in Fig. 3 is the expected unfacilitated Fick's Law flux of CO$_2$ across the film based on a CO$_2$ permeability $D_1H_1$ of $3.80 \cdot 10^{-10}$ mole $\cdot$ cm$^{-1} \cdot$ atm$^{-1} \cdot$ s$^{-1}$ determined by an extrapolation procedure given in a separate study [7]. The facilitation factors $F$ at diffusion path lengths of 600 $\mu$m and 1000 $\mu$m are shown in Fig. 4. As should be expected, the facilitation factor is observed to increase monotonically with increasing diffusion path length. That the uncatalyzed reaction is far from equilibrium is seen by comparing the corresponding maximum equilibrium facilitation factors (i.e. for $x_1 \to \infty$) which range from 5.6 to approx. 80 for this same range of conditions.
Facilitation in the flux of CO₂-enzyme-catalyzed reactions

In order to investigate the near-equilibrium regime for this system, it is necessary to increase the Damköhler number to 100 or more. This was achieved by the introduction of carbonic anhydrase which catalyzes the hydration reaction.

The flux of CO₂ at a diffusional path length of 200 μm and an enzyme concentration of 0.08 mg/ml (based on 80% activity of enzyme preparation) is shown in Fig. 5 at various partial pressure driving forces. The experiments were conducted over a period of 8–12 h and on three different occasions. For each of the three runs, a fresh NaHCO₃ solution was prepared and a new carbonic anhydrase sample used. The enzyme is seen to produce a very significant effect on the CO₂ flux; at a partial pressure driving force of about 0.1 atmos., the CO₂ flux is at least 10-fold higher than that observed in the absence of the catalyst. The lower solid straight line in Fig. 5 is the estimated CO₂ flux in absence of any facilitation reactions. The solid curve adjacent to the data points, in this and subsequent figures represents the solution to the
enzyme-catalyzed diffusion reaction equations, Eqn 27, by the boundary layer approximation (see Appendix II).

In the limit of zero partial pressure driving force, the approximate method of Ward [2] is of great use, for it provides the limiting slope of the CO$_2$ flux at zero partial pressures. An interesting observation is that as the limit of zero concentration of the transferred species is approached, the carrier, normally a mediator in the transport of CO$_2$, becomes the principal transport mode for CO$_2$. The expected concentration profiles in this limit will be presented below.

The next series of experiments were designed to investigate the relationship between the diffusion path length and enzyme concentration (or reaction rate constant). As indicated earlier in Eqn 27, for the enzyme-catalyzed reaction of CO$_2$ the diffusion–reaction resistance and the kinetic function expressions were

$$\alpha_c^2 = \frac{k_p E_0 L^2}{K_m D_1}, \quad \phi_c = \left( \tilde{C}_1 - m \frac{C^2_3}{1 - C_3} \right) / Q$$

The facilitation factor, $F$, depends, among other factors, on $\alpha_c$, therefore on the enzyme concentration $E_0$ and diffusion length $L$. But since $E_0$ and $L$ do not appear elsewhere in the equations, it would be expected that if the product $E_0 L^2$ is maintained constant, the facilitation factor $F$ should also be a constant even if the individual values of enzyme concentration and thickness are changed. The results of the experiments at two different path lengths and enzyme concentrations, but a constant value of $E_0 L^2$, are shown in Fig. 6. The solid lines adjacent to the experimental data are calculated fluxes at two different downstream partial pressures, $P_L$; the latter ranged from $5 \cdot 10^{-3}$ to $1.17 \cdot 10^{-2}$ atmos. in the experiments. The facilitation factor $F$ for these experiments is plotted in Fig. 7. It is noted that an increase in $\alpha_c$ (due either to increased $L$ or $E_0$) results in an increased facilitation factor $F$. The results also indicate that the constancy of $E_0 L^2$ does indeed result in the same measured facilitation factor.

