

$$v = V_M [S] / (K_M + [S])$$

$$V_M = k_{cat} [E_0]$$

k_{cat} = turnover number, maximum rate of substrate conversion per enzyme molecule

Note: for single substrate enzyme, $k_{cat} = k_2$

Most enzymes k_{cat} ranges from 1 to 10^4 /sec
(carbonic anhydrase, 10^6 /sec)

$$v = V_M [S] / (K_M + [S])$$

$K_{cat}/K_M = \eta$, catalytic efficiency

(reaction rate/binding affinity)

Upper limit (kinetic perfection) is restricted by diffusion of substrate so max. K_{cat}/K_M approx. $10^9 \text{ mol}^{-1} \text{ s}^{-1}$

carbonic anhydrase, acetylcholinesterase

Determination of K_M and V_M

Linearized models

Lineweaver-Burke

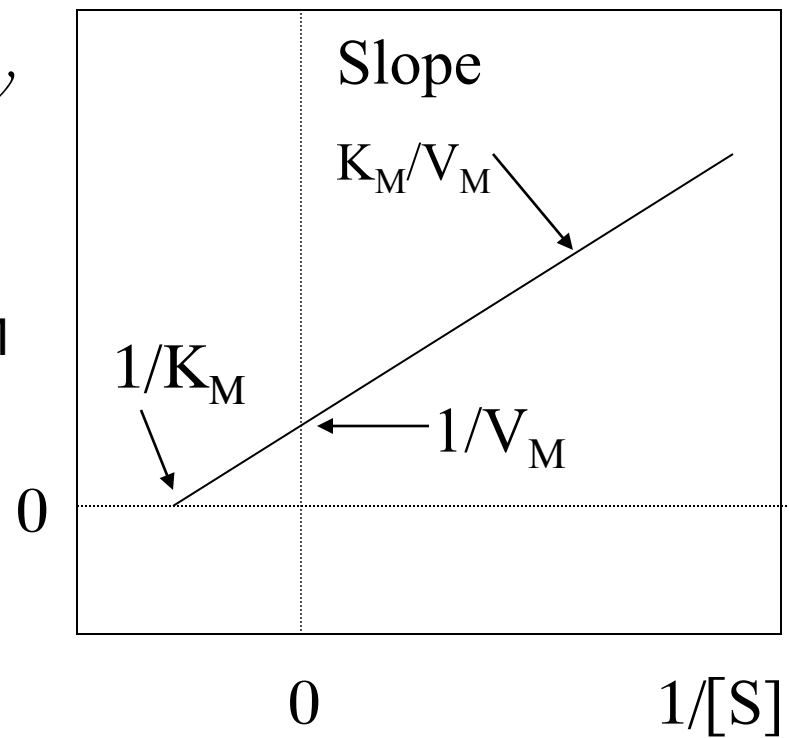
$$1/v = (K_M/V_M)(1/[S]) + 1/V_M$$

Limitations:

