Doctor 022

# Biochemistry

Sheet no. 22

## Enzyme kinetics


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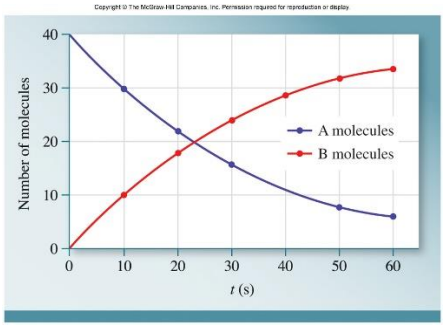
# Enzymes kinetics

**Kinetics deals with the rates of chemical reactions, enzymes kinetics is the study of the rates of enzymatic reactions (Enzymes kinetics=enzymes activity, kinetics of chemical reaction=rates of chemical reaction).**

**For the reaction (A to B), velocity (v) or rate of the reaction is the amount of B formed (or the amount of A consumed ) per unit time , t. That is,**

$$\text{Rate of reaction (velocity or v)} = -\frac{\Delta [A]}{\Delta t} \quad or \quad \frac{\Delta [B]}{\Delta t} = -k[A] = k[B]$$



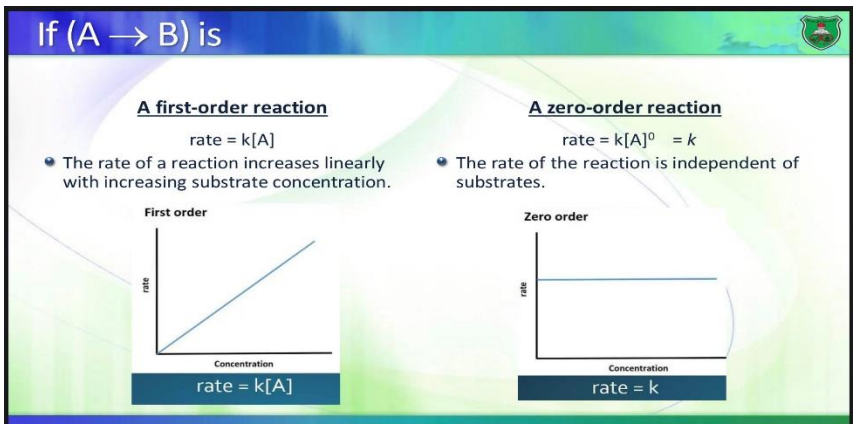

**The negative sign indicates that the concentration of A is decreasing numerator : the rate of change in [A] concentration.**

**Denominator: Time.**

**Note the (-) in the first equation . Notice they are equal.**

**This is known as the rate law , which describes how concentrations of reactants affect the rate of the reaction during a certain period .**

**Note : the rate is proportional to the concentration of A , and K is the rate constant. K has the unit of (time)$^{-1}$ ,usually sec$^{-1}$.**



**The first order reaction : by increasing the concentration of the substrate , the rate of the reaction increases .**

**The zero order reaction : Rate of the reaction does not change by changing the concentration of the substrate .**

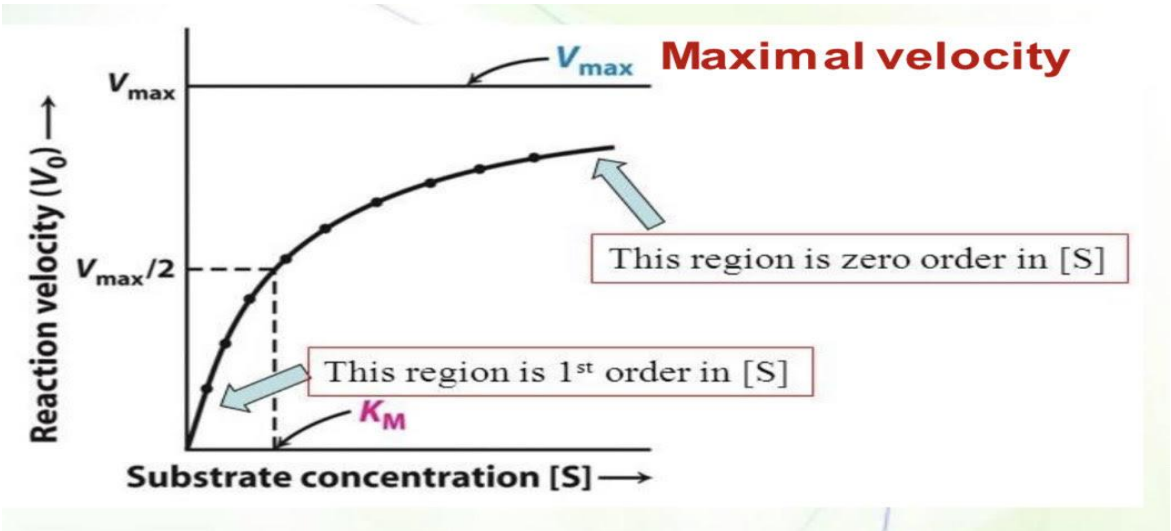
**Note the difference between the 2 reactions , the rate of first order reaction equals k[A] , while the rate of zero order reaction equals K (Independent of [A]. A^0=1**

