Kinetics of Enzyme Reactions 3

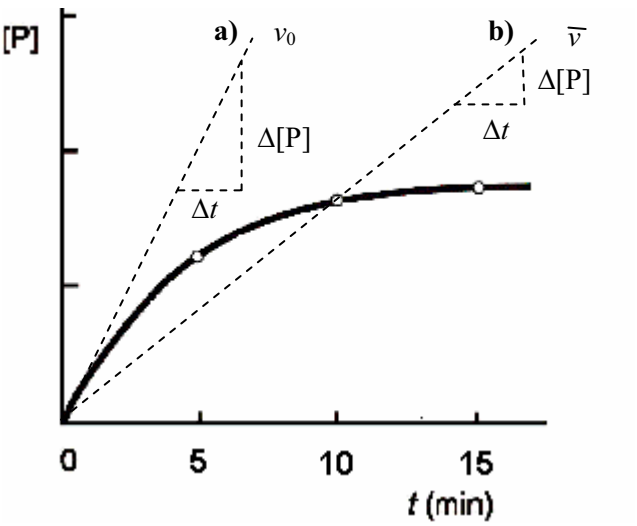
Rate and order of chemical reactions, kinetics of zero order and 1st order reactions. Mechanism of enzyme catalyzed reactions, activity and catalytic concentration of enzymes, factors affecting the enzyme activity, Michaelis plot, K_M, inhibitors.

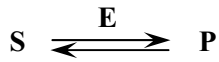
Quantification of Enzymes

Expression of enzyme quantity	Unit	Dimension
Catalytic (enzyme) activity	Katal (kat)	……
	International unit (U, IU)	μmol/min
Catalytic concentration	……	……
Mass concentration	g/L	g/L

1. Derive the relation between the values of catalytic activity in μkat and IU and vice versa.

Determination of Catalytic Activity





$$\text{catalytic concentration} = \frac{\Delta[\text{P}]}{\Delta t}$$

2. Characterize the main ways of catalytic activity measurement

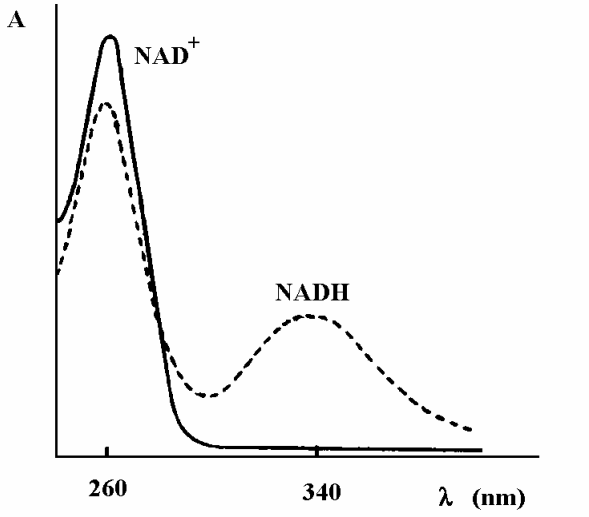
a) Kinetic method …….