Otto [21] observed that in the presence of 1 M NaHCO$_3$, the enzyme-catalyzed CO$_2$ reaction rate did not increase in direct proportion to the total enzyme concentration, $E_0$. If this is the case, then the kinetic function, $\phi_c$, would also contain terms in

![Fig. 6. Flux of CO$_2$ at a fixed Damköhler number (constant $E_0 L^2$) illustrating that the reaction rate increases linearly with enzyme concentration. Note different scales used to compensate for greater Fick's Law flux obtained with thinner membranes.](image)
enzyme concentration, $E_0$, and mere constancy of $E_0 L^2$ would not be sufficient to obtain a unique value of facilitation factor $F$. The fact that our experimental results conform to such a unique relationship leaves the discrepancy between Otto's results and ours still to be explained.

The relationship between the diffusion–reaction resistance, $\alpha_e$, and downstream CO$_2$ partial pressures was investigated further. The results reported in Fig. 8 are for a diffusional path length of 0.1 cm. (corresponding to a composite of five Gelman membranes) and at three carbonic anhydrase concentrations. At lower downstream CO$_2$ partial pressures, increasing enzyme concentration (or, in other words, $\alpha_e$) resulted in a corresponding increase in the CO$_2$ flux. Such an observation would indicate that the condition of chemical reaction equilibrium has not yet been attained. On the other hand, at higher downstream CO$_2$ partial pressures, the observed increase in flux at higher enzyme concentrations is comparatively small, indicating that chemical reaction equilibrium exists in most parts of the film. The drop in CO$_2$ flux due to an increased downstream CO$_2$ partial pressure $P_L$ is extremely significant; at an enzyme concentration of 0.8 mg/ml, changing $P_L$ from 0.0066–0.066 atmos., a 6% difference in the overall gradient, results in a reduction of the CO$_2$ flux from 0.38 to 0.18 cm$^3$/min.

DISCUSSION

In the uncatalyzed facilitation experiments several interesting features emerge from the fact that the system is in the near-diffusion regime as indicated by the rather
small observed facilitation factors. First, the downstream CO\textsubscript{2} partial pressure, \(P_L\), has negligible effect on the facilitation factor. Secondly, in this regime, calculations of facilitation show that fluxes are relatively insensitive to a number of parameters that affect it. For example, recall that the first-order near-diffusion solution, Eqn 28, did not contain any terms in carrier diffusivity. As a consequence, for the model solution indicated in Fig. 3 and 4, the facilitation factor changed by less than 2%, for a carrier diffusivity change of over 20%. The diffusion–reaction resistance parameter \(\alpha_1\) at a path length of 1000 \(\mu\)m is roughly 5.

Although one has exceeded the convergence limit of the near-diffusion regime analysis, the carrier diffusivity effect is nevertheless small. Yet another factor, the diffusivity of CO\textsubscript{2}, affects the model solution only slightly in this regime—a 20%, change in diffusivity resulting in a change in the facilitation factor by less than 1%.

The model solution indicated in Figs 3 and 4 are for the following parameter values:

\[
\begin{align*}
  k_1 & = 0.0375 \text{ s}^{-1} \\
  K' & = 0.105 \text{ gmole} \cdot \text{cm}^{-3} \cdot \text{atm}^{-1} \\
  n & = 11.46 \\
  D_1H_1 & = 3.80 \times 10^{-10} \text{ moles cm}^{-1} \cdot \text{atm}^{-1} \cdot \text{s}^{-1} \\
  D_1 & = 1.6 \times 10^{-5} \pm 0.2 \times 10^{-5} \text{ cm}^2/\text{s} \\
  D_3 & = 0.88 \times 10^{-5} \pm 0.1 \times 10^{-5} \text{ cm}^2/\text{s} \\
  \rho & = 0.01–0.04
\end{align*}
\]

