Enzymatic Kinetics

Michaelis Constant

Enzymatic kinetics

Kinetics of enzymatic reactions deals with the examination of velocity of reactions in connection with different factors such as:

- The concentration of reagents
- Temperature
- pH
- The presence of catalysators

The task of kinetics is:

- The settlement of character of reaction
- Mathematematic definition of the relationship between the velocity of reaction and its duration

The velocity of chemical reaction is:

- Proportional to the concentration of reagents
- Means the ratio of increase in the concentration of reaction product (or loss of substrate concentration) to the time when this increase (loss) appeared.

Kinetic character of reaction is defined by order of reaction

Order of reaction provides with the information about the relationship between the concentration as well as number of reagents and the velocity

We know the following orders:

- Zero order
- I st order
- II nd order
- n order

Reactions of zero order exhibit constant velocity independent from the concentration of substrate

Reactions of I st order – reactions where one or two substrates (the second is in excess and does not influence the velocity eg. hydrolysis of sucrose) take part, the velocity is proportional to the concentration of substrate

Time necessary to convert 50% of substrate is independent from the initial concentration of substrate.

Reactions of II nd order – reactions where two substrates take part, the velocity is directly proportional to the square of the substrate concentration

Half life time is reversely proportional to initial concentration of substrates.

The relationship between enzymatic reaction, velocity and the concentration of substrate

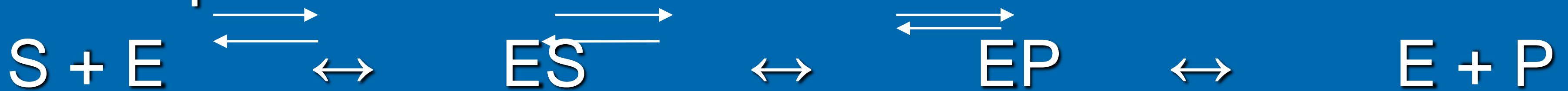
- The velocity of chemical reaction is defined as the amount of converted substrate in time unit or the amount of formated product in time unit. The unit is mol/s
- The increase in substrate concentration results in the increase of velocity of reaction. It is related to the fact that at the same time more molecules are converted and time when active center of enzyme remains empty (not connected to substrate) is shortened.
- It is not possible to increase the velocity unlimitedly via the increase in initial concentration of substrate.

Maximum velocity of enzymatic reaction

- Together with the increase in substrate concentration the frequency of connections in active center increases. The time of conversion of substrate into product is not changed but time of breaks between connections of new molecules of substrate is shortened.
- At high concentration of substrate breaks are shortened up to indispensable minimum and any increase in substrate concentration can not influence the velocity of reaction. At this moment maximum velocity is achieved.

Michaelis Constant

- The velocity of substrate conversion into product is dependent on the velocity of the following stages of this process



- Each of these intermediate reactions has its own reaction rate constant. The analysis of meaning of these intermediate reactions brought simplification of the issues leading to the determination:

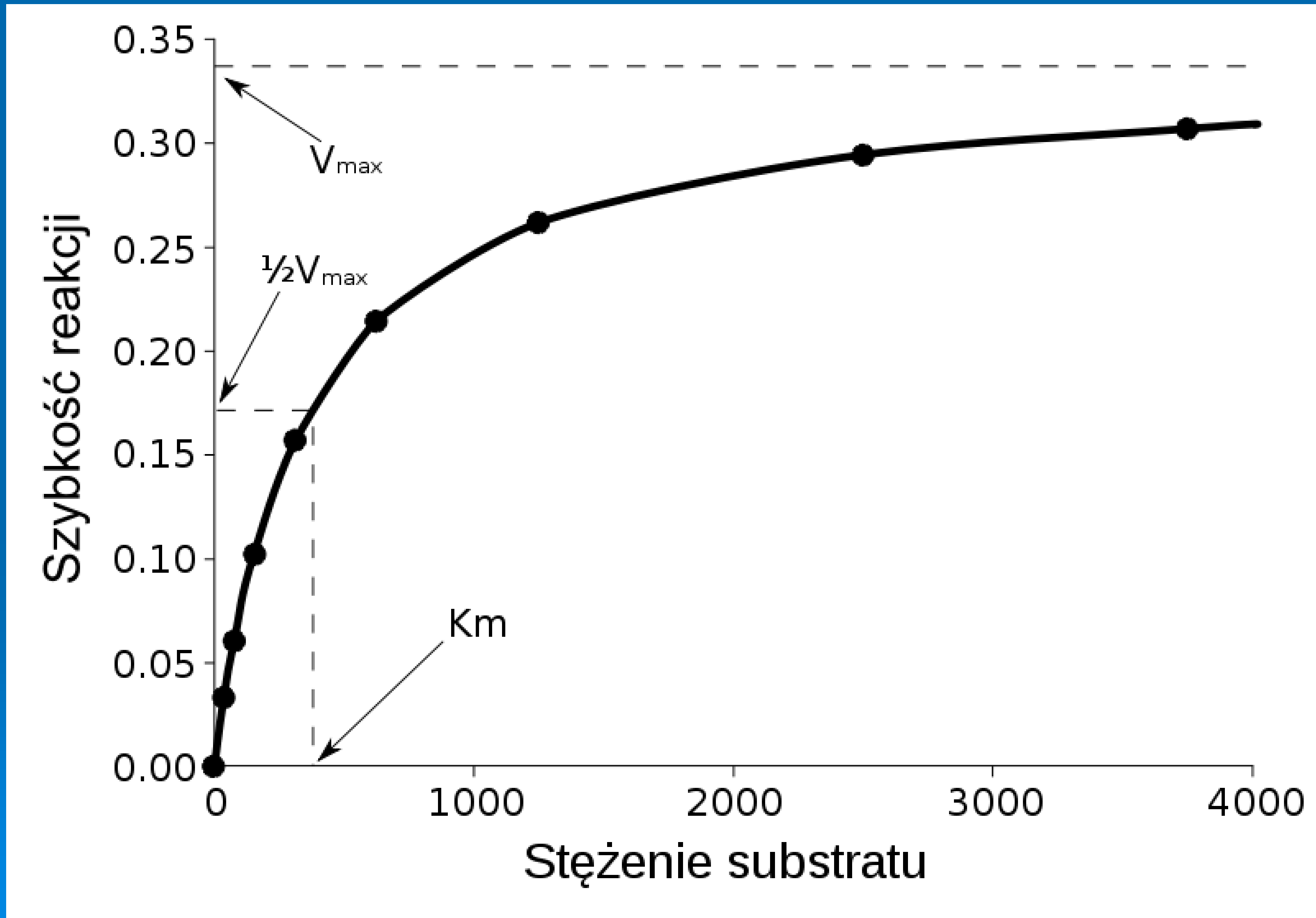
$K_M = [S] (V_{max} / v - 1)$, what if $v = \frac{1}{2} V_{max}$

Gives simple relationship: $K_M = [S]$

Michaelis Constant

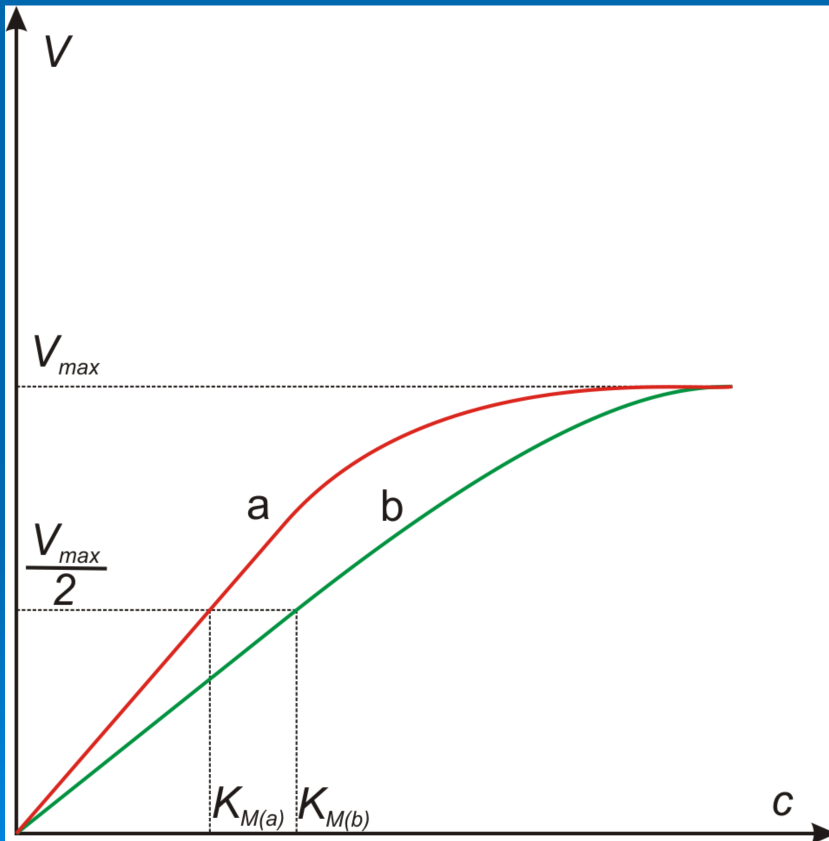
- Michaelis Constant numerically is equal to substrate concentration at which ½ of maximum velocity is achieved.
- As the only one reaction rate constant is defined in units : mol/dm^3 – it is the unit of concentration
- The size of this constant says about the affinity of enzyme tosubstrate. It is reversible proportional relationship. Higher value of constant is related to lower affinity of enzyme to substrate. If compared the affinity of enzyme to two substratres – higher value means lower affinity.