Overly sensitive at low $[S]$

need large $[S]$ near $1/V_M$

$1/v$



Determination of K_M and V_M

Linearized models

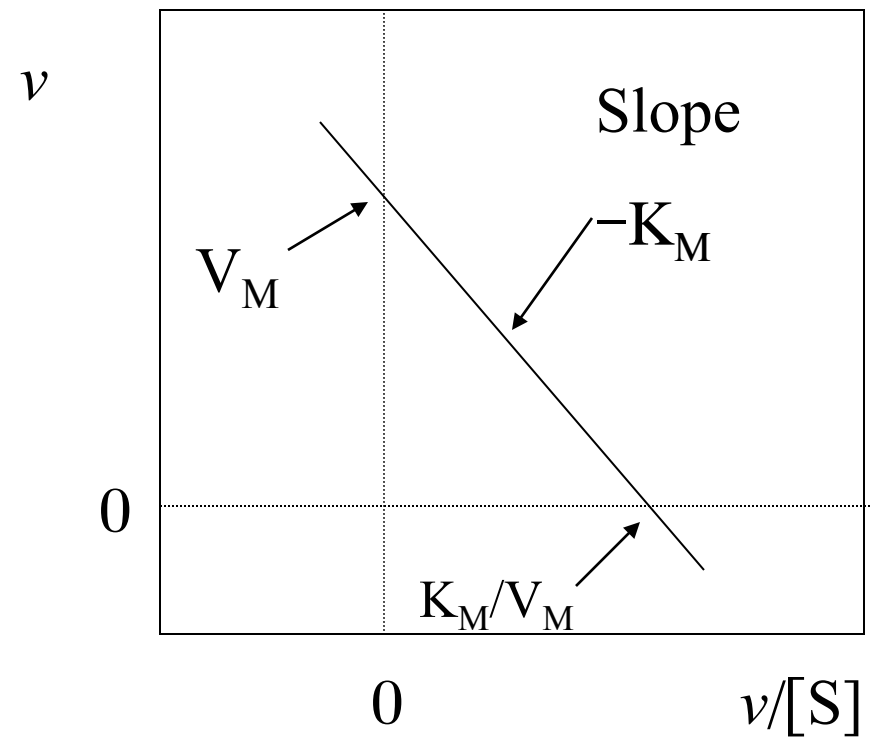
Eadie-Hofstee Linear Plot

v vs. v/S

$$v = -K_M (v/[S]) + V_M$$

Limitations:

overly sensitive at low $[S]$



Determination of K_M and V_M

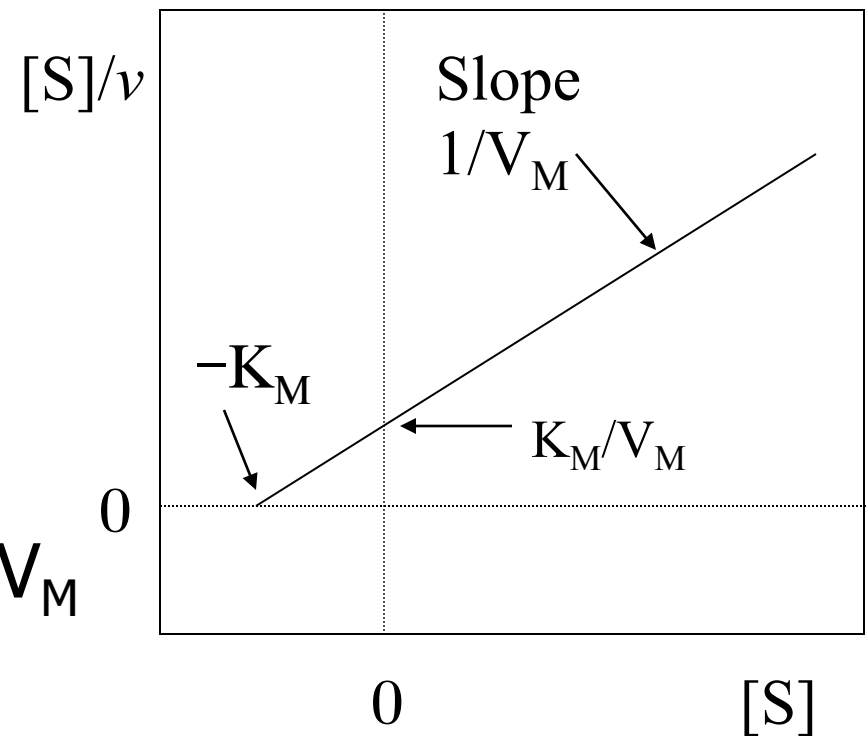
Linearized models

Hanes linearized plot
 $[S]/v$ vs $[S]$

$$[S]/v = ([S]/V_M) + K_M/V_M$$

Limitations:

Sensitivity at low v



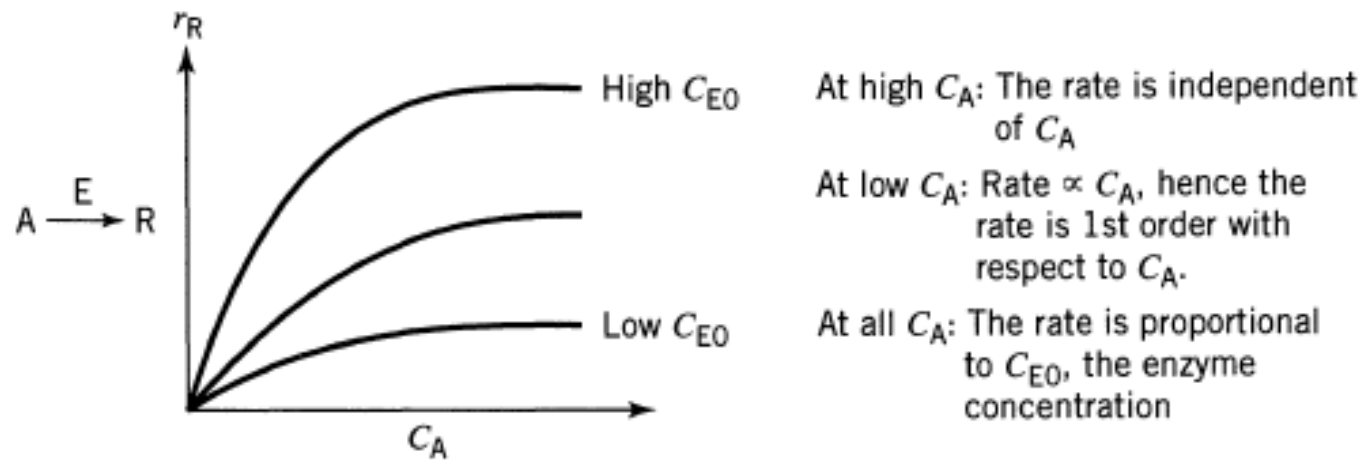
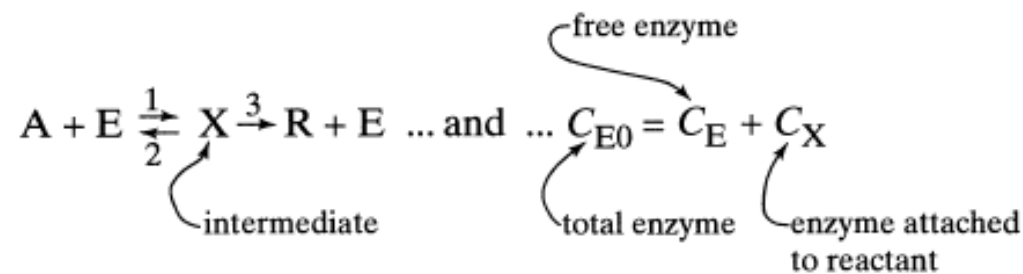


Figure 27.2 Typical rate-concentration curves for enzyme catalyzed reactions.

$$-r_A = r_R = k \frac{\overset{\text{total enzyme}}{C_{E0}} C_A}{\underset{\text{a constant, called the Michaelis constant}}{C_M + C_A}}$$



