COMPUTATIONAL CHEMISTRY GROUP PROJECT

SUBJECT CODE: CY40014

SEMESTER 1

AUTUMN 2021

JOINT M.Sc.-Ph.D. IN CHEMISTRY

INDIAN INSTITUTE OF TECHNOLOGY, KHARAGPUR

GROUP 10

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TITLE

Determination of K_m (Michaelis Menten constant), v_{max} (Maximum velocity of the enzyme catalysed reaction) and Lineweaver Burk plot from experimental values with the help of python program

OBJECTIVES

The objectives of this project is to write a python code which:

1. Takes the R_0 (initial rate of reaction) and S_0 (initial concentration of substrate) value set from the user.
2. Determines the K_m (Michaelis Menten Constant) of the enzyme.
3. Determines the v_{max} (maximum rate of reaction)
4. Plots and fits the Lineweaver Burk plot.
5. Determines the goodness of fit of the Lineweaver Burk plot.

CODE:

```
import matplotlib.pyplot as plt
import numpy as np

x = [float(x) for x in input("Enter all the values of S0 (in mM) with spaces in between two consequetive values: ").split()]
#print("List of S0 is: \n ", x)              #To view the list in output please remove the #
y = [float(y) for y in input("Enter all the values of R0 (in mM/s) with spaces in between two consequetive values: ").split()]
#print("List of R0 is: \n ", y)              #To view the list in output please remove the #

a=[1/i for i in x]
b=[1/j for j in y]


aave= np.mean(a)
bave= np.mean(b)
s1=0.0
s2=0.0
for i in range (len(a)):
    s1+=(a[i]-aave)*(b[i]-bave)
    s2+=(a[i]-aave)**2
m=s1/s2
c= bave-m*aave
#print("Slope=", m)            #To view the slope in output please remove the #
#print("Intercept=", c)        #To view the intercept in output please remove the #

y_fit= [m*i+c for i in a]
```

```
# Plotting the fitted data

plt.plot(a,b,"ok")
plt.plot(a, y_fit, "r")
plt.title (label="Lineweaver Burk Plot",loc='center', fontsize=20, color= "black", fontstyle = "italic")
plt.xlabel("1/$S_0$ (in $mM^{-1}$)")
plt.ylabel("1/$R_0$ (in s/mM)")

print("\n")
vmax= 1/c
print("vmax is ", round(vmax,4), " mM/s")                    # Obtaining vmax
Km= vmax*m
print("Michaelis Menten constant is ", round(Km,4), "mM")    # Obtaining Km

# Goodness of fit of linear curve

s3=0.0
s4=0.0
for i in range (len(a)):
    s3+= (y_fit[i]- bave)**2
    s4+= (b[i] - bave)**2
R_sq= s3/s4
print ("\n\nGoodness of fit(R^2) of the Lineweaver Burk plot is", round(R_sq,4))


# Pearson's correlation

s5=0.0
s6=0.0
s7=0.0
for i in range (len(a)):
    s5+= (a[i]-aave)*(b[i]-bave)
    s6+= (a[i]-aave)**2
    s7+= (b[i]-bave)**2
r=s5/np.sqrt(s6*s7)
print("\nPearson's r value is", round(r,4))
```

```
print("\nThe experimental data can be concluded to be precise if the Pearson's correlation and Goodness of fit values are near equ
```