![Fig. 9. Dimensionless concentration (CO\textsubscript{2}, HCO\textsubscript{3}–, H\textsuperscript{+}) and reaction (\(\phi\)) profiles within a membrane. Thickness \(L\), 0.5 cm; \(P_0\), 1.0 atmos.; \(\rho\), 0.01.](image-url)
The calculated concentrations of CO$_2$ ($\bar{C}_1$), HCO$_3^-$ ($C_3$), and H$^+$ ($C_5$), and the reaction rate within the film at an imposed CO$_2$ partial pressure, $P_0$, of 1.0 atmos. and 0.066 atmos. are shown in Figs 9 and 10, respectively. The calculations are for a film thickness of 0.5 cm, corresponding to $\alpha_1 = 23.4$. Several theoretical features of CO$_2$-HCO$_3^-$ facilitated transport phenomenon are evident, namely: (a) The reaction boundary layer phenomenon of Goddard et al. [8] is highly visible, as evidenced by finite reaction rates in the regions close to the boundaries. (b) At the lower $P_0$, the transport process inside the core region approaches a form of strictly carrier transport; the latter being defined as a transport process wherein the carrier is the sole mediator of transport process. (c) The Ward analysis [2] for the limit of “large carrier concentration” gives, at an upstream CO$_2$ partial pressure $P_0 = 0.066$ atmos. a facilitation factor, $F$, twice as large as one calculated here although $\langle \bar{C}_3 \rangle \approx 800$ $\langle \bar{C}_1 \rangle$. As such, for this particular case, one concludes that the “large carrier concentration” limit has not yet been achieved. (d) The pH across the film varies by 0.3 unit at the lower $P_0$ and 1.0 unit at the higher $P_0$; the minimum and maximum pH within the film being 7.56 and 9.08. The assumption regarding negligible H$^+$ and OH$^-$ concentration within the film made earlier in simplifying the model equations is therefore valid.

Inasmuch as the errors and deviation in the experimental data (±4 %) are significantly higher than the sensitivity of the model solution to its parameters, one cannot use the experimental data in the near diffusion regime to obtain accurate values of certain parameters, such as CO$_2$ and carrier diffusivities.

In the enzyme-catalyzed facilitation experiments, the solid lines adjacent to the data points represent solution to the model, Eqn 27. The model parameters were
chosen with a view of obtaining a best fit between model solution and experimental results for all the enzyme facilitation data reported in this study. A set of model parameters which resulted in an excellent agreement between model solution and experimental results for a given set of data did not necessarily result in similar agreement for a different set of data. The set of model parameter listed in Table II resulted in a model solution which agreed with all the experimental data to within $\pm 10\%$.

**TABLE II**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Parameter value “Best Fit”</th>
<th>Literature</th>
</tr>
</thead>
<tbody>
<tr>
<td>$k_p$, s$^{-1}$</td>
<td>$8 \cdot 10^5$</td>
<td>$8 \cdot 10^5$ [13]</td>
</tr>
<tr>
<td>$K_{m0}$, gmoles/cm$^3$</td>
<td>$12 \cdot 10^{-5}$</td>
<td>$12 \cdot 10^{-5}$ [13]</td>
</tr>
<tr>
<td>$K_H$, gmoles/cm$^3$</td>
<td>$5 \cdot 10^{-12} \pm 1 \cdot 10^{-12}$</td>
<td>$4.6 \cdot 10^{-13}$ [13]</td>
</tr>
<tr>
<td>$K_{m1}$, gmoles/cm$^3$</td>
<td>$Q = Q_1$</td>
<td>$1 \cdot 10^{-3} \pm 0.2 \cdot 10^{-3}$</td>
</tr>
<tr>
<td>$K'$, gmoles·cm$^{-3}$·atm$^{-1}$</td>
<td>$0.12 \pm 0.1$</td>
<td>0.105</td>
</tr>
<tr>
<td>$D_3$, cm$^2$/s</td>
<td>$0.80 \cdot 10^{-5} \pm 0.01 \cdot 10^{-5}$</td>
<td>$0.89 \cdot 10^{-5}$*</td>
</tr>
<tr>
<td>$D_1H_1$, gmoles·cm$^{-1}$·atm$^{-1}$·s$^{-1}$</td>
<td>$3.8 \cdot 10^{-10} \pm 0.1 \cdot 10^{-10}$ [7]</td>
<td></td>
</tr>
<tr>
<td>$D_1$, cm$^2$/s</td>
<td>$1.6 \cdot 10^{-5} \pm 0.1 \cdot 10^{-5}$</td>
<td></td>
</tr>
</tbody>
</table>

* Estimated in Physicochemical parameters.

