❖ Evaluation of Michaelis's Constant for Enzyme-Substrate Binding by Lineweaver-Burk Plot and Eadie-Hofstee Methods

The conventional approach to determine the Michaelis's constant (K_m) involve the plot of initial reaction rate vs initial substrate concentration. Then the Michaelis-Menten constant of enzyme-catalyzed reactions can simply be calculated once the maximum initial reaction rate is known. The typical Michaelis-Menten is used to fit the data is given below.

$$r_0 = \frac{r_{max}[S_0]}{K_m + [S_0]} \tag{275}$$

Where $r_{max} = k_2[E_0]$ is the maximum initial rate at $[E_0]$ enzyme concentration whereas $[S_0]$ is the initial substrate concentration. Finally, the concentration at which the initial rate in half of the maximum initial rate, will be equal to K_m.

This conventional method, however, suffers from serious limitations like the need for lots of data points until the initial rate becomes constant. Therefore, two other methods are quite popular to find Michaelis's constant without getting the actual plateau in the initial reaction rate. In this section, we will discuss two such methods for the evaluation of Michaelis's constant for enzyme-substrate binding.

➢ *The Lineweaver-Burk Plot to Evaluate Michaelis's Constant*

In order to understand the Lineweaver-Burk method for the determination of the maximum initial rate (r_{max}) and Michaelis's constant, we need to rearrange the general Michaelis-Menten equation first i.e. the reciprocal of equation (275) as given below.

$$\frac{1}{r_0} = \frac{K_m + [S_0]}{r_{max}[S_0]} \tag{276}$$

or

$$\frac{1}{r_0} = \frac{K_m}{r_{max}[S_0]} + \frac{[S_0]}{r_{max}[S_0]} \tag{277}$$

or

$$\frac{1}{r_0} = \frac{K_m}{r_{max}}\frac{1}{[S_0]} + \frac{1}{r_{max}} \tag{278}$$

Which is the equation of the straight line ($y = mx + c$). Therefore, if we plot the reciprocal of initial reaction rate vs the reciprocal of initial substrate concentration; the slope and intercepts will give K_m/r_{max} and $1/r_{max}$, respectively.

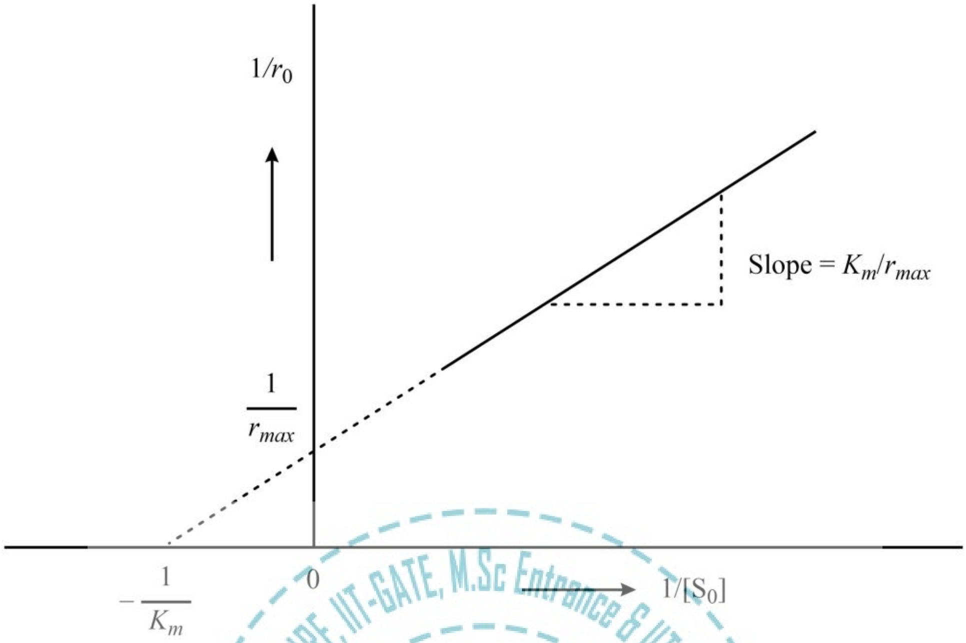

Figure 3. The Lineweaver-burk plot for enzyme-catalyzed reactions to evaluate the maximum initial rate (r_{max}) and Michaelis's constant.