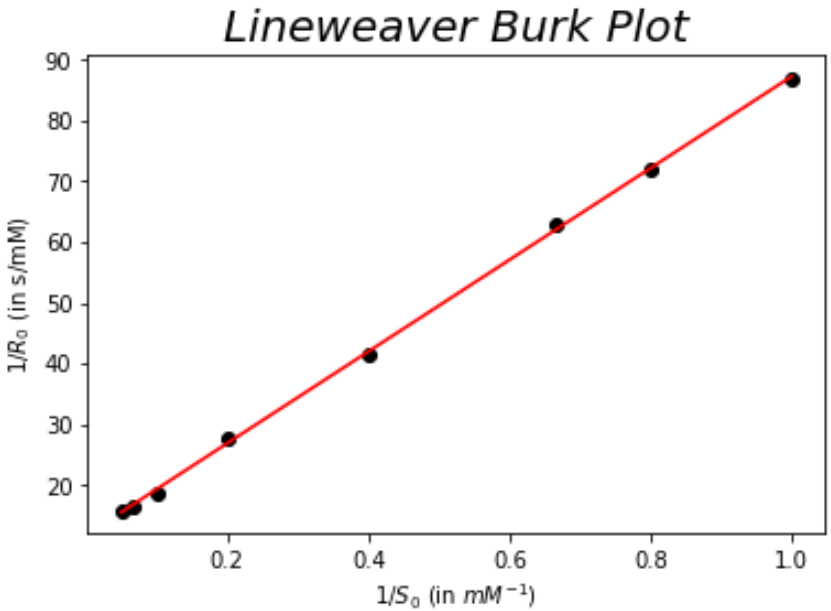
```
Enter all the values of S0 (in mM) with spaces in between two consequetive values: 1 1.25 1.5 2.5 5 10 15 20
Enter all the values of R0 (in mM/s) with spaces in between two consequetive values: 0.0115 0.0139 0.0159 0.024 0.036 0.0532


vmax is  0.0845  mM/s
Michaelis Menten constant is  6.3708 mM


Goodness of fit(R^2) of the Lineweaver Burk plot is 0.9996

Pearson's r value is 0.9998

The experimental data can be concluded to be precise if the Pearson's correlation and Goodness of fit values are near equal
```



Link to the Google Colab file:

https://colab.research.google.com/drive/1XRbDInn23TGW1809_lDsJYtzZbp0fDq6?usp=sharing

THEORY:

Enzyme kinetics and Michaelis–Menten equation:

In biological systems, enzymes act as catalysts and play a critical role in accelerating reactions, anywhere from 103 to 1017 times faster than the reaction would normally proceed. Enzymes are high-molecular weight proteins that act on a substrate, or reactant molecule, to form one or more products.

The principal features of many enzyme-catalysed reactions are as follows:

1. For a given initial concentration of substrate, $[S]$, the initial rate of product formation is proportional to the total concentration of enzyme, $[E]$.
2. For a given $[E]$, and low values of $[S]$, the rate of product formation is proportional to $[S]_0$.
3. For a given $[E]$, and high values of $[S]$, the rate of product formation becomes independent of $[S]_o$, reaching a maximum value known as the maximum velocity, v_{max}.

$$\mathrm{E} + \mathrm{S} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \mathrm{ES} \overset{k_2}{\rightarrow} \mathrm{E} + \mathrm{P}$$

$[E_0]$ = initial concentration of enzyme

$[E]$ = free enzyme concentration

$[ES]$ = concentration of enzyme substrate

$[p]$ = concentration of product

$[E_0] = [E] + [ES]$

Assumptions : 1. $[E] << [S]$

```
  R = k2 [ES] = d[P]/dt = - d[ES]/dt

2. Applying steady state approximation ,

(k-1 + k2) [ES] = k1[E][S]

[ES] = k1[E][S]/ (k-1 + k2)

Again, R= k2[ES]

        = k2k1[E][S]/(k-1+k2)

        = k2[E][S]/km

The ratio of rate constants is defined as Michalies Menten Constant.
 Km = ( k2 + k-1) / k1
```

Applying initial kinetics, [E]= [E0]-[ES]

```
[S0]=[S] as [S]>>[ES]

[ES]= [E0][S0]/(km+[S0])

R0 = k2[E0][S0]/(km+[S0])
```

Case 1: At low concentration of substrate, km >> [S0]

```
 At low concentration of substrate  R = k2[E0][S0]/Km

 So the reaction is 1st order with respect to substrate
```

