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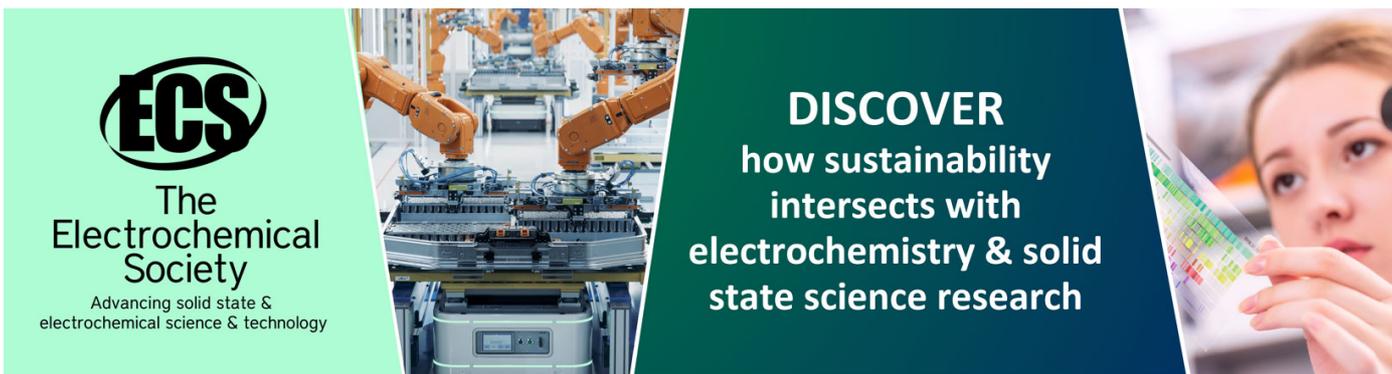
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Affirmation of the Separate Rate-law Method for Determining v_{\max} and K_M of the Michaelis Reaction

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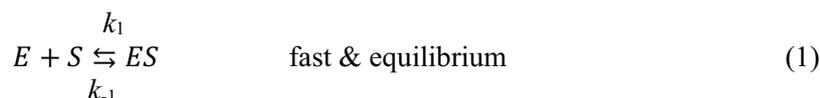
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Abstract. The maximum-velocity, v_{\max} , and the Michaelis-constant, K_M , are the essence of the Michaelis-Menten equation. The value of v_{\max} is determined when the reaction is in *zeroth*-order and K_M is in *first*-order. With a slight change in the notion of v_{\max} concept, both were used as the basis for a new determination method called Separate Rate-law Method (*SRIM*). As yet, the Lineweaver - Burk and Eadie - methods were the most commonly used. Both combine *zeroth*-order and *first*-order data. The purpose of this study was to validate the quality of all methods. The research was carried out theoretically. All were tested by applying them to the same literature data, and the results were compared. The assessment is based on the v_{\max} and K_M values obtained; doubling $[E]$ will double v_{\max} but the same K_M . The results show that the two present methods are inaccurate. Both give v_{\max} and K_M values even if the reaction is always *first*-order. Based on the integral method, two variants of the new method, $SRIM_{IM}$, and initial rate, $SRIM_{IRM}$, were also introduced. All new methods give more accuracy, which results in the method accuracy of $SRIM \approx SRIM_{IM} > SRIM_{IRM}$.

1. Introduction

According to Michaelis-Menten, enzyme-catalyzed reactions proceed by following the reaction mechanism in Eq. (1-2).



Using the Steady-State Approximation and after some steps, the rate law is written in Eq. (3).

$$v = \frac{k_2[E][S]}{K_M + [S]} \quad (3)$$

Where v , k_2 , $[E]$, $[S]$, and K_M are rate, enzyme rate constant, enzyme concentration, substrate concentration, and Michaelis constant. K_M is a measure of the enzyme's affinity for its substrate, and it has value in the range of 10^{-6} to 10^{-2} M. In experiments, the initial concentration of the enzyme is usually made very small compared to substrate concentration. In data analysis, the initial rate method (*IRM*) is the most commonly used.



