$$K_m' = \frac{[\mathrm{EH}][\mathrm{S}]}{[\mathrm{EHS}]}$$

$$K_1 = \frac{[\mathrm{EH}][\mathrm{H}^+]}{[\mathrm{EH}_2^+]} \tag{3.41}$$

$$K_2 = \frac{[\mathrm{E}^-][\mathrm{H}^+]}{[\mathrm{EH}]}$$

$$[\mathrm{E}_0] = [\mathrm{E}^-] + [\mathrm{EH}] + [\mathrm{EH}_2^+] + [\mathrm{EHS}], \quad v = k_2[\mathrm{EHS}]$$

We can derive the following rate expression:

$$v = \frac{V_m[\mathrm{S}]}{K_m'\left[1 + \frac{K_2}{[\mathrm{H}^+]} + \frac{[\mathrm{H}^+]}{K_1}\right] + [\mathrm{S}]} \tag{3.42}$$

or

$$v = \frac{V_m[\mathrm{S}]}{K_{m,\mathrm{app}}' + [\mathrm{S}]} \tag{3.43}$$

where $\quad K_{m,\mathrm{app}}' = K_m'\left[1 + \frac{K_2}{[\mathrm{H}^+]} + \frac{[\mathrm{H}^+]}{K_1}\right]$

As a result of this behavior, the pH optimum of the enzyme is between pK_1 and pK_2.

For the case of ionizing substrate, the following scheme and rate expression can be developed:

$$\begin{array}{l} \mathrm{SH}^+ + \mathrm{E} \underset{}{\overset{K_m'}{\rightleftharpoons}} \mathrm{ESH}^+ \xrightarrow{k_2} \mathrm{E} + \mathrm{HP}^+ \\ \quad \Downarrow\!\Uparrow K_1 \\ \mathrm{S} + \mathrm{H}^+ \end{array} \tag{3.44}$$

$$v = \frac{V_m[\mathrm{S}]}{K_m'\left(1 + \frac{K_1}{[\mathrm{H}^+]}\right) + [\mathrm{S}]} \tag{3.45}$$

Theoretical prediction of the pH optimum of enzymes requires a knowledge of the active site characteristics of enzymes, which are very difficult to obtain. The pH optimum for an enzyme is usually determined experimentally. Figure 3.14 depicts variation of enzymatic activity with pH for two different enzymes.

3.3.5.2. Temperature effects. The rate of enzyme-catalyzed reactions increases with temperature up to a certain limit. Above a certain temperature, enzyme activ-