- Enzyme-catalyzed reactions have hyperbolic plots.
- Initial velocity ($V_0$) varies with the substrate concentration [S] where the rate of catalysis rises linearly as the substrate concentration increases and then levels off and approaches a constant, maximal velocity (Vmax) at higher substrate concentrations.

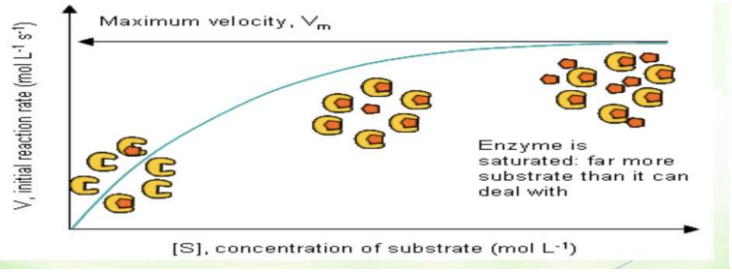
**For many enzymes , if we calculate the velocity of the reaction in comparison with the substrate concentration , we will notice that when the concentration is low ,the velocity increases linearly ( as in first order reaction ), but the more we increase the concentration , the rate of the reaction is becoming flat (hyperbolic plots), eventually we will reach maximum velocity , at this point the velocity of the reaction is independent of the substrate concentration ( as in zero order reaction ).**

**$V_0$ : varies with substrate concentration**

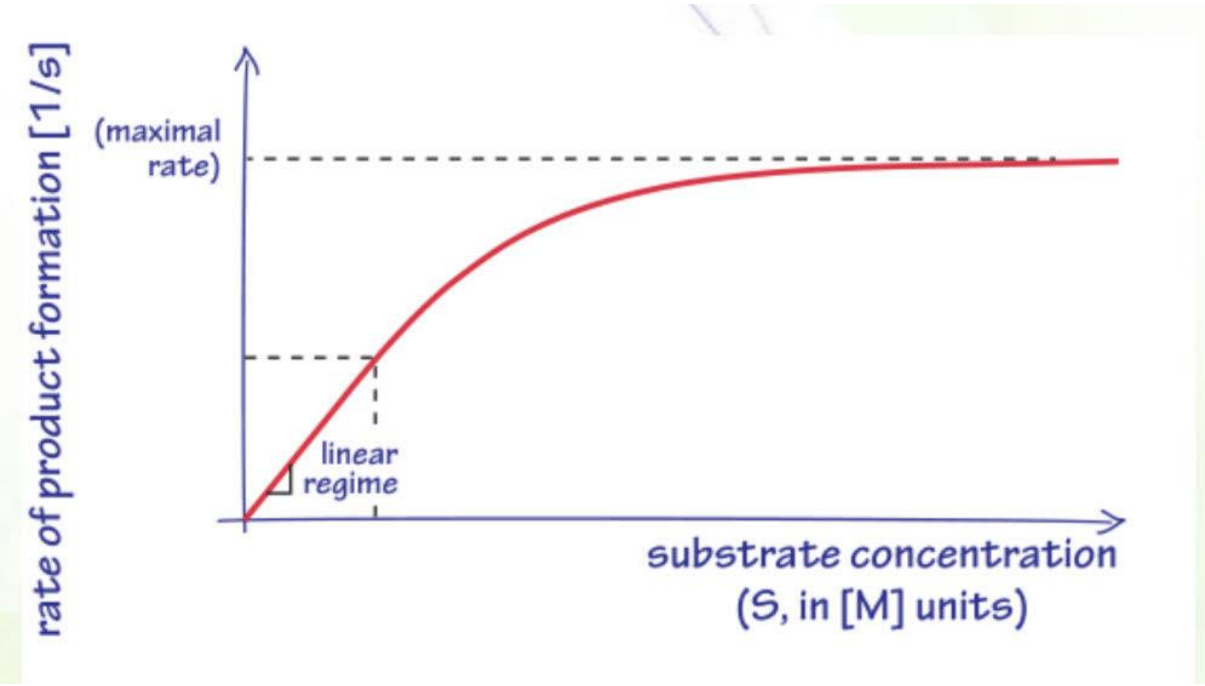
**$V_{max}$ : independent of the substrate concentration(it is a constant).**