In Eq. (3), the order with respect to $[E]$ is *first-order*, but the order with respect to $[S]$ is uncertain. If $[S] \gg K_M$, Eq. (3) becomes Eq. (4), in which the reaction is *zeroth-order* to $[S]$ and *first-order* to $[E]$.

$$v = k_2[E] \quad (4)$$

In this condition, all enzymes react with the substrate, and the reaction is in the maximum velocity or v will approach v_{max} . Then, Eq. (4) becomes Eq. (5).

$$v = v_{max} = k_2[E] \quad (5)$$

However, if $[S] \ll K_M$, Eq. (3) becomes Eq. (6), in which the reaction is of the *first-order* to $[E]$ and to $[S]$.

$$v = \frac{k_2[E][S]}{K_M} \quad (6)$$

Inserting Eq. (5) into Eq. (6) gives Eq. (7).

$$v = \frac{v_{max}[S]}{K_M} \quad (7)$$

Inserting Eq. (5) into Eq. (3) gives Eq. (8).

$$v = \frac{v_{max}[S]}{K_M + [S]} \quad (8)$$

Three problems arise. First, there is a need for a new method that followed the basic assumptions of Michaelis reaction. The method must be based on Eq. (5) and Eq. (7) and can be used as a standard method. Second, during the reaction process, the enzyme/catalyst concentration will be constant, but the substrate decreases. Depending on the ratio of the initial concentration of substrate and enzyme, the reaction can be zeroth-order at the beginning reaction; however, it then becomes first order at near-end reaction or always being the first-order reaction. There is no check for the real rate law at these two conditions in all of the present equations for Michaelis-Menten or catalyzed reaction fitting-constants determination. When both data are combined, this can cause problems. For example, if the first happens, it cannot give the correct values for v_{max} and K_M because the reaction changes from zeroth-order to first-order at the near-end. If the latter happens, it will contradict the essence of Eq. (5) and Eq. (7).

Third, v_{max} will be achieved if all enzymes/catalyst reacts with the substrate. However, the Michaelis reaction is *first-order* to enzyme/catalyst concentration. Thus, doubling enzyme/catalyst concentration will double the value of v_{max} as well. That is to say, v_{max} is not a constant. In the present equations, v_{max} is used to determine K_M without considering this fact. In addition, save for *first-order* reaction, *IRM* or the differential method, will give different rate constant values at a constant time of reaction.

The present methods for determining v_{max} and K_M are based on Eq. (8). However, it is difficult to determine both fitting-constants in this equation because the plot of v against $[S]$ is hyperbolic. To cope with, the linear form is usually used, such as that introduced by Lineweaver-Burk (*L-B*) [1] and by Eadie [2]-Hofstee (*E-H*) [3], Hanes [4], and Espenson. The most commonly used is *L-B* and *E-H*, as presented in Eq. (9-10), respectively.

$$\frac{1}{v_o} = \frac{1}{v_{max}} + \frac{K_M}{v_{max}} \frac{1}{[S]_o} \quad (9)$$

The linear plot of $(1/v_o)$ against $(1/[S]_o)$ gives $v_{max} = 1/\text{intercept}$ and $K_M = v_{max} / \text{slope}$.

$$v_o = v_{max} - \frac{v_o}{[S]_o} K_M \quad (10)$$

The linear plot of (v_o) against $(v_o/[S]_o)$ gives $v_{max} = \text{intercept}$ and $K_M = - \text{slope}$. Both equations use and share the outset and near-end data. Based on the above theoretical description, this study aims to introduce new methods based on Eq. (5) and Eq. (7) to prove that the present methods for v_{max} and K_M determination in Michaelis reactions are ambiguous and to introduce a better notion of v_{max} and the real constant in Michaelis reactions.

2. Method

2.1. Reaction model

This study theoretically used the enzymatic reaction data taken from the hydrolysis of phenylacetate catalyzed by enzyme acetylcholinesterase [6] and the hydrolysis of methyl-hydro cinnamate catalyzed by enzyme chymotrypsin [7]. These two hydrolysis data are presented in Table 1 and Table 2, respectively.