Case 2 : At high concentration of substrate , [S0] >> Km

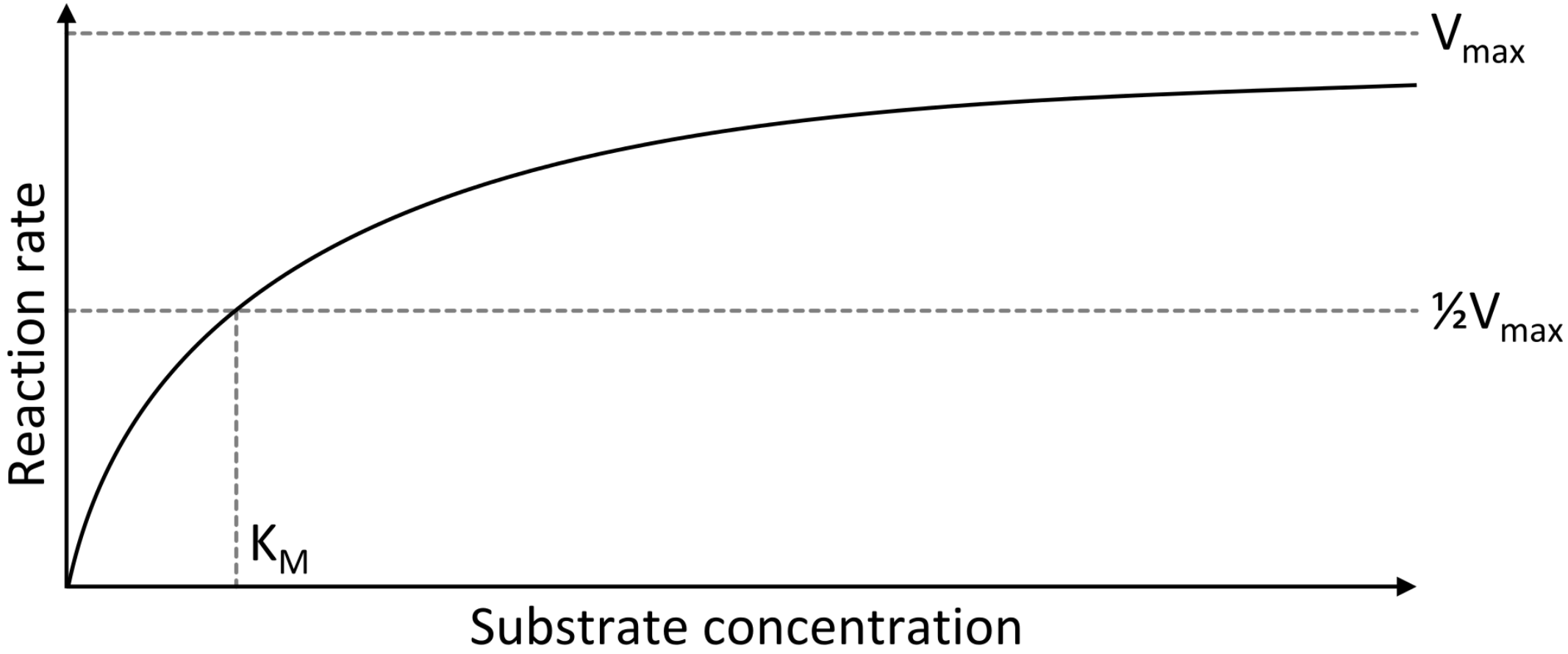
```
R = k2 [E0] = Rmax

So the enzyme catalyst reaction is zero order with respect to substate
```

Case 3 : At moderate concentration , Km = [S]

```
R = k2[E0]/2 = Rmax/2
```

So, the $[R]$ vs $[S_0]$ plot will comprise these three regions and will look like this



Lineweaver Burk plot :

In biochemistry, the Lineweaver–Burk plot (or double reciprocal plot) is a graphical representation of the Lineweaver–Burk equation of enzyme kinetics, described by Hans Lineweaver and Dean Burk in 1934.