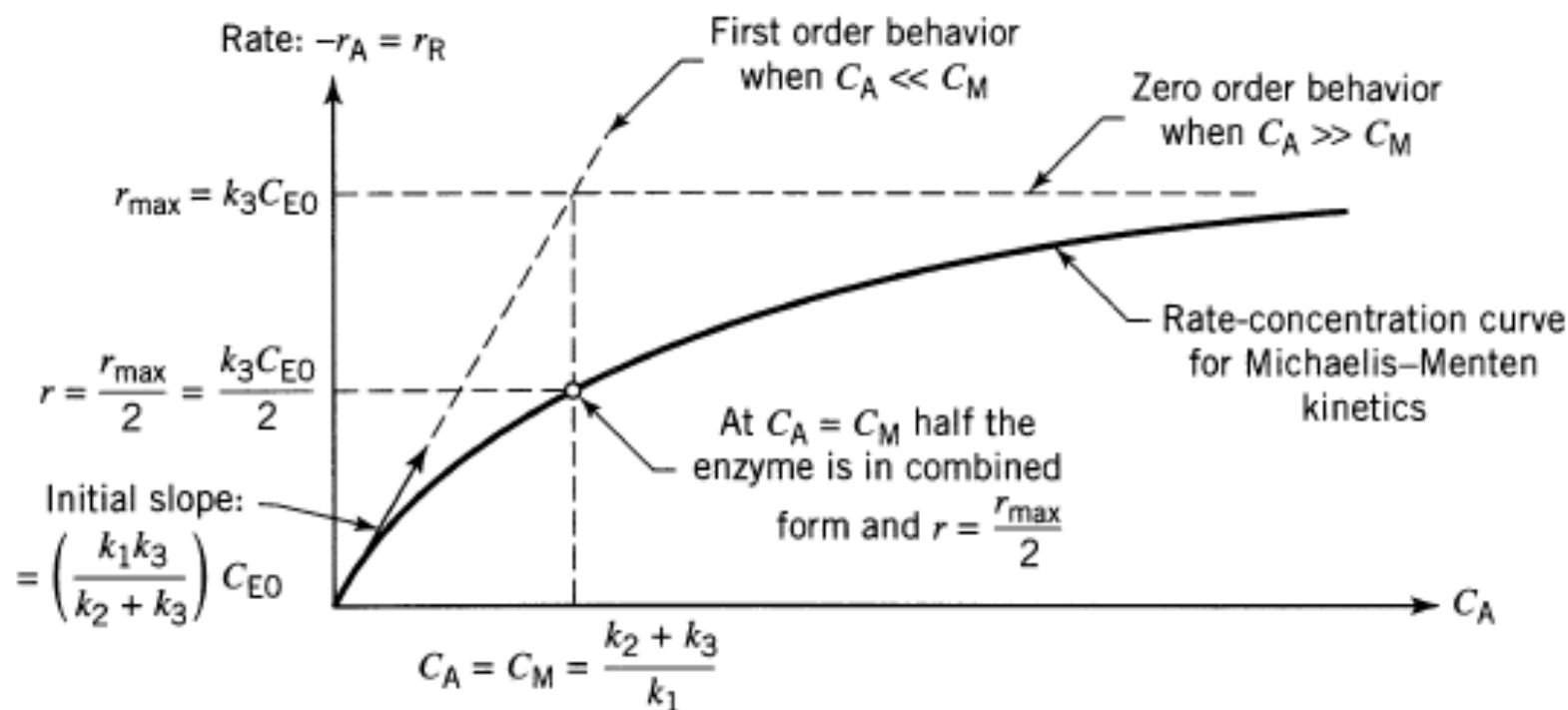


Figure 27.3 Special features of the M–M equation, Eq. 3.

- when $C_A = C_M$ half the enzyme is in free form, the other half combined.
- when $C_A \gg C_M$ most of the enzyme is tied up as complex X .
- when $C_A \ll C_M$ most of the enzyme is in free form.

How to evaluate the two rate constants of this important enzyme fermentation equation

Integration of the M-M equation gives

$$\underbrace{C_M \ln \frac{C_{A0}}{C_A}}_{\text{first-order term}} + \underbrace{(C_{A0} - C_A)}_{\text{zero-order term}} = k_3 C_{E0} t \quad (5)$$

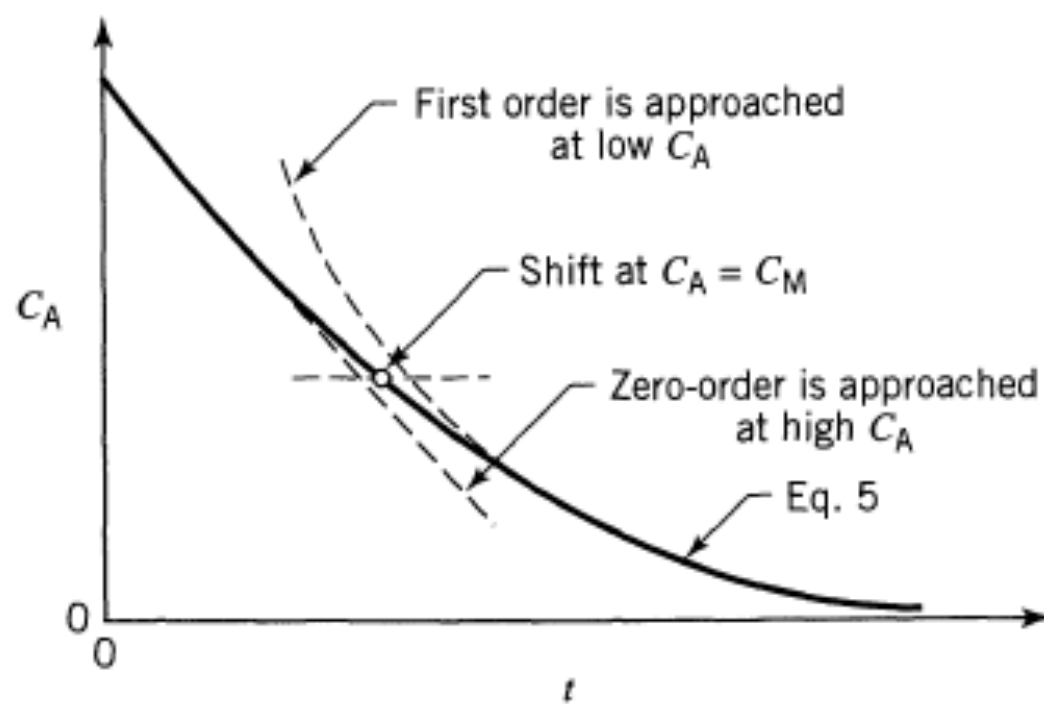


Figure 27.4 Concentration-time behavior of the M-M equation.