Table 1. Concentration data for fitting-constants determination in the hydrolysis of phenylacetate catalyzed by enzyme acetylcholinesterase [6].

| | | | | | | | | |
|-------------------------|------|------|------|------|------|------|------|------|
| [PhOAc], $10^{-4} M$ | 22.5 | 16.3 | 12.6 | 9.73 | 8.45 | 7.18 | 6.21 | 5.55 |
| $v_i, 10^{-7} M s^{-1}$ | 7.24 | 6.35 | 5.32 | 4.61 | 4.53 | 4.02 | 3.38 | 3.26 |

Table 2. Concentration data for fitting-constants determination in the hydrolysis of methyl hydro-cinnamate catalyzed by enzyme chymotrypsin [7].

| | | | | | | | |
|---------------------------------------|------|------|------|------|------|------|------|
| [Methyl hydro-cinnamate], $10^{-3} M$ | 30.8 | 14.6 | 8.57 | 4.60 | 2.24 | 1.28 | 0.32 |
| $v_i, 10^{-8} M s^{-1}$ | 20 | 17.5 | 15.0 | 11.5 | 7.5 | 5.0 | 1.5 |

The data can be divided into two parts and be assumed as the outset and near-end data. However, there is no data for studying the effects of changing enzyme or catalyst concentration. To cope with, the other model used is the reaction of acetone with bromine, catalyzed by H . Because there are two substrates, then the reaction occurred in conditions of excessive [Acetone] to [Bromine]. It is believed that in these conditions, the reaction has the rate law as presented in Eq. (11).

$$v = \frac{k_{obs}[H^+][Br_2]}{\kappa + [Br_2]} \quad (11)$$

Where $k_{obs} = k[\text{Acetone}]$ and κ are the observed rates constant and catalytic constant, respectively. When Eq. (12-13) was assumed, Eq. (11) is similar to Eq. (8). The absorbance unit data (measured at 400 nm) is presented in Table 3 [8, 9].

$$k_{obs} [H^+] = v_{max} \quad (12)$$

$$\kappa = K_M \quad (13)$$

2.2. Framework and Data Interpretation Technique.

2.2.1. Separate Rate-law Method (SRIM)

All data were analyzed using Eq. (9) and Eq. (10) and using the new equation. With slight changes in the notion of the maximum velocity concept, a new method, called Separate Rate-law Method or *SRIM*, for v_{max} determination is derived from Eq. (5).

Table 3. Absorbance data for fitting-constants determination in the reaction of acetone (0.8 *m*) with bromine 0.01 *m*) catalyzed by hcl (hcl 0.050 *M*, hcl 0.100 *M*, and hcl 0.200 *M*)

| <i>t/s</i> | [H ⁺] 0.050 <i>M</i> | | [H ⁺] 0.100 <i>M</i> | | [H ⁺] 0.200 <i>M</i> | | | | | | | |
|------------|----------------------------------|----------|----------------------------------|----------|----------------------------------|----------|-------|-------|-------|-------|-------|-------|
| | Outset | Near-end | Outset | Near-end | Outset | Near-end | | | | | | |
| 10 | 0.468 | 0.463 | 0.059 | 0.055 | 0.483 | 0.473 | 0.073 | 0.066 | 0.455 | 0.437 | 0.109 | 0.096 |
| 20 | 0.463 | 0.458 | 0.055 | 0.052 | 0.473 | 0.463 | 0.066 | 0.059 | 0.437 | 0.420 | 0.096 | 0.084 |
| 30 | 0.458 | 0.454 | 0.052 | 0.050 | 0.463 | 0.454 | 0.059 | 0.053 | 0.420 | 0.403 | 0.084 | 0.074 |
| 40 | 0.454 | 0.449 | 0.050 | 0.047 | 0.454 | 0.444 | 0.053 | 0.048 | 0.403 | 0.387 | 0.074 | 0.065 |
| 50 | 0.449 | 0.444 | 0.047 | 0.044 | 0.444 | 0.434 | 0.048 | 0.043 | 0.387 | 0.372 | 0.065 | 0.057 |
| 60 | 0.444 | | 0.044 | | 0.434 | | 0.043 | | 0.372 | | 0.057 | |