It is plotted $\frac{1}{R_0}$ against $\frac{1}{[S_0]}$

$R_0 = \frac{k_2[E_0][S_0]}{K_m+[S_0]} = \frac{R_{max}.[S_0]}{K_m+[S_0]}$

$\frac{1}{R_0} = \frac{K_m+[S_0]}{R_{max}[S_0]}$

so, $\frac{1}{R_0} = \frac{K_m}{R_{max}[S_0]} + \frac{1}{R_{max}}$

This equation is of type y= mx+c

where,

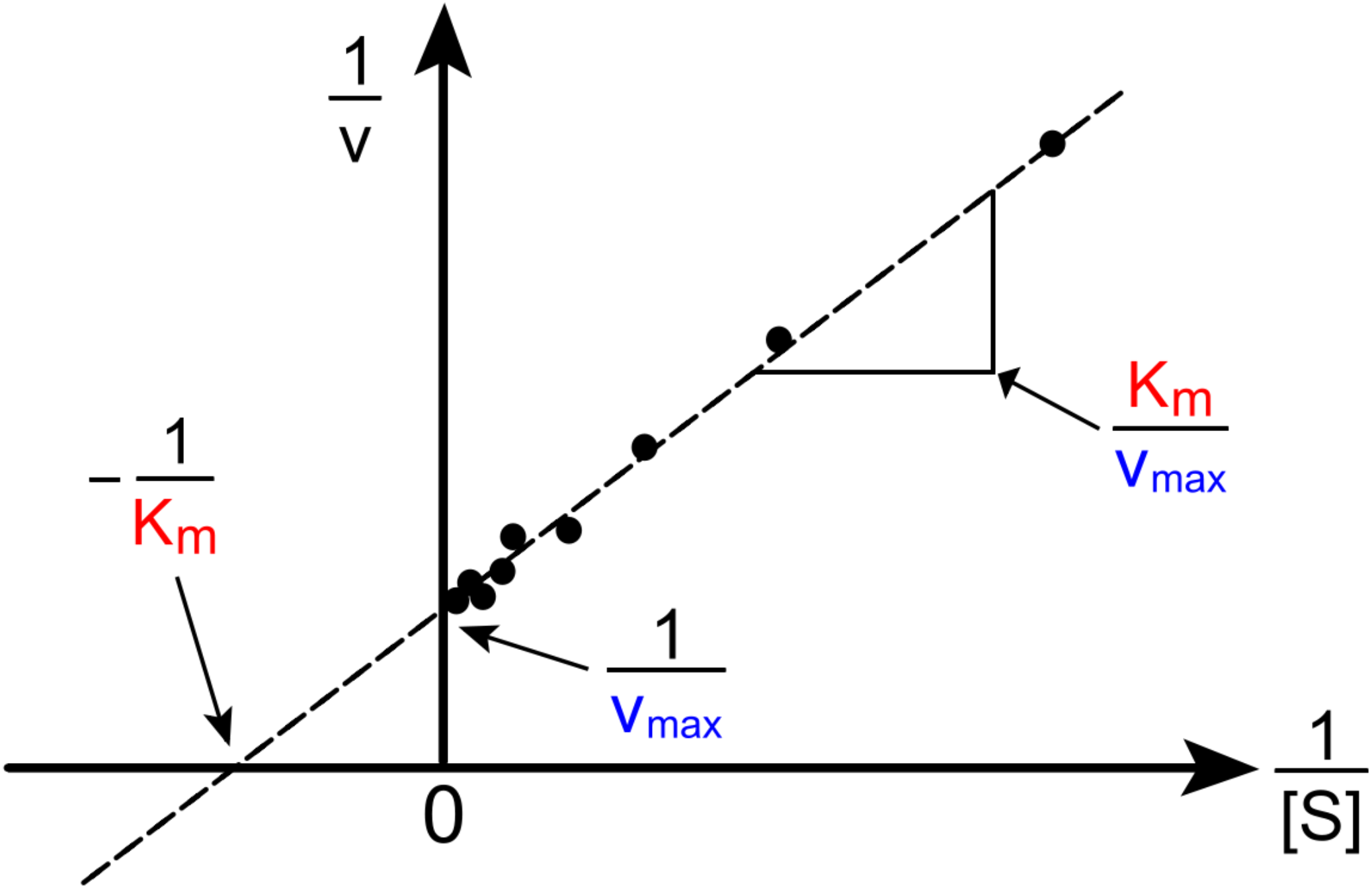
c= Intercept= $\frac{1}{R_{max}}$

m= Slope = $\frac{K_m}{R_{max}}$

$V_{max} or R_{max}$: Maximum velocity of the reaction.

K_m: Michaelis-Menten constant or enzyme affinity. The lower the K_m higher the affinity.

This is the Lineweaver Burk Plot.