**The hyperbolic plot is known as a saturation plot(becomes platue) because the enzyme becomes "saturated" with the substrate, i.e, each enzyme molecule has a substrate molecule associated with it .**




**-At a fixed concentration of enzyme, V is almost linearly proportional to [S] when [S] is small.**
**However, V is nearly independent of [S] when [S] is large. o**
**-The maximal rate, Vmax, is achieved when the catalytic sites on the enzyme are saturated with substrate.**
**-Vmax reveals the turnover number of an enzyme.**
**Turnover number :The number of substrate molecules converted into products by an enzyme molecule in a unit time when the enzyme is fully saturated with substrate .**



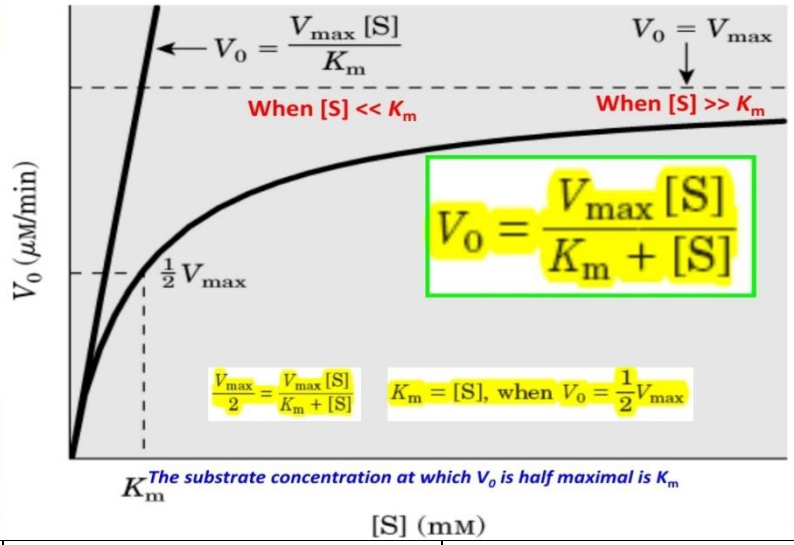
# The Mechaelis-Menten equation

The Michaelis-Menten equation is a quantitative description of the relationship between the rate of an enzyme catalysed reaction (Vo), substrate concentration [S], a rate constant (KM) and maximal velocity (Vmax).

$$V_0 = V_{max} \frac{[S]}{[S] + K_M}$$

The velocity of the reaction depends on its efficiency (Vmax) and the substrate concentration [S]

*Note: $K_m$ and $V_{max}$ (efficiency of enzyme)are constants.




| When [S]>>$K_m$ : | When [S]<<$K_m$ : | When [S] = $K_m$ : |
|---|---|---|
| Here $K_m$ is insignificant (بدون معنى)<br><br>For example<br>$K_m$ =1, [S]= 100<br>Vo= $V_{max}$ × 100/ (1+100) ≈ 1× $V_{max}$<br><br>The Vo is independent of the [S] when it is highly concentrated; so, when increasing the [S] there is no significant change in the velocity because the Vo has reached the $V_{max}$ | For example:<br>$K_m$ =1 , [S]= 0.1<br>Vo= $V_{max}$ × 0.1/(1+0.1)<br><br>The low substrate concentration has big effect on the velocity of the reaction; so any small changes in the [S] would have dramatically change in Vo .<br><br>Direct relationship between [S] and Vo, Increasing [S] increases Vo | For example<br>$K_m$ =1 , [S]= 1<br>Vo= $V_{max}$ × 1/(1+1) = ½ $V_{max}$<br><br>It is obvious that the [S] has an effect on the Vo |