Note: For *IRM*, the end of the one-time interval is considered as the initial value for a new (and the same) time interval.

$$v = -d[S]/dt = v_{max} \quad (14)$$

$$-[S]_o d \frac{[S]/[S]_o}{dt} = v_{max} \quad (15)$$

Introducing $\beta =$ fraction of substrate in the solution $= [S]/[S]_o$ into integral form of Eq. (15) gives Eq. 16.

$$-([S]_o) \int_1^\beta d\beta = v_{max} \int_0^{t_\beta} dt \quad (16a)$$

$$[S]_o(1 - \beta) = v_{max}t_\beta \quad (16b)$$

$$v_{max} = ([S]_o(1 - \beta_{zeroth})) / t_\beta \quad (16)$$

The equation for K_M determination is derived from Eq. (7).

$$v = -d[S]/dt = \left(\frac{v_{max}}{K_M}\right)[S] \quad (17a)$$

$$-d([S]/[S]) = \left(\frac{v_{max}}{K_M}\right) dt \quad (17)$$

Introducing $\beta = [S]/[S]_o$ into integral form of Eq. (17) gives Eq. (19)

$$-\int_1^\beta d(\beta)/\beta = \left(\frac{v_{max}}{K_M}\right) \int_0^{t_\beta} dt \quad (18)$$

$$-\ln \beta = \left(\frac{v_{max}}{K_M}\right) t_\beta \quad (19a)$$

$$K_M = -\frac{v_{max}t_\beta}{\ln \beta_{first}} \quad (19)$$

Eq. (16) can only be used if the rate is *zeroth*-order. For example, In Table 4, for $[H^+] = 0.050 M$, $[S]_0 = 0.468$, $\beta = (0.463/0.468 = 0.9893)$, and for $[H^+] = 0.100 M$, $[S]_0 = 0.483$, $\beta = (0.473/0.483) = 0.9793$. Eq. (19) can only be used if the reaction is *first*-order. Differ with *zeroth*-order reaction, here β is constant. The value used is not $\beta = (0.055/0.059)$, but the variance of the 5 values of β (for $[H^+] = 0.050 M = 0.9431$ and for $[H^+] = 0.1000 M = 0.8996$).

The variant of *SRIM* uses *IRM* (then called *SRIM_{IRM}*) and *IM* (then called *SRIM_{IM}*). Like *SRIM*, both *SRIM_{IRM}* and *SRIM_{IM}* can only be used when the reaction has a different rate law at the outset and near-end.

2.2.2. Data analysis

All methods are then used to determine the v_{\max} and K_M of the five reaction models. The results are presented in Table 4 through Table 8.

2.2.3. Data interpretations

A method is valid if v_{\max} is determined when the reaction is *zeroth*-order and K_M when the reaction is *first*-order. The v_{\max} value is relative to the enzyme (catalyst) concentrations. However, K_M value must be the same. A method will be ambiguously notified if it combines the outset and near-end data, regardless of the same or different reaction order.

3. Results and discussion

3.1. First case

SRIM is an integral method; therefore, it is interesting to compare *SRIM* and *SRIM_{IM}* together with *SRIM_{IRM}*. The advantage of *IRM* is that it directly gives the correct reaction order. However, the rate constant, k , value is uncertain. Save for *zeroth*-order reactions, k values are always different from *IM*. The correct value should be consulted with the Integral Method (*IM*) [10]. However, it is difficult to differentiate between integers and half-integers and even between sequences of integer order with *IM*. The actual value obtained from this method can be equated using the correction factor in Eq. (20-21). However, both of these equations will only be valid at a fixed β condition [11].