It is also worthy to mention that if the intercept is further extrapolated, it will lead to the intercept on the *x*-axis that equals to $-1/K_m$.

➢ ***The Eadie-Hofstee Plot to Evaluate Michaelis's Constant***

In order to understand the Eadie-Hofstee method for the determination of the maximum initial rate (r_{max}) and Michaelis's constant, we need to rearrange the general Michaelis-Menten equation first i.e. the reciprocal of equation (275) as given below.

$$\frac{1}{r_0} = \frac{K_m + [S_0]}{r_{max}[S_0]} \tag{279}$$

or

$$\frac{1}{r_0} = \frac{K_m}{r_{max}[S_0]} + \frac{[S_0]}{r_{max}[S_0]} \tag{280}$$

or

$$\frac{1}{r_0} = \frac{K_m}{r_{max}}\frac{1}{[S_0]} + \frac{1}{r_{max}} \tag{281}$$

Multiplying both sides by r_0, we get

$$\frac{r_0}{r_0} = \frac{K_m}{r_{max}}\frac{r_0}{[S_0]} + \frac{r_0}{r_{max}} \tag{282}$$

Rearranging for $r_0/[S_0]$, we get

$$\frac{r_0}{r_0} - \frac{r_0}{r_{max}} = \frac{K_m}{r_{max}}\frac{r_0}{[S_0]} \tag{283}$$

$$\frac{r_0}{[S_0]} = \frac{r_0}{r_0}\frac{r_{max}}{K_m} - \frac{r_0}{r_{max}}\frac{r_{max}}{K_m} \tag{284}$$

$$\frac{r_0}{[S_0]} = \frac{r_{max}}{K_m} - \frac{r_0}{K_m} \tag{285}$$

$$\frac{r_0}{[S_0]} = -\frac{1}{K_m}r_0 + \frac{r_{max}}{K_m} \tag{286}$$

Which is the equation of the straight line ($y = mx + c$). Therefore, if we plot the ratio of initial reaction rate to initial substrate concentration vs the initial reaction rate; the slope and intercepts will give $-1/K_m$ and r_{max}/K_m, respectively.

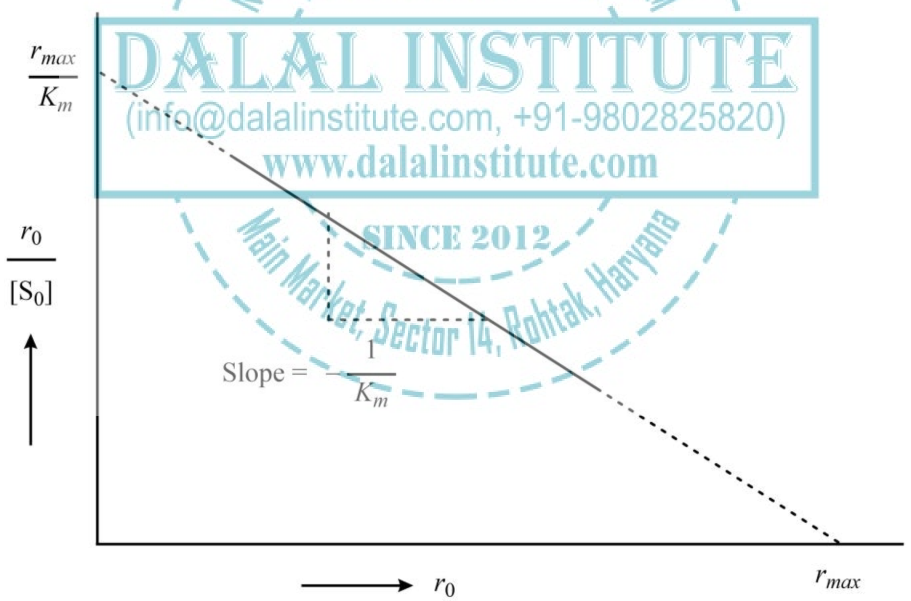

Figure 4. The Eadie-Hofstee plot for enzyme-catalyzed reactions to evaluate the maximum initial rate (r_{max}) and Michaelis's constant.

