## COMPARISON WITH EXPERIMENTAL DATA

The data of Richardson and Ayers (1959) for glass beads and air and Heertjes and McKibbins (1956) for silica gel and air were compared with calculated heat transfer coefficients for the total surface area. A value of $\epsilon_0 = 0.38$ was used as a typical void fraction for a fixed bed. Figure 1 shows this comparison with reasonable agreement between calculated and experimental coefficients.

## NOTATION

$C_p$ = specific heat
$D$ = diffusivity
$D_e$ = equivalent diameter of channel
$D_p$ = particle diameter
$d$ = particle cluster diameter
$g$ = acceleration due to gravity
$h_c$ = channel heat transfer coefficient
$h_p$ = heat transfer coefficient based on total particle surface
$h_{pc}$ = particle cluster coefficient based on channel area
$k$ = mass transfer coefficient
$N_{Re}$ = $D_e u_e/\nu$, Reynolds number
$N_{Sc}$ = Schmidt number
$u_e$ = actual velocity in packing channels
$u_s$ = superficial velocity

**Greek Letters**

$\alpha$ = thermal diffusivity of fluid
$\epsilon$ = overall bed void fraction
$\epsilon_0$ = void fraction of bed for particle clusters
$\xi$ = ratio of average channelling length to particle diameter
$\mu$ = fluid viscosity
$\nu$ = kinematic viscosity of fluid
$\rho$ = fluid density
$\rho_s$ = solids density
$\psi$ = dimensionless temperature or concentration
$\varphi$ = particle shape factor

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# On Kinetic Behavior at High Enzyme Concentrations


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Currently there are considerable interest and activities in potential applications of enzymes in various forms as industrial catalysts, some of which involve a large concentration of enzymes. For details one may refer to the review paper by Carbonell and Kostin (1972). For enzyme kinetics at high enzyme concentrations Carbonell and Kostin also refer to the work of Cha (1970), who compared what he called the true rate equation with a number of approximate rate equations which includes among others the Michaelis-Menten equation.

The purpose of this note is twofold: (1) to show that the rate equation which was developed by Reiner (1969) and subsequently used by Cha (1970) as the true rate is based on a misconception and is entirely equivalent to the Michaelis-Menten equation in the form of present day use (or more correctly Briggs-Haldane) and hence the comparisons made by Cha (1970) are incorrect, and (2) to bring attention to the fact that when the total enzyme concentration is high relative to the initial substrate concentration the pseudo-steady state (PSS) assumption which leads to the Michaelis-Menten (or Briggs-Haldane) equation may not hold, and therefore a valid comparison would be with the true rate which must be computed without the PSS assumption. This comparison is made using the data reported in the literature on the hydrolysis of acetyl-L-phenyl-alanine ethyl ether by chymotrypsin (Gutfreund and Hammond, 1959) at various concentration ratios of enzyme to the initial substrate. It is very important to fully realize the limitations imposed by the PSS assumption as currently there are being developed many potential applications involving use of concentrated enzymes.

## RATE EQUATIONS

The reaction under consideration is the simplest one substrate-one enzyme reaction in a batch reactor,

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftarrows}} ES \xrightarrow{k_2} E + P \qquad (1)$$

Since the mechanism involves four species, four rate equations can be written,

$$d(E)/dt = -k_1(E)(S) + (k_{-1} + k_2)(ES) \qquad (2)$$

$$d(S)/dt = -k_1(E)(S) + k_{-1}(ES) \qquad (3)$$

$$d(ES)/dt = k_1(E)(S) - (k_{-1} + k_2)(ES) \qquad (4)$$

$$d(P)/dt = k_2(ES) \qquad (5)$$

with the presumed initial conditions $(E)_{t=0} = (E_t)$, $(S)_{t=0} = (S_0)$ and $(ES)_{t=0} = (P)_{t=0} = 0$.
There are two conservation equations

$$(E_t) = (E) + (ES) \quad (6)$$

$$(S_0) = (S) + (ES) + (P) \quad (7)$$

Therefore, only two rate equations are independent, and one can choose any two from the above four with the exception of the pair $d(E)/dt$ and $d(ES)/dt$.

As independent rate equations we first choose Equations (4) and (5) and arbitrarily eliminate $(P)$ and $(E)$ by substituting the conservation equations, Equations (6) and (7), into Equations (4) and (5)

$$d(S)/dt = -k_1(E_t)(S) + [k_1(S) + k_{-1}](ES) \quad (8)$$

$$d(ES)/dt = k_1(E_t)(S) - [k_1(S) + k_{-1} + k_2](ES) \quad (9)$$

and the rate of the product formation is

$$v_T = d(P)/dt = -[d(S)/dt + d(ES)/dt] = k_2(ES) \quad (10)$$

Equations (8) and (9) are two nonlinear ordinary differential equations and can be numerically integrated with the given initial conditions to yield $(S)$ and $(ES)$ as functions of time. These, along with the conservation equations, Equations (6) and (7), describe this enzyme system completely.