$$C_f = 1 \quad \text{for } n = 0 \quad (20)$$

$$C_f = -\frac{1-\beta}{\beta \ln \beta} \quad \text{for } n = 1 \quad (21)$$

With Eq. (20), for *zeroth*-order, $k_{IRM} = k_{IM}$, and so on, $v_{\max-IRM} = v_{\max-IM}$. However, in Table 8, for $[H^+] = 0.05 M$, $v_{\max-IRM} \neq v_{\max-IM}$, the percentage difference is 14.53 %. The percentage difference of v_{\max} values between *SRIM* and *SRIM_{IM}* relative to *SRIM_{IM}* is 7.67%; this is slightly higher than the maximum allowable of 7%. The reason is that although *SRIM_{IRM}* gives the correct order, the regression coefficient is 0.0265, which means there almost no correlation. For the same case, *SRIM_{IM}* gives a regression coefficient of 0.9996 (Table 6.).

In this study, *SRIM* is taken as the standard method. It is only because the *SRIM* is directly derived from Eq. (5) and Eq. (7). Actually, *SRIM* is impractical to use. The order must determine in advance by *IM*. However, from these steps, v_{\max} and K_M can be directly determined by *SRIM_{IM}*. As shown in Table 8, the percentage difference between *SRIM_{IM}* and *SRIM* relative to *SRIM* for v_{\max} was 3.99% and for K_M was 2.49%. Therefore, it is better to use *SRIM_{IM}* as the standard. The only drawback is to obtain a definite order of the reactions, and two different integral equations must be tested; *zeroth*-order and *first*-order. However, this can be overcome by using Eq. (22).

$$t_i = \beta^{(i-1)(1-n)} t_\beta \quad (22)$$

Where β , t_β , t_i , i , and n are the remaining reactant fractions (must be kept constant), first time observation, following observations, observation, and reaction order, respectively [12, 13]. For example if $n = 0$ and $\beta = 0.5$ then $t_1 = t_\beta$, $t_2 = 1/2 t_\beta$, and $t_3 = 1/4 t_\beta$. Then, if observations are made simultaneously, the rate will not be the same; it is faster. Thus, for $n = 1$, t_i will always be the same with t_β .

3.2 Second case

There are three misconceptions in the *L-B* and *E-H* methods. First, both equations are based on *IRM*. *IRM* is best for determining reaction order but not for rate constants. There are two different laws.

Second, they do not consistently adhere to the basic concept of determining v_{\max} and K_M . The value of v_{\max} must be determined when the reaction is *zeroth-order* (at the outset) and K_M when the reaction is *first-order* (at near-end). However, neither method requires an examination of the real rate law. Instead, they use the combination of the outset and near-end data. There are values for v_{\max} and K_M , even if the reaction is *half-order* at the outset and *first-order* at the end (Table 4) or always *first-order* (Table 7). In Table 8, the v_{\max} value obtained by all methods is almost the same. Theoretically, the value obtained by *L-B* and *E-H* should be lower compared to the new method. With the new method, v_{\max} is determined when the reaction is *zeroth-order*, the reaction at its maximum velocity. This is because both methods are basically based on *IRM* (or differential method). There may be problems in using *IRM* for constant time interval observation data. In Table 5, the v_{\max} value by the *SRIM*_{IRM} is higher. As expected, the K_M values obtained by the new methods are relatively higher than those of *L-B* and *E-H*. The K_M value depends on v_{\max} .