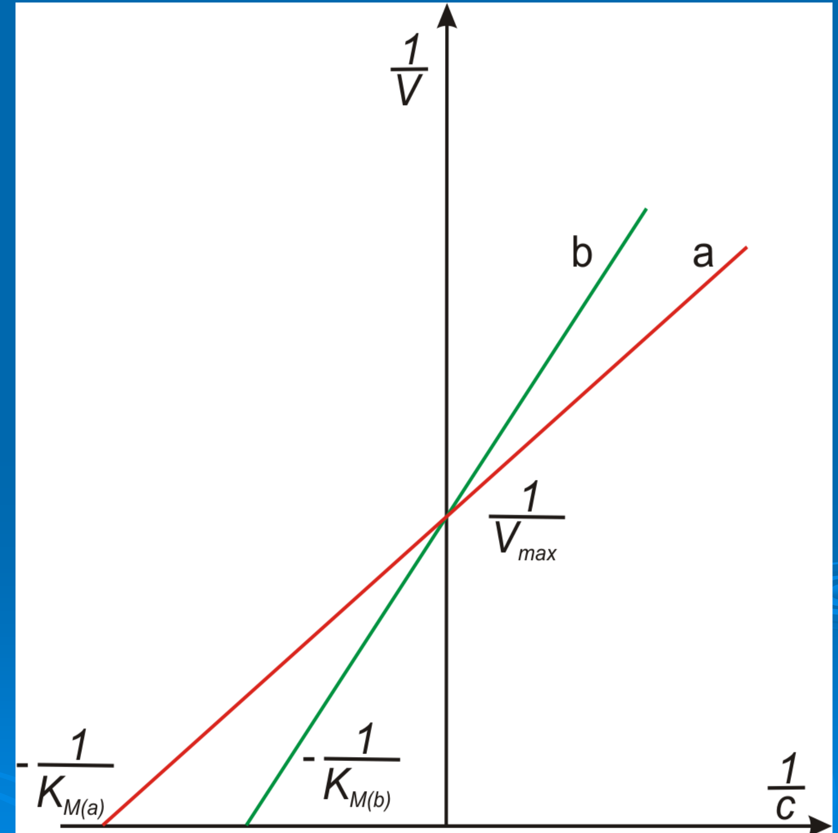
The determination of Michaelis Constant



The relationship between velocity of reaction (*V*) and substrate concentration (*c*) for the enzyme with different affinity for two substrates a and b in accordance to:

Michaelis-Menten and Lineweaver-Burk





The principle of method

- Invertase (β-fructo-furanosidase) belongs to hydrolases that cleave glycosidic bond in sucrose and breaks it down into glucose and fructose. This process is called inversion and is related to the name of enzyme. Optimum pH for this enzyme is ranged between 4-7, but at pH = 10 it is completely inactive.

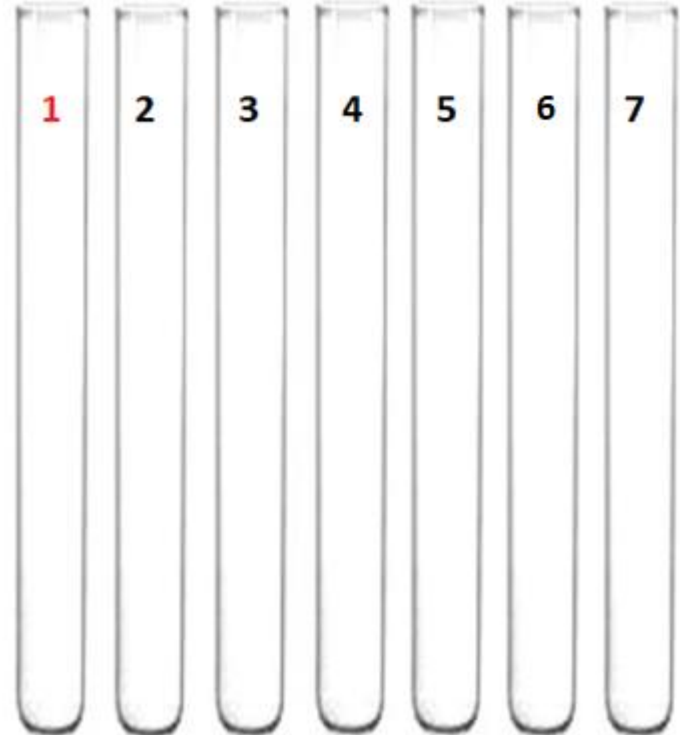
- The activity of invertase can be measured based on the methods for the detection of reducing sugars because together with the progress of hydrolysis the amount of single sugars (glucose and fructose) in the incubation mixture increases.

