

The substrate concentration resulting in the maximum reaction rate can be determined by setting  $d\nu/d[S] = 0$ . The  $[S]_{\max}$  is given by

$$[S]_{\max} = \sqrt{K'_m K_{S_i}} \quad (3.39)$$

### Example 3.2

The following data have been obtained for two different initial enzyme concentrations for an enzyme-catalyzed reaction.

$v([E_0] = 0.015 \text{ g/l})$ (g/l-min)	[S] (g/l)	$v([E_0] = 0.00875 \text{ g/l})$ (g/l-min)
1.14	20.0	0.67
0.87	10.0	0.51
0.70	6.7	0.41
0.59	5.0	0.34
0.50	4.0	0.29
0.44	3.3	
0.39	2.9	
0.35	2.5	

- a. Find  $K_m$ .
- b. Find  $V_m$  for  $[E_0] = 0.015 \text{ g/l}$ .
- c. Find  $V_m$  for  $[E_0] = 0.00875 \text{ g/l}$ .
- d. Find  $k_2$ .

**Solution** A Hanes–Woolf plot (Fig. 3.12) can be used to determine  $V_m$  and  $K_m$ .

$$\frac{[S]}{v} = \frac{K_m}{V_m} + \frac{1}{V_m} [S]$$

[S]/ $v$ ( $E_0 = 0.015$ ) (min)	[S]/ $v$ ( $E_0 = 0.00875$ ) (min)	[S] (g/l)
17.5	30	20.0
11.5	20	10.0
9.6	16	6.7
8.5	15	5.0
8.0	14	4.0
7.6		3.3
7.3		2.9
7.1		2.5

From a plot of  $[S]/v$  versus  $[S]$  for  $E_0 = 0.015 \text{ g/l}$ , the slope is found to be  $0.6 \text{ min/g/l}$  and  $V_m = 1/0.6 = 1.7 \text{ g/l min}$ . The  $y$ -axis intercept is  $K_m/V_m = 5.5 \text{ min}$  and  $K_m = 9.2 \text{ g [S]/l}$ .

Also,  $V_m = k_2 E_0$  and  $k_2 = 1.7/0.015 = 110 \text{ g/g enzyme-min}$ . The Hanes–Woolf plot for  $E_0 = 0.00875 \text{ g/l}$  gives a slope of  $1.0 \text{ min/g/l}$  and  $V_m = 1.0 \text{ g/l-min}$ ;  $k_2 = V_m/E_0 = 1.0/0.00875 = 114 \text{ g/g enzyme-min}$ .