Table 4. Results of data analysis of hydrolysis of phenylacetate catalyzed by acetylcholinesterase

| Method | | A | B | r | Order | V_{\max} | v_{\max} / K_M | K_M |
|--------------|----------|-----------------------|------------------------|---------|-------|-----------------------|------------------|-----------------------|
| | Outset | -4.6786 | 0.55 | 0.9948 | 0.5 | | | |
| | Near-end | -3.7877 | 0.83 | 0.9856 | 1 | | | |
| <i>L - B</i> | All data | 824855.5 | 1254.81 | 0.9927 | - | 1.21×10^{-6} | 1254.81 | 1.52×10^{-3} |
| <i>E - H</i> | All data | 1.17×10^{-6} | -1.44×10^{-3} | -0.9654 | | 1.17×10^{-6} | | 1.44×10^{-3} |

Third, however, the main misconception of the *L-B* and *E-H* methods concerns their equations. If $v = v_{\max}$ then both Eq. (15) and Eq. (16) must give:

$$K_M = 0 \quad (23)$$

Eq. (20) states that v_{\max} and K_M cannot be determined using a combination of outset and near-end data [11]. In actual, the used combined data is against Eq. (8). If $v_o = v_{\max}$, Eq. (8) also gives Eq. (20). These means that the value of v_{\max} and K_M must be determined separately; v_{\max} when $[S] \gg K_M$ (*zeroth-order* to $[S]$) and K_M when $[S] \ll K_M$ (*first-order* to $[S]$).

3.3. Third case.

Unusually, in Table 4, the order of $[S]$ is *half-order*. Such cases occur when the enzyme or substrate is a weak acid or base. According to Eq. 7, $[S]$ is *first-order*; however, the estimation using the modified method in the present study results in *half-order*. Therefore, the Michaelis reaction rate-law presented in Eq. 7 should be re-considered. In Table 8, v_{\max} changes with the change in $[H^+]$. However, dividing v_{\max} for $[H^+] 0.100 M$ by that at $[H^+] 0.100 M$ gives a value of about 2. Since the order must be integer or half-integer, the order to $[H^+]$ is $n = 1$ which is the most general order for a catalyst or enzyme. The division results also give relatively the same value for k_{obs} (the observed catalyst constant), where $k_{\text{obs}} = k_c [H^+][\text{Acetone}]$. These facts mean that the term maximum velocity is not a suitable notion for v_{\max} ; the better is the maximum relative velocity. The real constant is the observed catalyst constant k_{obs} (or generally k_c for a single substrate).

Table 5. Results of data analysis of hydrolysis of methyl hydro-cinnamate catalyzed by chymotrypsin

| Method | A | | B | | r | n | V_{max} | V_{max} / K_M | K_M / V_{max} | K_M |
|---------------|-----------------------|------------------------|---------|--------|------|-----------|-----------------------|-----------------------|-----------------|-----------------------|
| | A | B | A | B | | | | | | |
| Outset | -6.359 | 0.22 | 0.22 | 0.9906 | 0 | | | | | |
| Near-end | -4.901 | 0.84 | 0.9993 | 1 | | | | | | |
| SRI/M_{IRM} | | | | | | | 4.38×10^{-7} | 1.26×10^{-5} | | 3.49×10^{-2} |
| $L - B$ | 4373009.1 | 19939.679 | 1.0000 | - | 2.29 | 10^{-7} | | 19939.679 | | 4.56×10^{-3} |
| $E - H$ | 2.30×10^{-7} | -4.60×10^{-3} | -1.0000 | | 2.30 | 10^{-7} | | | | 4.60×10^{-3} |

Table 6. Results of statistic analysis data of reaction of acetone with bromine catalyzed by $[H^+]$