For the data presented, the model solutions based on the kinetic mechanisms of Kernohan [13] and Haldane [26] cannot be distinguished. The ionization pH of the enzyme carbonic anhydrase appears to be about 1 unit less than that reported by Kernohan [13]. A significant conclusion of this study seems to be that the facilitation experiments presented here may not be used either to investigate the kinetic mechanism of a reaction scheme or to determine, with any degree of accuracy, certain model parameters. A non-linear regression analysis of the flux data would be of little use in obtaining accurate model parameter value since only a significant change in the model parameters will show any change in the calculated flux. For example, the HCO$_3^-$ diffusivity has to be changed by at least ten percent to obtain a 2 %, or so, change in the calculated flux. The errors in measured flux being of the order of $\pm 4\%$, no accurate information regarding the bicarbonate diffusivity can be obtained. Suffice it to say that for the model described here, a reasonable fit is obtained between the model solution and the experimental results.

A final comment regarding some observations by Otto and Quinn [6] is in order. At a diffusion path length of 630 $\mu$m and a K$^+$ concentration of 1 and 2 M, Otto and Quinn report that the ratio of the observed flux to the calculated equilibrium flux reaches a maximum at an enzyme concentration of 0.5 mg/ml; at higher enzyme concentrations, the said ratio levels out. This behavior is contrary to the predictions of the diffusion-reaction equations used in this study. Otto and Quinn attribute their observation to some abnormal enzyme phenomena, such as changes in the enzyme orientation at high concentrations. Their results on inhibition of the enzyme by HCO$_3^-$ indicate that the rate of reaction of CO$_2$ in enzyme solutions increases monotonically, though not linearly, with the enzyme concentration. As a result, the facili-
tation in the flux of CO₂ should also increase monotonically with increasing enzyme concentration. The results reported here indicate that the flux of CO₂ at a given set of parameter values such as film thickness, upstream and downstream CO₂ partial pressure always increased with increasing enzyme concentration.

In terms of the separation of permeable substances across membranes, it should be noted that whereas the facilitation factor, $F$, (and therefore the extent of separation of gases) increases at high diffusion path lengths (or Damköhler numbers) and lower interfacial concentrations, the corresponding value of the absolute CO₂ flux decreases. An optimum condition is therefore obtained when the facilitation factor, $F$, as well as the CO₂ flux are high. For the system studied here one such optimum condition appears to be at a diffusion path length of 200 μm or less, CO₂ partial pressure, of about 1/6 atmos. at the gas–liquid interface, and the presence of NaHCO₃ (1 M) and enzyme carbonic anhydrase (0.5 mg/ml or more). The CO₂ flux under these conditions would be roughly $3 \cdot 10^{-8}$ gmole·cm⁻²·s⁻¹; the flux of O₂ under identical conditions is roughly $1.2 \cdot 10^{-10}$ gmole·cm⁻²·s⁻¹, giving a CO₂/O₂ separation factor of about 250. Alternatively, if air at one atmosphere (CO₂: 0.033 %) was maintained at the gas–liquid interface, the ratio of CO₂ flux to the N₂-O₂ flux would be about 1 compared to only 1/60 across a corresponding water film.

SUMMARY AND CONCLUSIONS

HCO₃⁻ and CO₃²⁻ entrapped within a liquid film were found to facilitate, or enhance, the flux of carbon dioxide across the liquid film as a result of the following reversible reaction within the film: CO₂ + CO₃²⁻ + H₂O ↔ 2HCO₃⁻. The facilitation studies were carried out in a NaHCO₃ solution; the reaction scheme then involves a total of nine species and six kinetic steps. An analysis based on assuming the validity of the macroscopic diffusion and kinetic equations at every point within the film and the following further conditions accounted for all aspects of the experimental data: (a) Electrical effects are absent and the membrane is electrically neutral, (b) diffusion process may be described by binary diffusivities and mobility interactions are absent, (c) at the liquid–gas interface, the partial pressure in the gas phase is related to the concentration in the liquid phase via a Henry’s law constant. Of the six kinetic steps, only the two involving CO₂ are rate limiting; the remaining four being extremely rapid and therefore assumed to be at chemical equilibrium. The mathematical treatment of the diffusion–reaction equations were simplified on the basis of the observations that (a) $D_{\text{HCO}_3^-} \cong D_{\text{CO}_3^{2-}}$; (b) $H^+ \ll \text{Na}^+$; (c) $\text{OH}^- \ll \text{HCO}_3^- + 2\text{CO}_3^{2-}$, (d) NaHCO₃ fully dissociated.