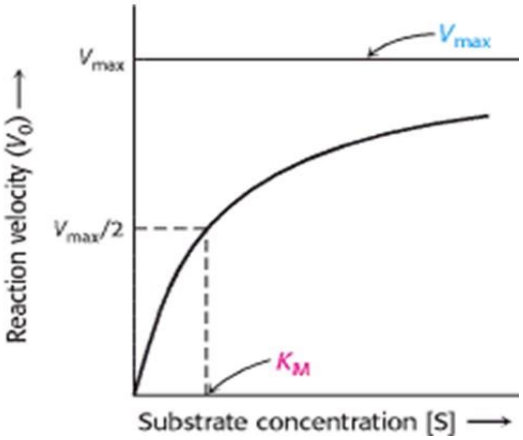
## The Mechaelis-Menten constant $K_M$:

- $K_M$ is the concentration of substrate at which half the active sites are filled.
- When [S] = $K_M$, then $V_o$ = Vmax/2

Another definition: $K_M$ is the concentration of the substrate when the Vo=½ $V_{max}$

- Therefore, it provides a measure of enzyme affinity towards a substrate.
- It is constant for different enzymes

$$V_0 = V_{max} \frac{[S]}{[S] + K_M}$$




**The lower the $K_M$ of an enzyme towards a substrate is, the higher its affinity to the same substrate is.**

How to calculate $K_M$?

- For a reaction "Enzymatic reaction":

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

Rate of dissociation

$$K_M = \frac{k_{-1} + k_2}{k_1}$$

Rate of association

- $k_1$: the constant for the ability of the enzyme to bind a substrate (Affinity)
- $K_{-1}$: the constant for the reverse reaction when the enzyme releases the substrate- remember the interaction between the substrate and enzyme is reversible (not covalent interactions)-
- $K_2$: the constant for the ability of the **ES** complex to be converted to E and **P** (product).

**Since $k_{-1} >> k_2$),**

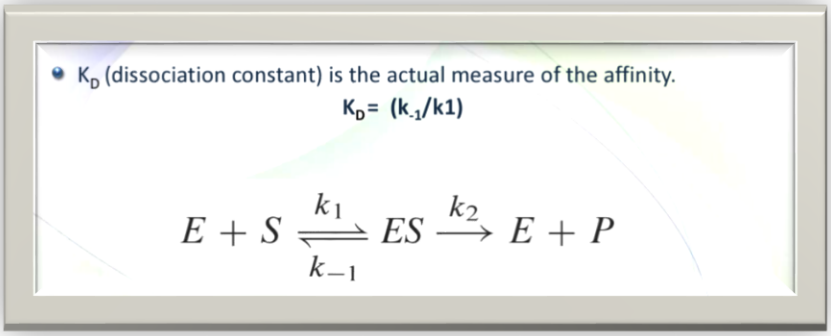
**$K_M = k_{-1}/k_1$**

$K_M$ is related to the rate of dissociation of a substrate from the enzyme to the rate of enzyme-substrate association, or the strength of the interaction between the enzyme and the substrate

**$K_M$ *describes* the affinity** of an enzyme for its substrate but is NOT an accurate measure of affinity because the $K_2$ should be taken in consideration, however, it is very small factor but it would be significant.

Notes:

(1).E→ E + P, is irreversible in this reaction but it would be reversible in another enzymes, (2).$K_2$ is much higher than $K_{-1}$



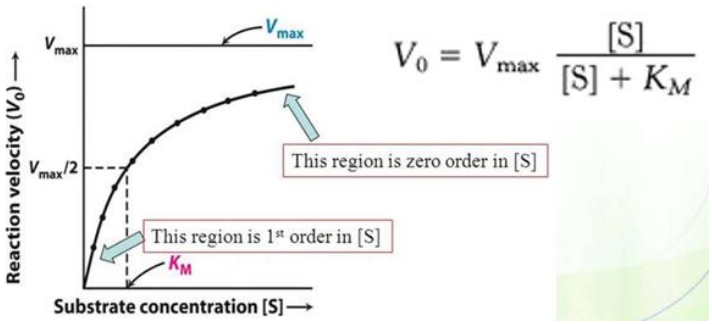

**Dissociation constant: Is the rate of dissociation divided by the rate of association.**

**$K_D$ has an inverse relationship with K1, where in = $K_D$(k-1/k1), $K_D$ decreases k1 increases. Meaning that if the dissociation constant decreases affinity increases.**

## Reaction order in relation to Km:

- **At very low substrate concentration, when [S] is much less than Km, V0= Vmax.[S]/(KM + [S]); that is, the rate is directly proportional to the substrate concentration and is affected by how well a substrate binds to an enzyme.**
- **At high substrate concentration, when [S] is much greater than Km, V0= Vmax; that is, the rate is maximal, independent of substrate concentration or how well an enzyme binds to the substrate.**