| Method | $[H^+]$ (M) | Outset | | | | Near - end | | | |
|--------|-------------|-------------|------------------------|---------|-------------|------------------------|------------------------|---------|-----------------------|
| | | A | B | r | n | A | B | r | n |
| IRM | 0.050 | -3.2653 | 0.16 \approx 0 | 0.0265 | 0 | -1.9664 | 1.22 \approx 1 | 0.4323 | 1 |
| | 0.100 | -3.0057 | 0.01 \approx 0 | 0.0072 | 0 | -2.0485 | 0.96 \approx 1 | 0.9957 | 1 |
| | 0.200 | -2.3841 | 1.05 \approx 1 | 0.9692 | 1 | -1.9439 | 0.97 \approx 1 | 0.9950 | 1 |
| IM | $[H^+]$ (M) | n_{trial} | | | n | n_{trial} | | | |
| | | A | B | r | A | B | r | n | |
| | | 0.473 | -4.74×10^{-4} | -0.9996 | 0 | 0.061 | -2.89×10^{-4} | -0.9962 | 1 |
| | 0.050 | -0.749 | -1.04×10^{-3} | -0.9995 | 1 | -2.780 | -5.65×10^{-3} | -0.9974 | |
| | 0.100 | 0.493 | -9.74×10^{-4} | -0.9999 | 0 | 0.078 | -6.00×10^{-4} | -0.9968 | |
| | 0.200 | -0.706 | -2.13×10^{-3} | -0.9998 | 0 | -2.510 | -1.06×10^{-2} | -0.9999 | |
| | | 0.471 | -1.66×10^{-3} | -0.9995 | 0 | 0.117 | -1.04×10^{-3} | -0.9957 | |
| | | -0.747 | -4.04×10^{-3} | -1.0000 | 1 | -2.086 | -1.30×10^{-2} | -1.0000 | |
| Method | $[H^+]$ (M) | $L - B$ | | r | $[H^+]$ (M) | $E - H$ | | r | |
| | | A | B | | | A | B | | |
| | | 1904.1 | 84.084 | | | 0.7818 | 0.050 | | 4.91×10^{-4} |
| | 0.100 | 897.151 | 48.4317 | 0.9601 | 0.100 | -1.08×10^{-3} | -4.73×10^{-2} | -0.9999 | |
| | 0.200 | 481.77 | 43.8227 | 0.9610 | 0.200 | -1.96×10^{-3} | -7.54×10^{-2} | -0.9012 | |

Table 7. Effects of catalyst concentrations changes on the reaction of acetone with bromine by *SRIM*, *SRIM_{IRM}*, *SRIM_{IM}*, *L-B*, and *L-H*

| $[H^+]$ (M) | Methods | v_{max} Abs. | v_{max}/K_M | K_M Abs | $1/v_{max}$ | v_{max} Abs | K_M/v_{max} | K_M Abs | v_{max} M | K_M M |
|----------------|------------------------------|-----------------------|--|--|-------------|---------------|--|--|--|-----------------------|
| 0.050 | <i>SRIM</i> | 5.01×10^{-4} | 8.55×10^{-2} | | | | | | 3.13×10^{-6} | 5.35×10^{-4} |
| | <i>SRIM_{IRM}</i> | 5.43×10^{-4} | 1.08×10^{-2} | | | | | | 3.39×10^{-6} | 3.14×10^{-4} |
| | <i>SRIM_{IM}</i> | 4.74×10^{-4} | 5.65×10^{-3} | | | | | | 2.96×10^{-6} | 5.24×10^{-4} |
| | <i>L - B</i> <i>E - H</i> | 1904.1 | 84.084 | 5.25×10^{-4} 4.91×10^{-4} | 1904.1 | 84.084 | 4.41×10^{-2} 3.01×10^{-2} | 3.28×10^{-6} 3.07×10^{-6} | 2.76×10^{-4} 1.88×10^{-4} | |
| 0.100 | <i>SRIM</i> | 1.00×10^{-3} | 9.45×10^{-2} | | | | | | 6.25×10^{-6} | 5.91×10^{-4} |
| | <i>SRIM_{IRM}</i> | 9.87×10^{-4} | 8.94×10^{-3} | | | | | | 6.17×10^{-6} | 6.90×10^{-4} |
| | <i>SRIM_{IM}</i> | 9.74×10^{-4} | 1.06×10^{-2} | | | | | | 6.09×10^{-6} | 5.74×10^{-4} |
| | <i>L - B</i> <i>E - H</i> | 897.151 | 1.12×10^{-3} 1.08×10^{-3} | 48.4317 | 897.151 | 48.4317 | 5.39×10^{-2} 4.73×10^{-2} | 7.00×10^{-6} 6.75×10^{-6} | 3.37×10^{-4} 2.96×10^{-4} | |
| 0.200 | <i>L - B</i> | 481.77 | 2.08×10^{-3} | 43.8227 | 481.77 | 43.8227 | 9.10×10^{-2} | 1.30×10^{-5} | 5.69×10^{-4} | |
| | <i>E - H</i> | | 1.96×10^{-3} | | | | 7.54×10^{-2} | 1.23×10^{-5} | 4.71×10^{-4} | |