1
v
$-\frac{1}{K_m}$
$\frac{K_m}{V_{max}}$
$\frac{1}{V_{max}}$
0
1
[S]

DISCUSSION:

Features of the code:

1. The code first takes input from the user.
2. Then reciprocal of the data sets are taken and curve fitting is performed on these data
3. We get a fitted straight line with positive slope which is the Lineweaver Burk plot.
4. The slope and intercept are obtained from where we get the K_m and v_{max} values.
5. Lastly Goodness of fit and Pearson's r value are calculated.

Uses of the code:

1. The user can instantly determine K_m values and v_{max} with experimental values without actual calculation which is time and energy consuming. The graph also can be obtained and analysed without manual drawing.
2. The code also finds out the goodness of fit and Pearson's r value , both of which helps us to understand the accuracy of the experimental data because more accurate data will result in a better fitted line in the graph.
3. By having a look at the K_m and v_{max} of various enzymes we can get an idea about some features of the enzymes and compare their behavoirs. This is discussed in the following sections in details.

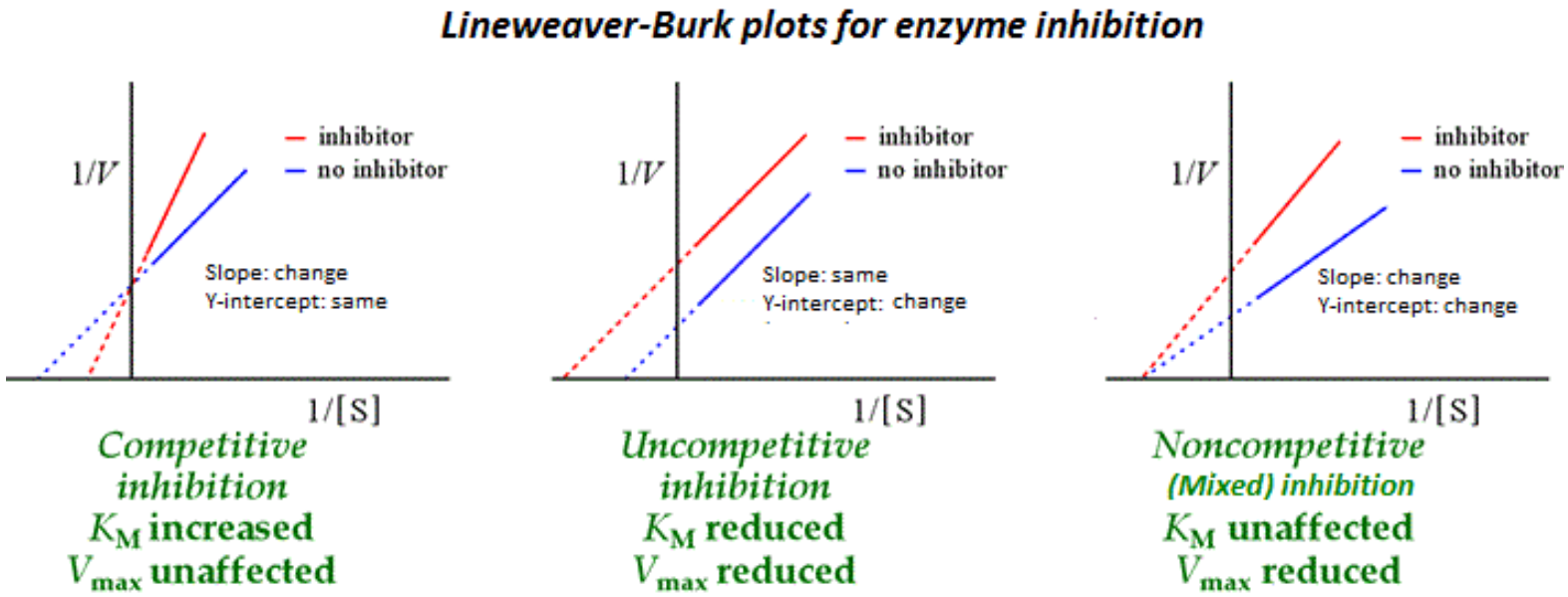
APPLICATIONS :

1. The analysis of enzyme behavior using Michaelis-Menten kinetics is a staple of the biochemistry laboratory. We can measure kinetic properties of enzymes with precision and distinguish between competitive, non-competitive, and uncompetitive inhibition.