Unfortunately this equation cannot be plotted directly to find the values of the constants k_3 and C_M . However, by manipulation we find the following form which can be plotted, as shown in Fig. 27.5, to give the rate constants

$$\frac{C_{A0} - C_A}{\ln \frac{C_{A0}}{C_A}} = -C_M + k_3 C_{E0} \cdot \frac{t}{\ln \frac{C_{A0}}{C_A}} \quad (6)$$

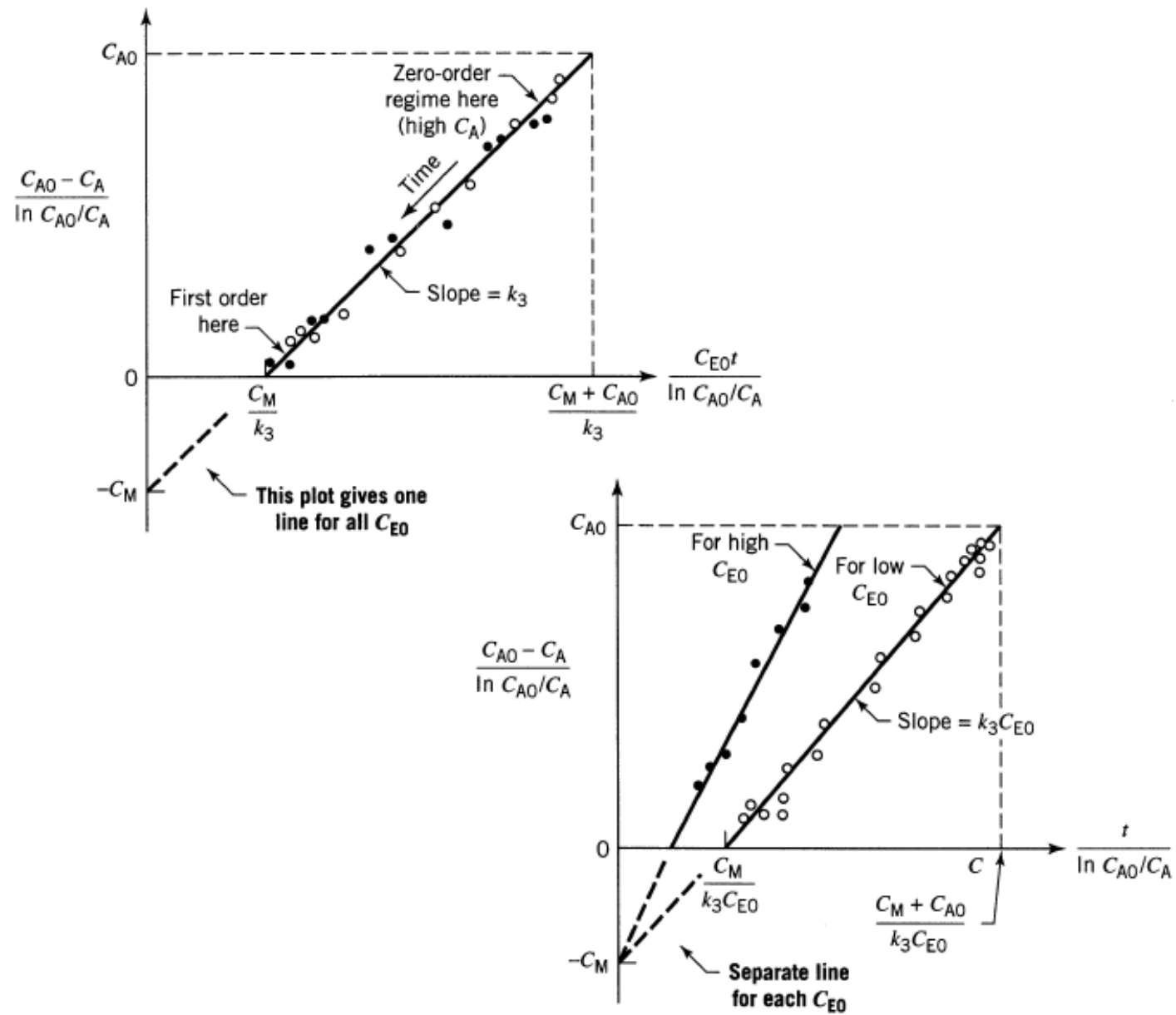


Figure 27.5 Either plot can be used to test and fit the M-M equation (Eq. 6) from batch reactor data.