**To sum up, Km is the indication of affinity, so if Km increases affinity decreases.**




**For further understanding, the velocity of the reaction depends more on the substrate concentration if the substrate concentration was much lower than Km, and if it becomes high in comparison with Km the velocity of the reaction will depend less on it (as we mentioned previously).**

**(إذا ما فهمت الفكرة جرب مرة عوض التركيز 0.1 ومرة 100 وقارن فرق التأثير.)**

**A student asked: When the substrate concentration is equal to Km?**

**Doctor's answer: When the velocity of the reaction equals half of the Vmax.**

**Note:**

- **The Km values of enzymes range widely (mostly, 10-7 to 10-1).**
- **Each substrate has a unique Km for a given enzymatic process, but Vmax is related to the enzyme and is the same for the same reaction of more than one substrate.**

table 8–6

$K_m$ for Some Enzymes and Substrates

| Enzyme | Substrate | $K_m$ (mM) |
|---|---|---|
| Catalase | $H_2O_2$ | 25 |
| Hexokinase (brain) | ATP | 0.4 |
| | D-Glucose | 0.05 |
| | D-Fructose | 1.5 |
| Carbonic anhydrase | $HCO_3^-$ | 26 |
| Chymotrypsin | Glycyltyrosinylglycine | 108 |
| | *N*-Benzoyltyrosinamide | 2.5 |
| β-Galactosidase | D-Lactose | 4.0 |
| Threonine dehydratase | L-Threonine | 5.0 |

**This table is not for memorizing just for understanding.**

- **Catalase has Km=25 mM which means that at 25 mM the velocity of the reaction catalysed by catalase is half the max.**

- **Hexokinase when associated with glucose has Km equals to 0.05 meaning that the affinity is very high compared to 25 mM, in the case of hexokinase (has low Km) we need a low substrate concentration to reach half the max.**

- **Notice that hexokinase has several substrates: ATP, glucose and fructose (hexokinase phosphorylate six common sugars), and they don't have the same Km meaning that the affinity differs as well (the affinity to ATP and fructose is less than the affinity to glucose).**

  **NOTE : The highest the KM→ the more substrate concentration is needed to reach the Vmax→ the affinity is low.**

# Same enzyme, different substrates, different reactions

**Example: Hexokinase-enzyme that phosphorylates glucose**

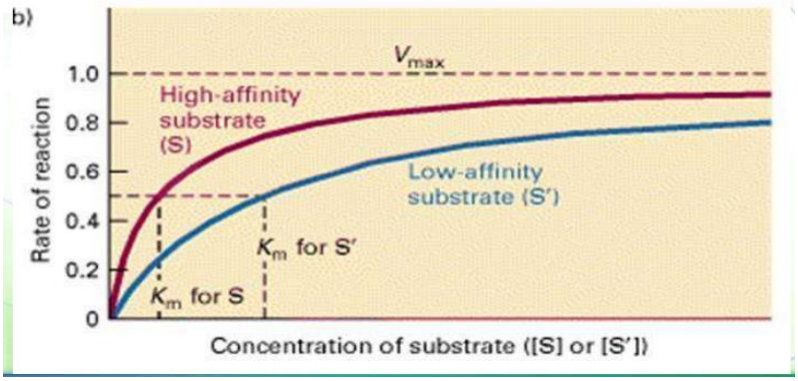
$$\text{Glucose} + \text{ATP} \longrightarrow \text{Glucose} - 6\text{-P} + \text{ADP} + \text{H}^+$$

**-A reaction is catalyzed by an enzyme with substrate S (high affinity) and with substrate S' (low affinity).**

**-Vmax is the same with both substrates, but KM is higher for S', the low-affinity substrate.**

**-for ATP the affinity is lower and for Glucose the affinity is higher, so to reach half of $V_{max}$ you need a lower amount of Glucose compared to ATP.**

**-Affinities are different and the $K_m$s are different but $V_{max}$ doesn't change because it depends on the efficiency of the enzyme not the concentration of the substance.**