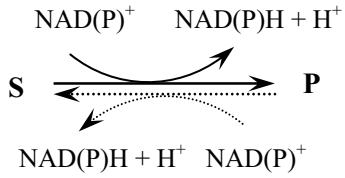
b) Constant time method ……

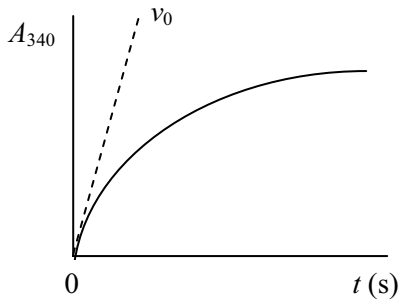
c) Immunochemical determination ……

3. Lactate dehydrogenase has the catalytic activity 2 μkat. How many molecules of lactate will be formed from pyruvate in one minute, if an excess of substrate is present? ($7.23 \cdot 10^{19}$)
4. How much product will be formed in an enzymatic reaction in 10 minutes, if the enzyme activity is 10 μkat? What experimental conditions must be kept to achieve the amount theoretically calculated? (6 mmol)
5. Serum (0.1 mL) was added into a reaction mixture. After 10 minutes, exactly $6 \cdot 10^{-3}$ mmol of the product were determined. What is the catalytic concentration of the enzyme? Is the result different from the activity determined by the kinetic method? (100 μkat/L)
6. The reaction mixture contains 2.5 mL of a buffer, 0.2 mL of NAD^+ solution, 0.1 mL of serum and 0.2 mL of lactate solution. The reaction proceeded exactly for 10 minutes and 0.0012 mol/L of NADH was measured in the mixture. Calculate the catalytic activity and the catalytic concentration of lactate dehydrogenase. (6 nkat, 60 μkat/L)
7. Calculate the activity of catalase, if 6.72 μL O_2 was released in 10 minutes. In the reaction mixture was an excess of H_2O_2 and the reaction proceeded under normal conditions. (1 nkat)

Optical (UV) Test







$$\text{Catalytic concentrat ion} \sim \frac{\Delta A_{340}}{\Delta t}$$

Use:

a) Determination of enzyme activities – NAD(P)-dependent dehydrogenases
– coupled determination of other enzyme activities

b) Determination of substrate concentration – e.g. lactate

8. How will the absorbance at 340 nm change during the lactate determination when using the optical test?

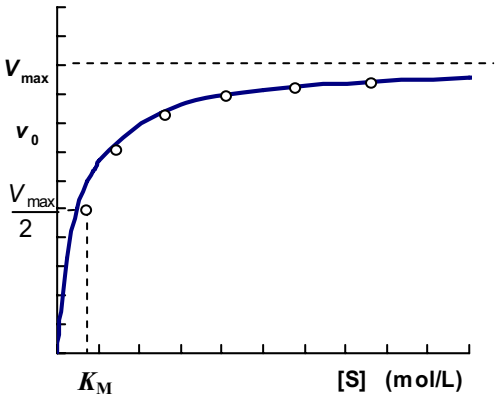
Factors Affecting Rate of Enzyme Reactions

I. Substrate Concentration

The course of a mono-substrate enzyme reaction:

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

Michaelis Plot (Saturation Curve)



For steady state: **[ES]** = constant

Equation of Michaelis-Menten

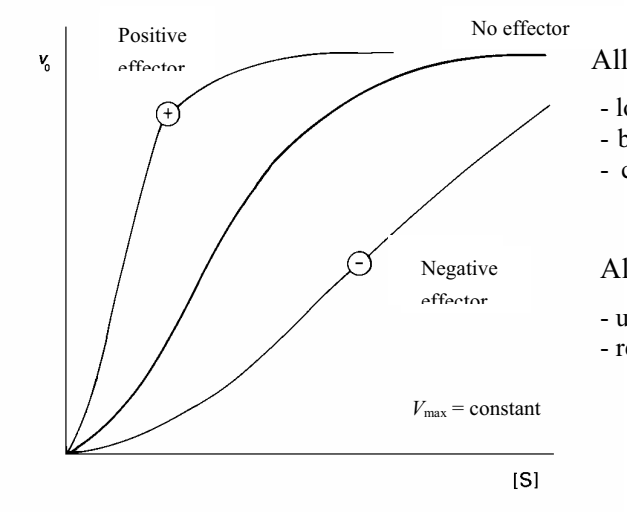
$$v_0 = V_{max} \cdot \frac{[S]}{K_M + [S]}$$

[S] … initial concentration of substrate

V_{max} … maximal initial velocity

9. Compare: kinetic curve: dependence of …..... on ……
   saturation curve: dependence of …… on ……
10. Suggest an experiment, which provides the data for the construction of the saturation curve.
11. What is the dimension of K_M?
12. Characterize the three parts of the Michaelis-Menten plot for the values: a) [S] << K_M b) [S] >> K_M. What are the reaction orders under these conditions?
13. Characterize the point on the saturation curve for the value [S] = K_M.
14. Calculate the [S]/K_M ratio, if the initial reaction rate v_0 is: a) 90 % V_{max}; b) 99 % V_{max}. (a) 9; b) 99)
15. For which of the following substrates S_1, S_2 a S_3 does an enzyme with a broad substrate specificity have the highest affinity (K_{M1} = 400 μmol/L, K_{M2} =1000 μmol/L, K_{M3} = 60 μmol/L)?
16. The enzyme β-galactosidase has K_M(lactose) = 400 μmol/L. What concentration of lactose has to be used to maintain a zero-order kinetic? (≥100 K_M, i.e. ≥40 mmol/L)
17. The K_M of an enzyme was increased twice in comparison with the normal value due to a mutation. What concentration of substrate must be used not to change the reaction rate?
18. L-Asparagin is necessary for proteosynthesis in some cancer cells. The enzyme asparaginase converting Asn to Asp and ammonia is administered during the treatment of some leukaemia types. The decrease of Asn in circulation occurs consequently and the proliferation of cells is lowered. Which of the asparaginase forms will be the most suitable for the treatment if the Asn concentration in blood is 0.2 mM?
    a) K_M = 0.2 mM; V_{max} = 0.1 mM/h
    b) K_M = 0.2 mM; V_{max} = 0.5 mM/h
    c) K_M = 2 mM; V_{max} = 0.1 mM/h
    d) K_M = 0.1 mM; V_{max} = 0.5 mM/h
19. Explain the term substrate specificity using hexokinase and glucokinase as examples.