   Competitive inhibition increases the K_m, or lowers substrate affinity. The K_m inhibited is αK_m. Graphically this can be seen as the inhibited enzyme having a larger x-intercept. The slopes of competitively inhibited enzymes and non-inhibited enzymes are different.

With **pure noncompetitive inhibition** v_{max} is lowered with inhibition. v_{max} inhibited is αv_{max}. This can be seen on the Lineweaver–Burk plot as an increased y-intercept with inhibition, as the reciprocal is plotted. Pure noncompetitive inhibition does not effect substrate affinity, therefore K_m remains unchanged. Graphically this can be seen in that enzymes with pure noncompetitive inhibition intersect with non-inhibited enzymes at the x-axis.

v_{max} decreases with **uncompetitive inhibition**. v_{max} inhibited is αv_{max}. This can be seen on the Lineweaver–Burk plot as an increased y-intercept with inhibition, as the reciprocal is plotted. This relationship is seen in both uncompetitive inhibition and pure competitive inhibition. Substrate affinity increases with uncompetitive inhibition, or lowers K_m. The inhibited K_m is K_m/α. Graphically this means that enzymes with uncompetitive inhibition will have a smaller x-intercept than non inhibited enzymes.Despite the x-intercept and y-intercept of uncompetitive inhibition both changing, the slope remains constant. Graphically uncompetitive inhibition can be identified in that the line of inhibited enzyme is parallel to non-inhibited enzyme.



2. Ferulic acid (FA) production has become a frequent focus on today's research due to its antioxidant properties. Studies were performed to determine the kinetics of FA production in a mixed culture fermentation by applying the Michaelis–Menten kinetic model.

3. The Michaelis–Menten equation is mainly used to characterize the enzymatic rate at different substrate concentrations, but it is also widely applied to characterize the elimination of chemical (the first-order kinetics) compounds from the body.

A good example is ethyl alcohol (follow zero order kinetics in human body) ; alcohol dehydrogenase becomes saturated with normal doses of alcohol beverages; for example, 0.5‰ blood concentration is more than 20 times higher than the Km-value of ethanol for alcohol dehydrogenase. Because of this saturation, ethyl alcohol is eliminated at a constant rate about 0.1 g/h/kg or 7 g/h in human.

4. K_{cat} is the turnover number, or reactions per unit time. The lower the K_{cat} the slower the reaction.

   $K_{cat} = V_{max}/[Enzyme]$.

   Turnover number can be determined from V_{max} if the concentration of Enzyme is measured, thus speed of reaction can be predicted.

5. The constant K_{cat}/K_m (catalytic efficiency) is a measure of how efficiently an enzyme converts a substrate into product. Diffusion limited enzymes, such as fumarase, work at the theoretical upper limit of $10^8 – 10^{10} M^{-1} s^{-1}$, limited by diffusion of substrate into the active site.

   For example Catalytic efficiency of fumerase is $1.6 \times 10^8 M^{-1} s^{-1}$

FUTURE POTENTIAL OF THE CODE

1. This code can be modified to be useful for determining type of inhibition in case of enzyme inhibition reactions. By comparing the two K_m and v_{max} obtained from the two sets of data and implementing if else statements we can find out if the inhibition is competitive, uncompetitive or non competitive as explained in the Applications section.

2. If the Enzyme concentration is determined, then the code can be extended to get k_{cat} and speed of reaction can be compared.

3. From k_{cat} and K_m, catalytic efficiency also can be calculated.

ACKNOWLEDGEMENTS

We hereby express our gratitude to Prof. Sabyashachi Mishra, Associate Professor, Department of Chemistry, IIT Kharagpur for his valuable advice and guidance which helped us a lot to complete this project.

We also thank our friends and family for all their support and help.

REFERENCES

https://en.wikipedia.org/wiki/Lineweaver%E2%80%93Burk_plot

https://en.wikipedia.org/wiki/Michaelis%E2%80%93Menten_kinetics

https://www.wikiwand.com/en/Lineweaver%E2%80%93Burk_plot

https://www.geeksforgeeks.org/matplotlib-pyplot-title-in-python/