**-If an enzyme binds to another substrate generating different product(s), then Vmax will be different.**

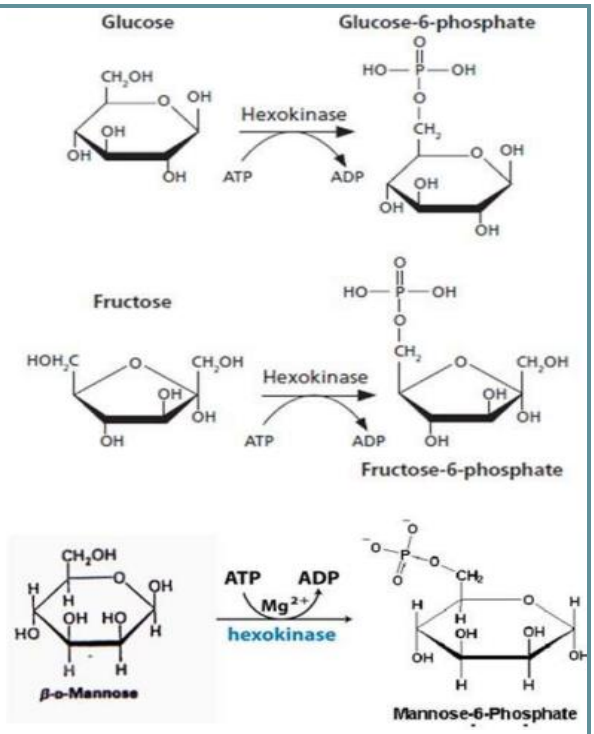
**For example, hexokinase phosphorylates glucose, fructose, and mannose at different Vmax values.**

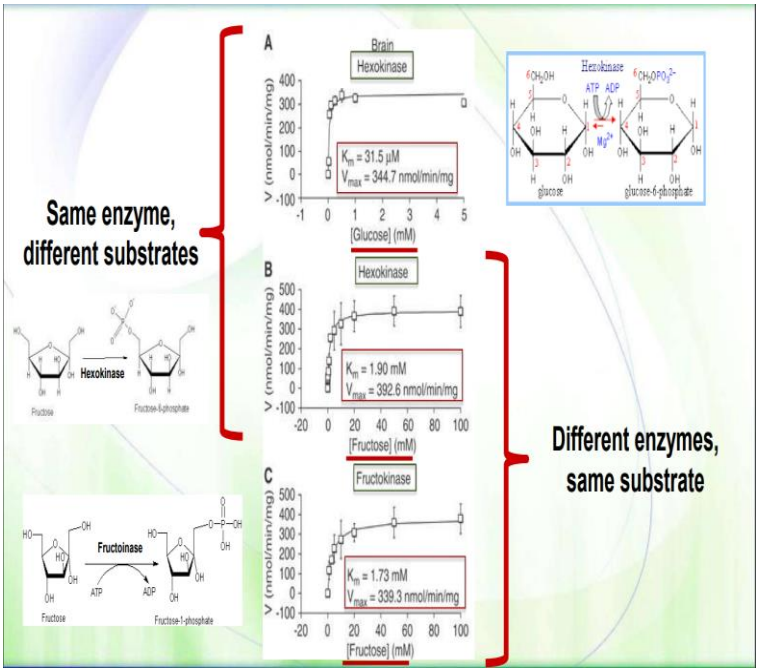
| Hexose | $K_M$ (μM) | $V_{max}$ (nmol/(min × mg)) |
|---|---|---|
| Glucose | 59 ± 10 | 26 ± 2 |
| Mannose | 32 ± 2 | 13 ± 1 |
| Fructose | 4436 ± 2275 | 34 ± 5 |

**annose has the highest affinity (lowest KM value).**

**Fructose has the highest efficiency (highest Vmax).**

(The numbers are not to be memorized, for all those fanatics our there)




## Same enzyme(Hexokinase), different substrates (glucose-fructose):

If we compare between glucose and fructose in terms of:

**-Affinity:** Glucose(KM=31.5 uM) > fructose(KM=1.9mM).

Remember: the highest the KM value, the lowest the affinity.

**Note the units (uM—mM).**

**-efficiency:** fructose(Vmax= 392.6 nmol/min/mg) > glucose (Vmax=344.7 nmol/min/mg).

## Different enzymes (Hexokinase-fructokinase) ,same substrate(fructose):

If we compare between Hexokinase and Fructokinase in terms of:

**-affinity:** Hexokinase(KM=1.9mM) > Fructokinase(KM=1.73mM).

Remember: the highest the KM value, the lowest the affinity.

**-efficiency:** Hexokinase(Vmax 392.6 nmol/min/mg) > fructokinase (Vmax= 339.3 nmol/min/mg).

## Example:

A biochemist obtains the following set of data for an enzyme that is known to follow Michaelis-Menten kinetics. Approximately, Vmax of this enzyme is … & KM is …?