Enzyme carbonic anhydrase was used as a catalyst in the CO₂ reactions. In analyzing the enzyme catalyzed reactions it was assumed that the rate of CO₂ conversion was directly proportional to the total enzyme concentration.

The solutions to the mathematical model indicate that with increasing Damköhler number the extent of facilitation increases monotonically and approaches the limit of equilibrium facilitation. The experimental study conformed to such a prediction and no abnormal behaviour in the kinetics of enzyme carbonic anhydrase were noticed.

Two different kinetic inhibition schemes for the enzyme catalyzed reactions
gave numerical results that were almost identical (within \( \pm 2\% \)). In light of this, reasonable correspondence between model calculations and experimental data should be viewed with some caution as evidence for a particular model. Also, precise information regarding model parameter values and/or the determination of a reaction kinetic scheme should not be expected from such an experimental arrangement.

In considering the physiological significance of this study, one must allow for a number of differences in the physiological transfer of CO\(_2\). The diffusion path length of a biological cell being considerably smaller than thicknesses studied here, the enzyme carbonic anhydrase would play a major role in the transfer of CO\(_2\). The physiological reaction medium contains, besides the HCO\(_3^-\) ion, several other species, such as HPO\(_4^{2-}\) and Cl\(^-\) which influence the reactions and catalysis. A direct application of the experimental results presented here to the estimation of CO\(_2\) flux under physiological conditions without accounting for the differences in composition may be erroneous. However, the mathematical treatment presented here should be of great use in such a context.

APPENDIX I

Justification of simplifications of model equations

All calculations shown below are for a 1 M NaHCO\(_3\)-Na\(_2\)CO\(_3\) solution, that is, \( c_6^0 = 1 \) gmole/l.

(i) In conducting the facilitation studies, a strong dissociating salt (for example, NaHCO\(_3\)) is added to obtain significant HCO\(_3^-\) concentration. Thus, one may safely assume that \( k_5 \approx \infty \) or that \( C_0 \ll C_4 \). Eqn 24a thus simplifies to

\[
C_4 \approx 1, \quad \text{at all } x \text{ values (1.1)}
\]

(ii) Typical ranges of CO\(_3^{2-}\) and HCO\(_3^-\) concentration (\( C_2 \) and \( C_3 \)) are: \( C_2, 0.98 \pm 0.20; C_3, 0.01-0.40 \). The ranges of OH\(^-\) and H\(^+\) concentration (\( C_7 \) and \( C_5 \)) are calculated from Eqn 22 to be: \( C_7, 1.1 \cdot 10^{-4}-5 \cdot 10^{-7}; C_5, 1.4 \cdot 10^{-10}-3 \cdot 10^{-8} \). In view of this, one may neglect the concentration of OH\(^-\) and H\(^+\) from the zero charge density constraint, Eqn 25b, to give

\[
\int_0^1 (2C_2 + C_3)dx \approx \int_0^1 C_4 dx \approx 1
\]

Further, since it has been shown in a preceding section that \( D_2 \approx D_3 \) the above constraint reduces to

\[
2C_2 + C_3 \approx 1, \quad \text{at all } x \text{ values (1.2)}
\]

(iii) Finally, the flux equation, Eqn 26, may be simplified since (a) \( D_6 C_6 \approx 0 \), at all \( x \) values and (b) \( C_9 \ll 10^{-2} C_1 \).