Saturation Curve of Allosteric Enzymes



Allosteric effector

- low molecular compound (often intermediate or product)
- binding into another region different from the active site
- change of enzyme conformation → change of activity

Allosteric enzyme

- usually more subunits (often regulatory and catalytic)
- regulatory functions in metabolism

II. Enzyme Concentration

In general: $v_0 = k \cdot [\mathrm{E}]_t$

For a fully saturated enzyme ($[E]_t = [ES]$): $\boldsymbol{v_0 = V_{max} = k_{cat} \cdot [E]_t}$

where $[E]_t = [E] + [ES]$;

$k_{cat} = V_{max} / [E]_t$ **molecular activity** *(turnover number)*

20. Construct the saturation curves for the three different enzyme concentrations ($[E_1] < [E_2] < [E_3]$) of the same enzyme and the same reaction type. How will K_M, V_{max} values change?
21. The molecular activity of carbonic anhydrase is $6 \cdot 10^5$ s^{-1}. How many molecules of carbonic acid were formed, if the reaction was catalyzed by one molecule of the enzyme for 10 milliseconds? The enzyme is fully saturated. (6 000)
22. The molecular activity of lysozyme is 0.5 s^{-1}. How many glycosidic bonds would be hydrolyzed by 1 μmol of the enzyme in 1 minute? Assume that the enzyme would be saturated. ($0.5\ \mu mol = 3.01 \cdot 10^{17}$)

III. Temperature

IV. pH

V. Activators

23. In which way is the enzyme activity affected by: a) temperature; b) pH; c) activators? Give examples.
24. What is *Taq* polymerase, at which method it is used?

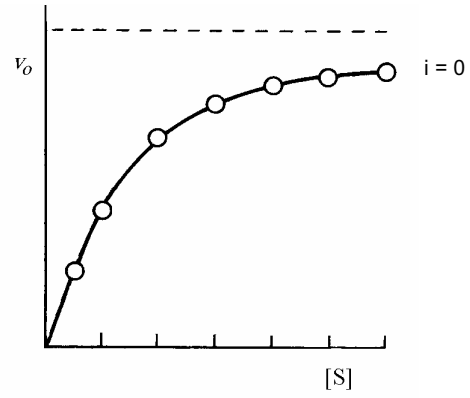
VI. Inhibitors

25. Explain the difference between the reversible and irreversible inhibition.
26. Give examples of irreversible inhibition.
27. What types of irreversible inhibition are distinguished?
28. Complete into the table, how are changed the values of K_M and V_{max} and give examples of inhibitors.

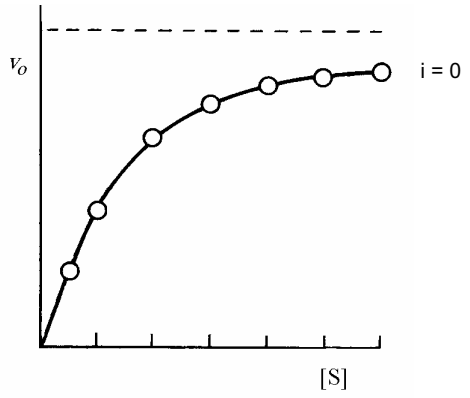
Parameter	Competitive inhibition	Non-competitive inhibition
K_M		
V_{max}		
Inhibitor		

29. Draw the course of competitive and non-competitive inhibition. Mark the values K_M, V_{max} into the plots.

a) competitive inhibition



b) non-competitive inhibition



30. Explain the terms: proenzyme, isoenzyme and isoform.