A) 5000 & 699

B) 699 & 5000

C) 621 & 50

D) 94 & 1

E) 700 & 8

| Substrate Concentration (µM) | Initial velocity (µmol/min) |
|---|---|
| 1 | 49 |
| 2 | 96 |
| 8 | 349 |
| 50 | 621 |
| 100 | 676 |
| 1000 | 698 |
| 5000 | 699 |

Answer: E

**Explanation:**

Vmax= 700 → highest substrate concentration.

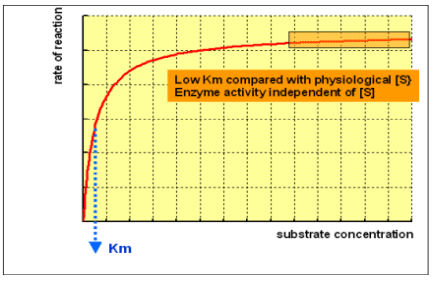
KM=8 → remember that KM= [S] when V0 = ½ Vmax

Vmax=700 → V0=350 → [s] = 8 =KM

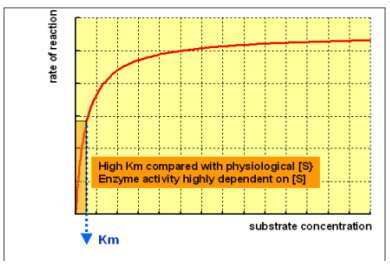
## Importance of KM

-If KM is lower than physiological concentration of S

The enzyme is normally saturated with substrate and will act at a constant rate, regardless of variations in the concentration of substrate.



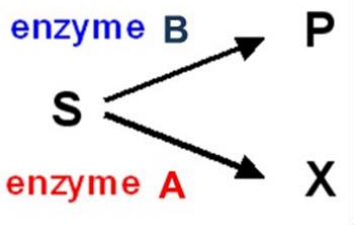

-If KM is higher than physiological concentration of S

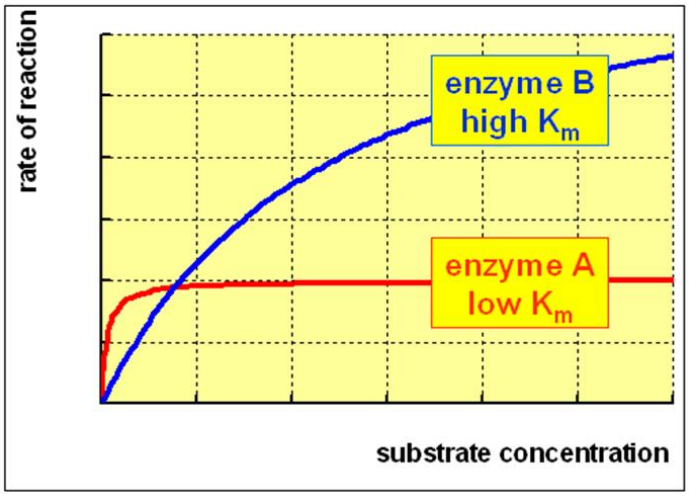


The enzyme is not saturated with substrate and its activity will vary as the concentration of substrate varies and the rate of formation of product will depend on the availability of substrate.

## Metabolic pathway

If two enzymes, in different pathways, compete for the same substrate, then knowing the values of $K_M$ and Vmax for both enzymes permits prediction of the metabolic rate of the substrate and the relative amount that will flow through each pathway under various conditions.




**-If we have very low substrate concentration (S) will be converted to (X) more then (P).**

Let's take GLUCOSE as an example, glucose is a source of energy in our body and it is present in different concentrations in the different states of the body.

It's stored in the liver and usually consumed in the muscles, when we are fasting the glucose concentration is low so the liver will not store it, instead the muscles will take it, but when we are sleeping the liver will store it.

The preference here was upon the concentration of glucose when it was high the liver's enzymes work to store it, but in case of low concentration the muscle's enzymes will work to get the glucose

which reaction is favorable when:

[ S ] is very low ?

[ S ] is very high ?

With regard to our example, enzyme B represents

the enzymes of the liver while enzyme A represents

the enzymes of the muscles.

