V_0 (mmol hydrolyzed/l-min)		
Free enzyme	Immobilized enzyme	S_0 (mol/l)
0.083	0.056	0.010
0.143	0.098	0.020
0.188	0.127	0.030
0.222	0.149	0.040
0.250	0.168	0.050
0.330	0.227	0.100
0.408	0.290	0.290

a. Determine the K_m and V_m for this reaction using both free and immobilized enzyme.

b. Do the data indicate any diffusion limitations in the immobilized enzyme preparation?

Solution From a double-reciprocal plot of $1/v$ versus $1/S$ for free enzyme (Fig. 3.22), $-1/K_m = -20$ and $K_m = 0.05\ M$. $1/V_m = 2$ and $V_m = 0.5$ mmol/l min. From a double-reciprocal plot of $1/v$ versus $1/S$ for the immobilized enzyme, $-1/K_m = -20$ and $K_m = 0.05\ M$. $1/V_m = 3$ and $V_m = 0.33$ mmol/l-min. Since the K_m values for free and immobilized enzymes are the same, there is no diffusion limitation.

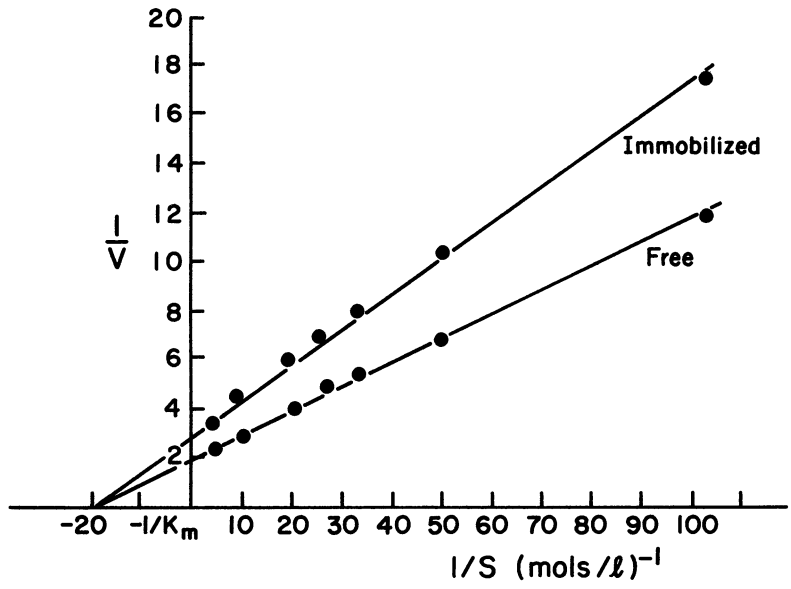

Figure 3.22. Double-reciprocal plots for free and immobilized enzymes (Example 3.4).