

Chemistry 2710 Spring 2004 Test 3 Solutions

1. Let k_f be the turnover number (pumping rate divided by volume). Then,

$$\begin{aligned}\frac{db}{dt} &= k_f(b_i - b) - 2kab, \\ \frac{dp}{dt} &= -k_f p + kab.\end{aligned}$$

2. We calculate $v/[\text{estradiol}]$ for each of the data points:

$10^4 v/[\text{estradiol}]$ ($\text{L g}^{-1} \text{min}^{-1}$)	1.82	3.21	3.85	5.29	6.05	6.67
v ($\text{nmol g}^{-1} \text{min}^{-1}$)	15.3	10.1	8.2	4.5	2.6	0.8

The data are plotted in Fig. 1. The data definitely show some curvature (points at the ends above the line, points in the middle below), so the data do not obey the Michaelis-Menten rate law.

A few quick notes:

- The full data set shows the same tendency, although there is a lot of scatter: Points at the ends are systematically above the line of best fit while points in the middle are systematically below. For hydroxylation at C-4, the reaction definitely shows complex kinetics. I suspect that hydroxylation at C-2 is also complex, but that the parameters are such that the deviation from linearity is just a little less obvious.
- There is a tendency in enzymology to accept the hypothesis that the data fit the Michaelis-Menten rate law even when slight curvature can be detected in the linearized plots, as in this case.
- Because enzymes are so sensitive to their environment, curvature in Eadie-Hofstee plots often arises from experimental artifacts (problems controlling ionic strength, secondary effects of substrate on enzyme conformation at high concentrations, etc.) rather than from mechanistic issues.

3. We start by rewriting

$$v = \frac{\frac{v_{\max}^0}{1 + [\text{I}]_0/K_4} [\text{S}]}{[\text{S}] + K_S \frac{1 + [\text{I}]_0/K_3}{1 + [\text{I}]_0/K_4}}.$$

This puts the rate law in the Michaelis-Menten form with

$$v_{\max} = \frac{v_{\max}^0}{1 + [\text{I}]_0/K_4}, \quad (1a)$$

$$\text{and } K_M = K_S \frac{1 + [\text{I}]_0/K_3}{1 + [\text{I}]_0/K_4}. \quad (1b)$$

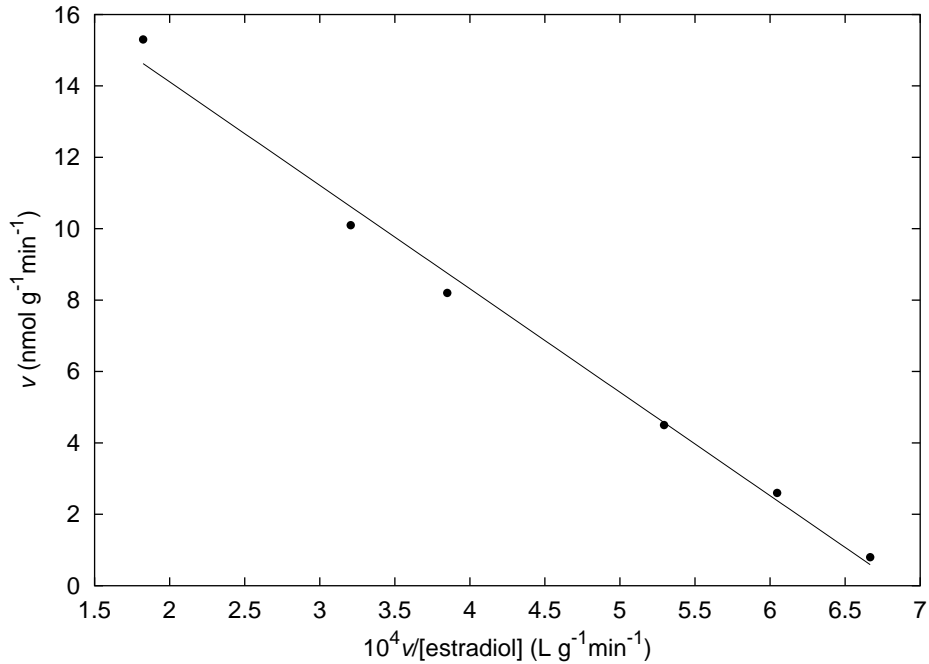


Figure 1: Eadie-Hofstee plot of the data from question 2.

We create an Eadie-Hofstee plot for each value of $[I]_0$. The intercept of each of these plots is v_{\max} and the slope is $-K_M$. We need two additional graphs to get the parameters.

- (a) Given v_{\max} at several different values of $[I]_0$, we can recover v_{\max}^0 and K_4 . Start by taking the reciprocal of equation 1a:

$$\frac{1}{v_{\max}} = \frac{1 + [I]_0/K_4}{v_{\max}^0} = \frac{1}{v_{\max}^0} + \frac{1}{K_4 v_{\max}^0} [I]_0.$$

A graph of $1/v_{\max}$ vs $[I]_0$ should therefore yield a straight line with slope $1/(K_4 v_{\max}^0)$ and intercept $1/v_{\max}^0$. From this graph, we therefore have

$$v_{\max}^0 = \frac{1}{\text{intercept}},$$

$$\text{and } K_4 = \frac{1}{(\text{slope}) v_{\max}^0} = \frac{\text{intercept}}{\text{slope}}.$$

- (b) Recovering K_S and K_3 by analyzing equation 1b is a trickier business. Start by rewriting

$$K_M (1 + [I]_0/K_4) = K_S (1 + [I]_0/K_3).$$

Since we know K_4 from the analysis of graph 3a, we can calculate the quantity on the left-hand side for each value of $[I]_0$. For convenience, let us define

$$y = K_M (1 + [I]_0/K_4).$$

We therefore plot y vs $[I]_0$. The result should be a straight-line graph of slope K_S/K_3 and intercept K_S . We therefore get K_S directly and can calculate K_3 by

$$K_3 = \frac{K_S}{\text{slope}} = \frac{\text{intercept}}{\text{slope}}.$$

4. Enzyme conservation:

$$\begin{aligned} e_0 &= e + f + g. \\ \therefore e &= e_0 - f - g. \end{aligned}$$

Apply EA to reaction steps 1 and 2:¹

$$k_1ea = k_1a(e_0 - f - g) = k_{-1}f. \quad (2a)$$

$$k_2fb = k_{-2}g. \quad (2b)$$

Rate of reaction:

$$v = k_{-3}g.$$

Solve equation 2a for f . Substitute into 2b and solve for g :

$$f = \frac{k_1a(e_0 - g)}{k_1a + k_{-1}}.$$

$$\therefore k_2b \frac{k_1a(e_0 - g)}{k_1a + k_{-1}} = k_{-2}g.$$

$$\therefore k_1k_2ab(e_0 - g) = k_{-2}g(k_1a + k_{-1}).$$

$$\therefore g = \frac{k_1k_2e_0ab}{k_1k_2ab + k_{-2}(k_1a + k_{-1})}.$$

Substitute into our equation for the rate of reaction:

$$v = \frac{k_1k_2k_{-3}e_0ab}{k_1k_2ab + k_{-2}(k_1a + k_{-1})}.$$

¹We could equally well apply the SSA to F and G.