# Uses of $K_M$

1 ) Determine the substrate preference of an enzyme.

if an enzyme has more than one substrate, the substrate with the lowest $K_M$ is probably the preferred physiological substrate. Like the pervious example

2 ) Distinguish isozymes , which are different enzymes catalyzing the same reaction. Hexokinase and Glucokinase are considered as isozymes, both of them phosphorylate glucose at carbon 6 , but they have different $K_M$ ( SAME REACTION – DIFFERENT ENZYMES ) ** More explanation next week **

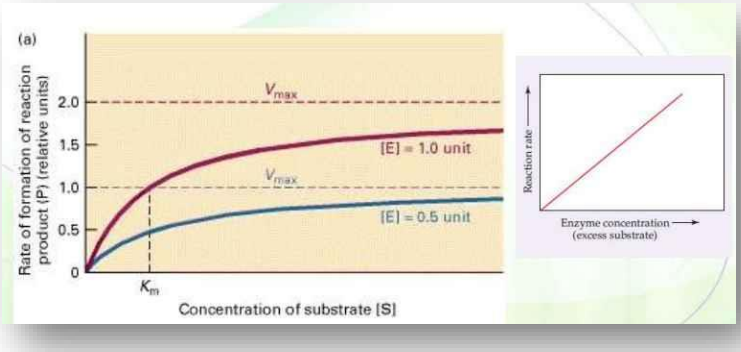
3 ) check for abnormalities in an enzyme.

Vmax and $K_M$ are supposed to be constant any change may cause mutation.

## Vmax and enzyme concentration

Doubling the concentration of enzyme causes proportional increase in the reaction rate, so that the maximal velocity Vmax is doubled; the $K_M$, however, is unaltered.

It's like adding a MOTOR to the car that will increase its velocity.




## Vmax & Kcat

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

For the enzymatic reaction

The maximal rate, Vmax is equal to the product of k2, also known as kcat, and the total concentration of the enzyme.

Vmax = k2 $[E]_T$ OR kcat = Vmax/ $[E]_T$

So Vmax depends on 1) the ability of the enzyme to convert the substrate to the product once the substrate binds to it *( Kcat)* . 2) [ ENZYME ].

**But Kcat is constant for any given enzyme.**

table 8–7

**Turnover Numbers ($k_{cat}$) of Some Enzymes**

| Enzyme | Substrate | $k_{cat}$ ($s^{-1}$) |
|---|---|---|
| Catalase | $H_2O_2$ | 40,000,000 |
| Carbonic anhydrase | $HCO_3^-$ | 400,000 |
| Acetylcholinesterase | Acetylcholine | 14,000 |
| β-Lactamase | Benzylpenicillin | 2,000 |
| Fumarase | Fumarate | 800 |
| RecA protein (an ATPase) | ATP | 0.4 |

Note the value of Kcat of CATALASE

It's 40000000 $S^{-1}$ that means

that each molcule of CATALASE can

catalyse 40000000 reaction per seconde.

Kcat, turnover number, is the concentration ( or moles ) of substrate molecules converted into product per unit time *per concentration ( or moles ) of enzyme*, **when fully saturated.**

So it describes how quickly an enzyme acts, ( how fast the ES complex proceeds to E + P )

In other words, the maximal rate, Vmax , reveals the turnover number of an enzyme if the total concentration of active sites $[E]_T$ is known.

Kcat itself is the turnover number not Vmax → Vmax gives an idication about turnover number.

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**You are working on the enzyme "Medicine" which has a molecular weight of 50,000 g/mol. You have used 10 µg of the enzyme in an experiment and the results show that the enzyme at best converts 9.6 µmol of the substrate per min at 25°C. The turnover number (kcat) for the enzyme is:**

**mol = g/MW**

**A. 9.6 s-1 B. 48 s-1 C. 800 s-1 D. 960 s-1 E. 1920 s-1**

**SOLUTION:**

**MW = 50,000 g/mol Weight = 10 µg**
**Vmax = 9.6 µmol of the substrate per min**

**Kcat (9.6/60)/(10 µg/50,000) = 800 $S^{-1}$**

**Example:**

**A 10-6 M solution of carbonic anhydrase catalyzes the formation of 0.6 M H2CO3 per second when it is fully saturated with substrate.**

**Kcat = Vmax/[E] = 0.6 /10-6 = 6 x 105 / sec**

**6x 105 x 60 sec/min = 3.6 x 107 / min**

**3.6 / 1x 107 = 2.7 x 10-6 min per reaction**

**Each catalyzed reaction takes place in a time equal to 1/k2, which is 2.7 µs for carbonic anhydrase.**

**The turnover numbers of most enzymes with their physiological substrates fall in the range from 1 to 104 per second.**

**ملاحظة: ما تبقى من المحاضرة سيتم شرحها وجاهيا**