It is also worthy to mention that if the intercept is further extrapolated, it will lead to the intercept on the *x*-axis that equals to r_{max}.

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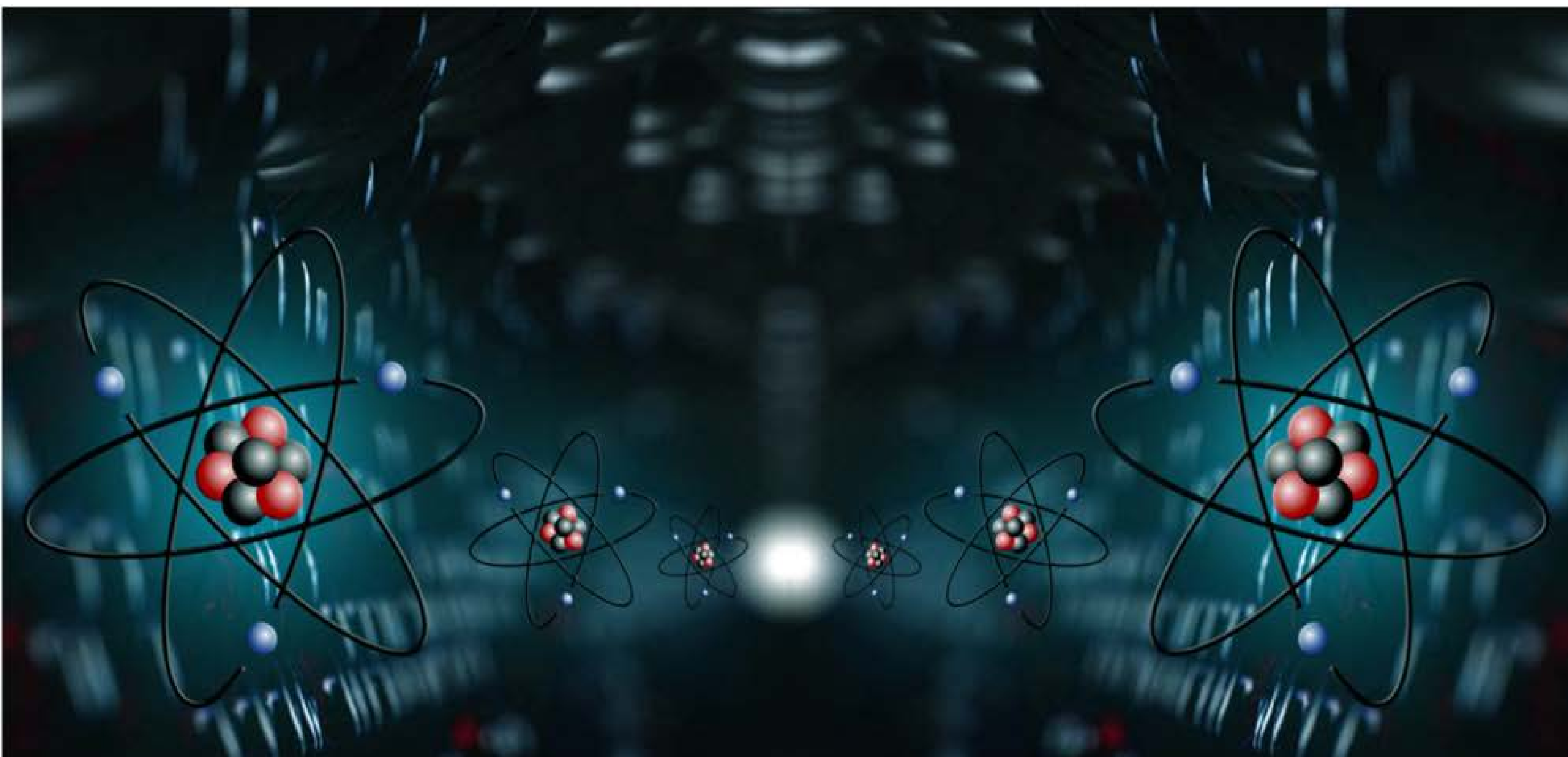

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