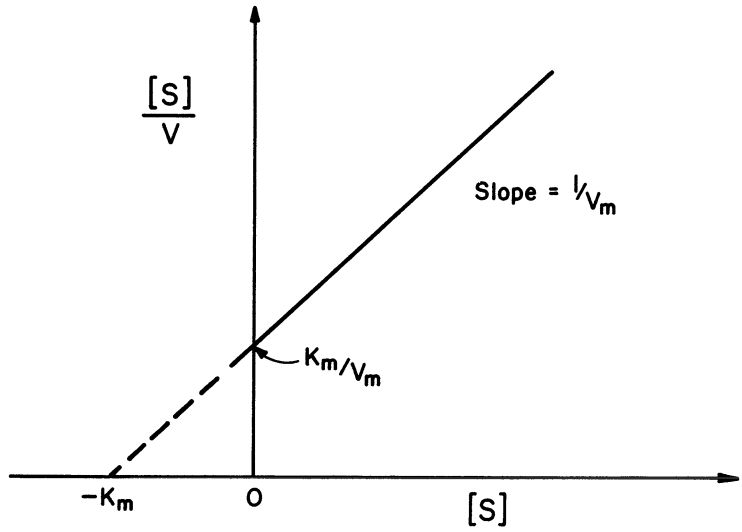

Figure 3.7. Hanes–Woolf plot.

or

$$V_m - \frac{[S_0] - [S]}{t} = \frac{K_m}{t} \ln \frac{[S_0]}{[S]} \tag{3.17}$$

A plot of $1/t \ln[S_0]/[S]$ versus $\{[S_0] - [S]\}/t$ results in a line of slope $-1/K_m$ and intercept of V_m/K_m.

3.3.3.5. Interpretation of K_m and V_m. While K_m (or K_m') is an intrinsic parameter, V_m is not. K_m is solely a function of rate parameters and is expected to change with temperature or pH. However, V_m is a function of the rate parameter k_2 and the initial enzyme level, $[E_0]$. As $[E_0]$ changes, so does V_m. Of course, k_2 can be readily calculated if $[E_0]$ is known. For highly purified enzyme preparations it may be possible to express $[E_0]$ in terms of mol/l or g/l.

When the enzyme is part of a crude preparation, its concentration is in terms of "units." A "unit" is the amount of enzyme that gives a predetermined amount of catalytic activity under specific conditions. For example, one unit would be formation of one µmol product per minute at a specified pH and temperature with a substrate concentration much greater than the value of K_m. The *specific activity* is the number of units of activity per amount of total protein. For example, a crude cell lysate might have a specific activity of 0.2 units/mg protein which upon purification may increase to 10 units/mg protein. Only enzyme that remains catalytically active will be measured. The enzyme may be *denatured* if it unfolds or has its three-dimensional shape altered by pH extremes or temperature during purification. The denatured enzyme will have no activity.