Thus,

\[
D_1 C_1 + D_2 C_2 + D_3 C_3 \approx p_{13} x + q_{13}
\]

A further transformation of the diffusion-reaction equations is made so as to relate the CO\(_2\) flux to its partial pressure: thus, Eqn 23 along with the simplifications proposed here is written as
\[
\frac{d^2 \tilde{C}_1}{dx^2} = \frac{k_1 L^2}{D_1} \phi
\]
\[
\tilde{C}_1 + gC_3 = p_{13} x + q_{13}
\]
where
\[
\tilde{C}_1 = \frac{\tilde{C}_1}{C_1(0)}
\]
\[
\phi = \left( \frac{C_1 - C_3^2}{1 - C_3} \right) \left( 1 + n \frac{1 - C_3}{C_3} \right)
\]
\[
g = \frac{1}{2} \frac{D_3 \dot{C}_6^0}{(D_1 H_1)P_0}
\]
\[
m = \frac{2C_6^0}{(KH_1)P_0} \quad n = \frac{k_2 K_6}{2k_4 K_4}
\]
The boundary conditions on Eqn 1.4 are
\[
\tilde{C}_1(0) = 1
\]
\[
\tilde{C}_1(1) = \rho \equiv P_L P/H_0
\]
\[
\frac{dC_3}{dx} = 0 \quad \text{at} \quad x = 0, 1.
\]

APPENDIX II

Boundary layer approximation

Kreuzer and Hoofd [9, 32] and Smith et al. [10] have presented a boundary layer approximation for solving the non-linear diffusion-reaction equations. The approximation presented by these authors was limited to reactions of the type: 
\[
A_1 + A_2 \rightleftharpoons A_3.
\]
Their approach is generalized herein so as to be applicable to any arbitrary kinetic function \( \phi \).

The governing equations for facilitated transport phenomenon in the presence of single chemical reaction may be written as
\[
\frac{d^2 C_i}{dx^2} = g_i x^2 \phi
\]
\[
g_i C_j - g_j C_i = p_{ij} x + q_{ij}
\]
\[
\int_0^1 (\text{Carrier})dx = C_3^0
\]
where
\[
g_i = \frac{D^*/D_1 v_1}{x^2} = k^*L^2/D^*, \phi = \omega/k^*C^*, C_i = \tilde{C}_i/C^* \text{ and } x = y/L.
\]
It can be shown that, for any set of given \( p_{ij} \) and \( q_{ij} \), there exist concentration functions \( \psi_i \) which satisfy the equilibrium relationship and the stoichiometric invariance relationships:
\[
(\phi)_{\psi_i} = 0
\]
\[
g_i \psi_j - g_j \psi_i = p_{ij} x + q_{ij}
\]
The perturbation of the "actual" concentration functions $C_i$ from the equilibrium concentration functions $\psi_i$ may be defined by a single parameter:

$$C_i = \psi_i + g_i \delta$$ (II.6)

The parameter $\delta$ behaves in a manner similar to the boundary layer correction of Goddard et al. [8]. Within the central portion of the film, the equilibrium concentration functions adequately describe the transport process and $\delta \to 0$. Within regions close to the boundaries, $\delta$ permits one to correct for deviations from equilibrium: however, such deviation may not necessarily be a result of finite $x$ alone.

The kinetic function $\phi$ is now expanded in a (first-order) Taylor series around the equilibrium concentration functions; thus,

$$\phi = \Phi + \eta^2 \delta$$ (II.7)

where $\Phi \equiv (\phi)_{\psi_i} = 0$, $\eta^2 = \sum \phi^j g_j$ and $\phi^j = \partial \phi / \partial C_j$ at $C_j = \psi_j$.