The principle of method

- Reducing sugars react with copper reagent and copper I is synthesized. In order to determine quantitatively the amount of copper I which was synthesized during the oxidation of sugars the reaction with phospho-molybdenum reagent should be performed. The intensity of obtained colour is proportional to the amount of reducing sugar in the sample.

Protocol

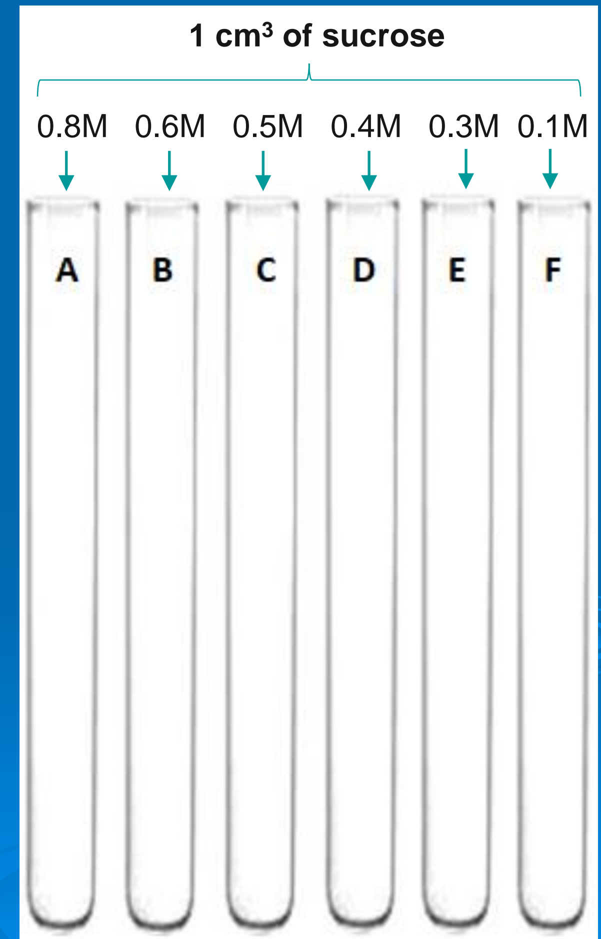
- Add 0,5cm3 of Copper reagent, 0,7cm3 of H2O dest and 0,1 cm3 0,05M NaOH to the set of 7 tubes marked from 1 to 7.
- In addition to tube no 1 add 0,1 cm3 of enzyme and 0,1 cm3 H2O dest – it is blank sample for colorimetric measurements.



Protocol

- Prepare set of 6 tubes marked from A to F and pipete sucrose of appropriate concentration:

  A – 1 cm3 **0,8M** sucrose
  B - 1 cm3 **0,6M** sucrose
  C - 1 cm3 **0,5M** sucrose
  D - 1 cm3 **0,4M** sucrose
  E - 1 cm3 **0,3M** sucrose
  F - 1 cm3 **0,1M** sucrose



Protocol

- Tubes A-F should be incubated in water bath for 5min in 37o C. After incubation add 1 cm3 of enzyme (invertase) to each tube and immediately incubate for further 15 min in similar conditions.

Note time **“0” – time** when substrate was mixed with enzyme and enzymatic reaction was initiated. It should be kept in mind that after adding the enzyme the concentration of substrate is half lower.

Protocol

- After 15 min. of incubation in water bath transfer appropriate amounts of solutions from set of tubes marked A-F to set of tubes marked 2-7:

A – 0,2 cm3 of incubation mixture to tube no **2**
B - 0,2 cm3 of incubation mixture to tube no **3**
C - 0,2 cm3 of incubation mixture to tube no **4**
D - 0,2 cm3 of incubation mixture to tube no **5**
E - 0,2 cm3 of incubation mixture to tube no **6**
F - 0,2 cm3 of incubation mixture to tube no **7**

Protocol

- All tubes 1-7 heat in boiling water bath for 8 min.
- Chill carefully under the stream of tap water and add to each tube 1cm3 of phosphomolybdenum reagent.
- Each tube should be diluted with distilled water approximately 10 times (ask the teacher).
- Measure absorbance at wave length of 610 nm against the solution from tube 1 (also similarly diluted).
- Give the results in the table.

The calculation of results

1. Draw the plot of dependencies between absorbance and the concentration of substrates after 15 min of incubation.
2. Calculate Michaelis Constant