An alternative way of describing this system is to pick the same set of rate expressions as above, and eliminate $(E)$ and $(S)$ instead of $(E)$ and $(P)$ by using the two conservation equations.

$$d(ES)/dt = k_1(E_t)[(S_0) - (P)] - [k_{-1} + k_2 + k_1(E_t) + k_1(S_0) - k_1(P)](ES) + k_1(ES)^2 \quad (11)$$

$$d(P)/dt = k_2(ES) \quad (12)$$

$$v_T = d(P)/dt = k_2(ES) \quad (13)$$

Once again, Equations (11) and (12) are two nonlinear ordinary differential equations and can be integrated numerically to yield the time histories of $(ES)$ and $(P)$. These, with the conservation equations, Equations (6) and (7), completely describe the time behavior of this enzyme system.

The difficulty involved in numerically solving the nonlinear differential equations, Equations (8) and (9), or (11) and (12), has been avoided under a certain condition, the PSS assumption.

## PSEUDOSTEADY STATE ASSUMPTION AND RATE EXPRESSIONS

The PSS assumption, which was proposed originally by Briggs and Haldane (1925) and is valid when $(S_0) >> (E_t)$ as shown rigorously by Miller and Alberty (1958) or more recently by Heineken et al. (1967), allows one to set $d(ES)/dt = 0$. The rate expression under this assumption is obtained by setting $d(ES)/dt = 0$ in Equation (12) and substituting the result in Equation (13),

$$(ES) = (E_t)(S)/[K + (S)] \quad (14)$$

$$v_M = d(P)/dt = k_2(E_t)(S)/[K + (S)] = V_{max}(S)/[K + (S)] \quad (15)$$

where $K = (k_{-1} + k_2)/k_1$. This is the so-called "Michaelis-Menten equation" in the form of current use. Equation (15) can be readily integrated to give an explicit relationship for the substrate

$$k_2(E_t)t = K \ln [(S_0)/(S)] + (S_0) - (S) \quad (16)$$

From this equation $(S)$ is known at any time, and hence $(ES)$ from Equation (14), $(E)$ from Equation (6), and $(P)$ from Equation (7). This completes the solution.

An alternative way is to apply the PSS assumption to Equation (11) and solve the resulting quadratic equation for $(ES)$,

$$2(ES) = [K + (E_t) + (S_0) - (P)] \pm \{[K + (E_t) + (S_0) - (P)]^2 - 4(E_t)[(S_0) - (P)]\}^{1/2} \quad (17)$$

When the time is allowed to approach infinite so that $[(S_0) - (P)]$ approaches zero, $(ES)$ must also approach zero. Hence, we take the negative sign. Equation (17) is equivalent to Equation (14) and is readily verified if one recognizes that $(S_0) - (P) = (S) + (ES)$. The rate expression is obtained by substituting Equation (17) into Equation (12).

$$v_S = d(P)/dt = k_2(ES) = k_2[K + (E_t) + (S_0) - (P) - \{[K + (E_t) + (S_0) - (P)]^2 - 4(E_t)[(S_0) - (P)]\}^{1/2}]/2 \quad (18)$$

Thus, we obtain a rate expression which appears to be different. However, since Equations (14) and (17) are equivalent, the rate expression of Equation (18) is also equivalent to the Michaelis-Menten equation (15), that is, $v_S = v_M$. However, the choice of Equation (11) leads to Equation (18) which cannot be integrated analytically, while the Michaelis-Menten equation leads to an analytical expression, Equation (16).

## REINER EQUATION

Reiner (1969) claims that in arriving at Equation (15) a conservation equation for the substrate had been ignored and sets out to correct this situation by writing what he calls is a conservation equation

$$(S_t) = (S) + (ES) \quad (19)$$

He then substitutes Equation (19) into Equation (12), solves the quadratic expression for $(ES)$ in terms of $(S_t)$ and substitutes the result into Equation (5) to obtain

$$v_R = d(P)/dt = \frac{1}{2}k_2[K + (E_t) + (S_t) - \{[K + (E_t) + (S_t)]^2 - 4(E_t)(S_t)\}^{1/2}] = -d(S)/dt = -d(S_t)/dt \quad (20)$$

which is the equation used by Cha (1970) as the true rate. As we have seen above, for a particular selection of a pair of rate equations it is unnecessary to use the substrate conservation equation in arriving at Equation (15), but this does not mean substrate conservation has been ignored or violated. The substrate conservation equation is still necessary to define the system completely. In fact, Equation (19) is not a conservation equation but merely a new definition for the sum of $(ES)$ and $(S)$. The correct conservation equation is given by Equation (10), that is,

$$(S_t) = (S) + (ES) = (S_0) - (P) \quad (21)$$

In fact, substitution of Equation (21) reduces Equation (20) to Equation (18), that is, $v_R = v_S$. In other words the Reiner equation is equivalent to the PSS rate expression given by Equation (18), which in turn was shown to be equivalent to the Michaelis-Menten equation. Thus, $v_M = v_R = v_S$. Consequently, the true rate used by Cha (1970) is not a true rate but merely a disguised form of a PSS rate expression equivalent to the Michaelis-Menten equation.