Substituting the concentration and kinetic functions, Eqns II.6 and II.7, into the diffusion-reaction equation, Eqn II.1, gives:

$$\frac{d^2 \psi_i}{dx^2} + g_i \frac{d^2 \delta}{dx^2} = g_i x^2 \eta^2 \delta$$ (II.8)

Since the parameter $\delta$ need be evaluated only in the "boundary" layer regions, Eqn II.8 is solved in regions close to the boundaries, that is, near $x = 0$ and $x = 1$. Within these regions it may be shown [9]

$$\left| \frac{d^2 \psi_i}{dx^2} \right| \ll \left| \frac{d^2 \delta}{dx^2} \right|$$

and that the kinetic function derivatives $\phi^j$ are constants, obtained by evaluation at the boundaries; thus,

Near $x = 0$, \( \frac{d^2 \delta}{dx^2} = \eta^2 x^2 \delta \) (II.9)

Near $x = 1$, \( \frac{d^2 \delta}{dx^2} = \eta^2 x^2 \delta \)

The solution to Eqn II.9 is

Near $x = 0$ \( \delta = \delta_0 e^{-\eta x} \)

Near $x = 1$ \( \delta = \delta_1 e^{-\eta x(1-x)} \) (II.10)

where the constants $\delta_0$ and $\delta_1$ are the values of $\delta$ at $x = 0$, 1, respectively; these are evaluated by using the non-transference boundary condition on carrier species: thus.

$$\psi'_3(0) = g_3 \delta \eta x$$

$$\psi'_3(1) = -g_3 \delta \eta x$$ (II.11)

The concentration functions for the transferred species, $\psi_1$ and $C_1$ at the two boundaries are then given by
\[ C_1 = \psi_1(0) + \frac{g_1}{g_3} \frac{1}{\eta x} \psi_3(0) \]

\[ C_1 = \psi_1(1) - \frac{g_1}{g_3} \frac{1}{\eta x} \psi_3(1) \quad (II.12) \]

A further simplification may be obtained by utilizing Eqns II.4 and II.5; thus
\[ \phi^1 \psi'_1 + \phi^2 \psi'_2 + \phi^3 \psi'_3 = 0 \quad (II.13) \]

and
\[ g_1 \psi'_3 - g_3 \psi'_1 = p_{13} \]
\[ g_2 \psi'_3 - g_3 \psi'_2 = 0 \quad (II.14) \]

Substituting Eqn II.14 into Eqn II.13 and utilizing the definition of \( \eta^2 \) gives
\[ \phi^1 (g_1 \psi'_3 - p_{13}) + \phi^2 g_2 \psi'_3 + \phi^3 g_3 \psi'_3 = 0 \]

or
\[ \psi'_3 = \frac{\phi^1}{\eta^2} p_{13} \quad (II.15) \]

Eqn II.15 in Eqn II.12 gives
\[ C_1 = \psi_1(0) + \frac{g_1}{g_3} \frac{\phi^1}{x\eta^3} \frac{p_{13}}{g_3} \]
\[ C_1 = \psi_1(1) - \frac{g_1}{g_3} \frac{\phi^1}{x\eta^3} \frac{p_{13}}{g_3} \quad (II.16) \]

Eqn II.6 along with:
\[ \phi = 0 \]
\[ g_i \psi_j - g_j \psi_i = p_{ij} x + q_{ij} \quad (II.17) \]
\[ \int_0^1 (\text{Carrier}) dx = C_3^0 \]

permits one to calculate, iteratively, the flux factor, \( p_{13}/g_3 \).

NOTES ADDED IN PROOF

(A) In the experiments to be described, \( \alpha \), the Damkholer number for the uncatalyzed reaction was varied between 1 and 5, and \( \alpha \), for the enzyme-catalyzed experiments was varied between 30-500.

(B) The decrease in flux in the limit of thin films shown by the difference between \( N_{i,0} \) and \( N_{i,0} \), reflects the decreased diffusivity and solubility of CO2 in 1 M NaHCO3 solution relative to pure water. The actual total "facilitated" flux through the bicarbonate solution, given by \( N_i \), only becomes greater than the CO2 flux through water when the film thickness exceeds 0.2 cm. In thicker films, the augmenta-
tion due to the CO₂ reaction with carbonate more than compensates for the decreased diffusivity and solubility of CO₂ in these solutions. Note that the total flux always decreases with increasing film thickness, even though the facilitation effect, $N_1 - N_1^0$, increases with thickness to the maximum value, $N_1^e - N_1^0$, shown by the arrow in Fig. 2.

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