Table 8. The effect of changing the $[H^+]$ on the reaction order and the values of v_{max} and κ in the reaction of acetone with bromine.

| Methods | $[H^+]$ M | v_{max} M s ⁻¹ | Ratio | n | k_{obs} s ⁻¹ | $\dot{X} \times 10^6$ | $\sigma\chi$ | PD % | $K_M \times 10^4$ M | Ratio | $\dot{X} \times 10^4$ | $\sigma\chi$ | PD % |
|---------------------------|--------------|-----------------------------|------------------|---|---------------------------|-----------------------|-----------------------|------|---------------------|-------|-----------------------|-----------------------|-------|
| <i>SRIM</i> | 0.100 | 6.25×10^{-6} | $2.00 \approx 2$ | 1 | 6.25×10^{-5} | 6.26 | 5.00×10^{-8} | 1 | 5.91 | 1.11 | 5.63 | 2.85×10^{-5} | 1 |
| | 0.050 | 3.13×10^{-6} | | | 6.26×10^{-5} | | | | 5.35 | | | | |
| <i>SRIM_{IRM}</i> | 0.100 | 6.17×10^{-6} | $1.82 \approx 2$ | 1 | 6.17×10^{-5} | 6.48 | 3.05×10^{-7} | 3.51 | 6.90 | 2.20 | 5.02 | 1.88×10^{-4} | 10.83 |
| | 0.050 | 3.39×10^{-6} | | | 6.78×10^{-5} | | | | 3.14 | | | | |
| <i>SRIM_{IM}</i> | 0.100 | 6.09×10^{-6} | $2.06 \approx 2$ | 1 | 6.09×10^{-5} | 6.01 | 8.50×10^{-8} | 3.99 | 5.74 | 1.10 | 5.49 | 2.50×10^{-5} | 2.49 |
| | 0.050 | 2.96×10^{-6} | | | 5.92×10^{-5} | | | | 5.24 | | | | |
| <i>L - B</i> | 0.100 | 7.00×10^{-6} | $2.13 \approx 2$ | 1 | 7.00×10^{-5} | 6.78 | 2.20×10^{-6} | 8.48 | 3.37 | 1.22 | 3.07 | 3.05×10^{-5} | 45.47 |
| | 0.050 | 3.28×10^{-6} | | | 6.56×10^{-5} | | | | 2.76 | | | | |
| <i>E - H</i> | 0.100 | 6.75×10^{-6} | $2.20 \approx 2$ | 1 | 6.75×10^{-5} | 6.45 | 3.05×10^{-6} | 8.31 | 2.96 | 1.60 | 2.42 | 5.40×10^{-5} | 57.02 |
| | 0.050 | 3.07×10^{-6} | | | 6.14×10^{-5} | | | | 1.88 | | | | |
| <i>L - B</i> | 0.200 | 1.30×10^{-5} | | | | | | | 5.69 | | | | |
| | <i>E - H</i> | 1.23×10^{-5} | | | | | | | 4.71 | | | | |

4. Conclusions

Three new methods for determining the fitting constant of the Michaelis-reaction have been introduced. The $SRIM$, $SRIM_{IRM}$, and $SRIM_{IM}$ methods are reliable for determining the fitting-constants of enzyme-catalyzed reaction, in which the order of the accuracy method for these three methods is $SRIM \approx SRIM_{IM} > SRIM_{IRM}$. The Lineweaver-Burk and Eadie-Hofstee methods are not recommended for the determination of the fitting-constants of an enzyme-catalyzed reaction. The better notion of the concept of v_{max} is the relative maximum rate, not the maximum rate. The true constant is k_{obs} (or k_c if the substrate is single).

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