Finally, the rate equation of Reiner cannot be integrated readily.

## COMPARISONS MADE BY CHA

Using Equation (23) as the true velocity Cha (1970) made comparisons with other approximations and concluded that the time-honored Michaelis-Menten equation results in an intolerably high error at high enzyme and low substrate concentrations. However, there are two reasons which make the work of Cha (1970) incorrect. As shown above, the so-called true velocity is merely a disguised form of the PSS rate expression equivalent to the Michaelis-Menten equation, and secondly he did not use the Michaelis-Menten equation in the form of current use but a variation which is obtained by replacing the substrate concentration, $(S)$ by the sum of the substrate and enzyme complex concentration $(S_t) = (ES) + (S)$, that is,

$$v_C = k_2(E_t)(S_t)/[K + (S_t)] \qquad (24)$$

By doing this an additional error has been imposed arbitrarily, and Equation (21) is no longer the Michaelis-Menten equation. Furthermore, a number of approximations proposed and claimed to be better than the Michaelis-Menten equation by Cha is based on the so-called true rate which was shown above to be incorrect.

## KINETICS AT HIGH INITIAL CONCENTRATION RATIOS OF ENZYME TO SUBSTRATE

When the initial concentration ratio of enzyme to substrate is appreciable or high, the real difficulty is the validity of the PSS assumption, which may not hold. The sufficient condition for validity of this assumption is $(S_0) >> (E_t)$. Otherwise, one cannot set $d(ES)/dt = 0$. Thus the only valid comparison is between the two rate expressions, one with and the other without the PSS assumption. The magnitude of error involved with the PSS assumption for a simple Michaelis-Menten mechanism of Equation (1) has been examined before (Miller and Alberty, 1958; Heineken et al., 1967) for some fixed values of parameters. Hommes (1962) and Walter and Morales (1964) gave maps for some combinations of rate constants. The discrepancies between the true and the PSS solutions are analyzed here. Here we demonstrate the effect of the initial concentration ratio of enzyme to substrate $\alpha = (E_t)/(S_0)$ on the time histories of substrate using the hydrolysis of acetyl-L-phenylalanine ether by chymotrypsin (Gutfreund and Hammond, 1959). For this system $k_1 = 10^6$, $k_2 = 10$/sec, $K = 10^{-4}$, and $k_{-1} = 90$/sec, and for simplicity when we set arbitrarily the initial substrate concentration to equal the Michaelis constant, that is, $(S_0) = K$. The agreement between the PSS and exact solutions is quite good when the initial enzyme concentration is less than 1% of the initial substrate concentration, that is, $\alpha < 0.01$, and so indistinguishable that no attempt was made to show this in Figure 1. However, as this ratio becomes large the error in the PSS solution relative to the exact solution becomes intolerably high. Notice that the error involved is particularly high in the early portion of time, indicating that the initial rate approach could be quite poor when the enzyme concentration is high relative to the initial substrate concentration.

## DISCUSSION

It has been shown that a certain misconception in derivation of rate expressions for the simplest mechanism has led to misleading and erroneous analysis of enzyme kinetics at high enzyme concentrations. The Michaelis-Menten rate expression is completely equivalent to the Reiner equation and is valid only when the PSS assumption is valid. The PSS assumption holds when the initial enzyme concentration relative to the initial substrate concentration $\alpha = (E_t)/(S_0)$ is negligible. When this ratio $\alpha$ is appreciable the PSS assumption may not hold, and one must work with the complete set of rate expressions.

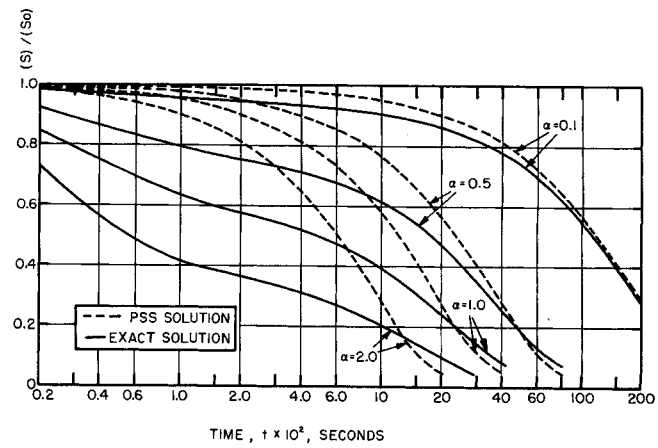


**Fig. 1. Hydrolysis of acetyl-l-phenylalanine ether by chymotrypsin.**


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## NOTATION

$\alpha$ = $(E_t)/(S_0)$
$(E)$ = free enzyme concentration
$(E_t)$ = total enzyme concentration
$(ES)$ = concentration of enzyme complex
$k_i$ = rate constants
$(P)$ = product concentration
$(S)$ = substrate concentration
$(S_0)$ = initial substrate concentration
$t$ = time
$v_C$, $v_M$, $v_R$, $v_S$, $v_T$ = Cha, Michaelis-Menten, Reiner, superficial, and true rates, respectively

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