3.3.3. Experimentally Determining Rate Parameters for Michaelis–Menten Type Kinetics

The determination of values for K_m and V_m with high precision can be difficult. Typically, experimental data are obtained from *initial-rate experiments*. A batch reactor is charged with a known amount of substrate $[S_0]$ and enzyme $[E_0]$. The product (or substrate concentration) is plotted against time. The initial slope of this curve is estimated (i.e., $v = d[P]/dt|_{t=0} = -d[S]/dt|_{t=0}$). This value of v then depends on the values of $[E_0]$ and $[S_0]$ in the charge to the reactor. Many such experiments can be used to generate many pairs of v and [S] data. These could be plotted as in Fig. 3.3, but the accurate determination of K_m from such a plot is very difficult. Consequently, other methods of analyzing such data have been suggested.

3.3.3.1. Double-reciprocal plot (Lineweaver–Burk plot). Equation 3.12b can be linearized in double-reciprocal form:

$$\frac{1}{v} = \frac{1}{V_m} + \frac{K_m}{V_m}\frac{1}{[S]} \tag{3.13}$$

A plot of $1/v$ versus $1/[S]$ yields a linear line with a slope of K_m/V_m and y-axis intercept of $1/V_m$, as depicted in Fig. 3.5. A double-reciprocal plot gives good estimates on V_m, but not necessarily on K_m. Because the error about the reciprocal of a data point is not symmetric, the reader should be cautious in applying regression analysis (least squares) to such plots. Data points at low substrate concentrations influence the slope and intercept more than those at high substrate concentrations.

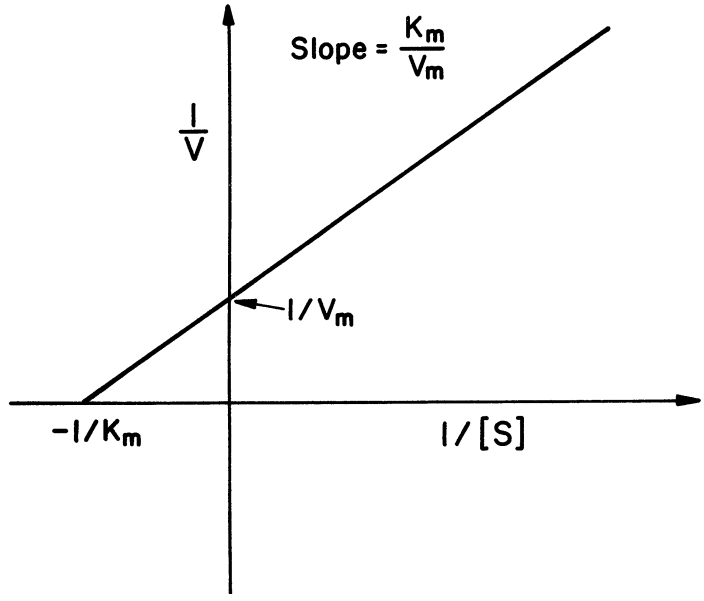

Figure 3.5. Double-reciprocal (Lineweaver–Burk) plot.