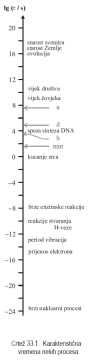


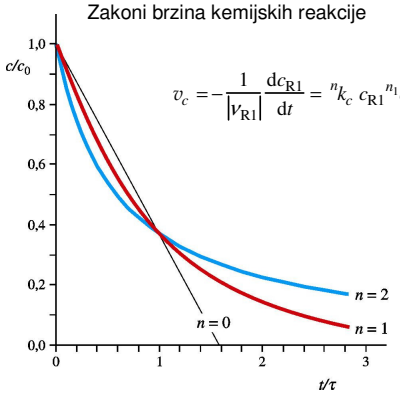
Crtež 33.1. Karakteristična vremena nekih procesa

DEFINICIJE POJMOVA

- brzina konverzije
- brzina kemijske reakcije
- brzina trošenja / nastajanja
- molekularnost
- red reakcije
- koeficijent (konstanta) brzine reakcije
- mehanizam kemijske reakcije

$$v_c = \frac{1}{\nu_B}\frac{\mathrm{d}c_B}{\mathrm{d}t}$$

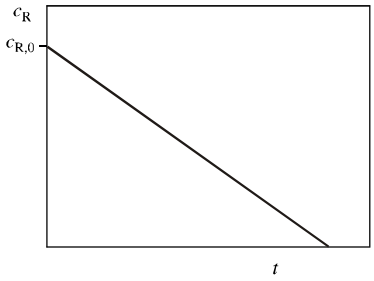
Zakoni brzina kemijskih reakcije



$$v_c = -\frac{1}{|\nu_{R1}|}\frac{\mathrm{d}c_{R1}}{\mathrm{d}t} = {}^{n}k_c\, c_{R1}{}^{n_1} c_{R2}{}^{n_2}$$

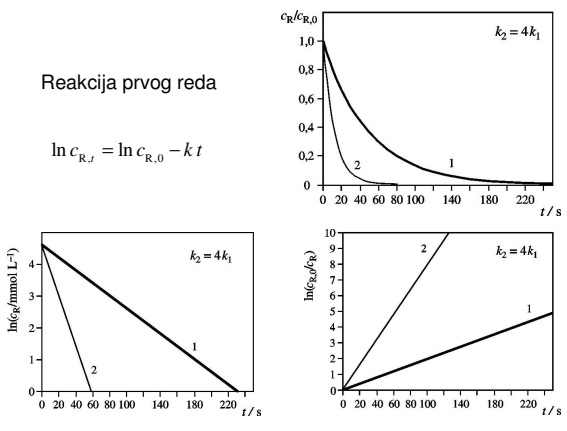
Reakcija nultog reda

$$c_{R,t} = c_{R,0} - k\,t$$



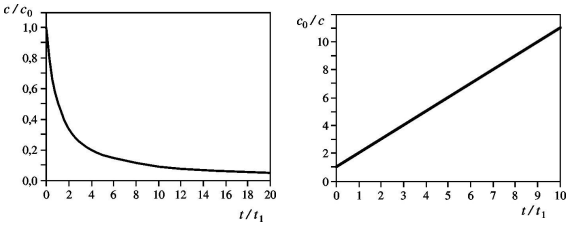
Reakcija prvog reda

$$\ln c_{R,t} = \ln c_{R,0} - k\,t$$



Reakcija drugog reda

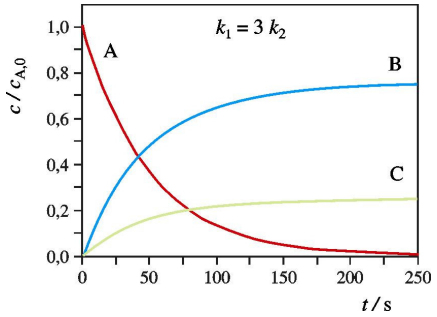
$$\frac{1}{c_{R,t}} = \frac{1}{c_{R,0}} + k\,t$$



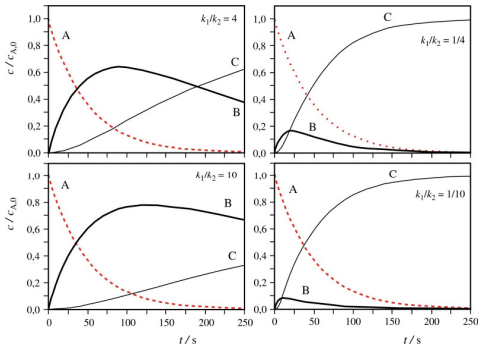
MEHANIZAM REAKCIJE

- usporedne ili paralelne reakcije
- uzastopne ili konsekutivne reakcije
- povrative ili reverzibilne reakcije

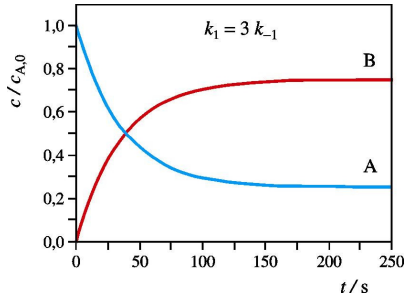
usporedne ili paralelne reakcije



uzastopne ili konsekutivne reakcije



povrative ili reverzibilne reakcije

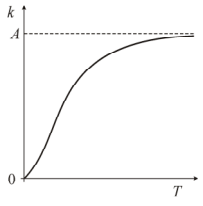


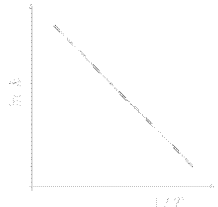
Temperaturna ovisnost brzine reakcije

Slika 33.2 Svante Arrhenius švedski kemičar (1859 - 1927) i dobitnik Nobelove nagrade za kemiju 1903. za teoriju elektrolitne disocijacije.

$$k = A \cdot \exp(-E_a / RT)$$

$$\ln k = \ln A - \frac{E_a}{RT}$$





$$k = A \cdot \exp(-E_a / RT)$$

$$\ln k = \ln A - \frac{E_a}{RT}$$

Teorije brzina reakcija

- Teorija sudara
- Teorija prijelaznog stanja

ENZIMSKA KINETIKA

$$S \xrightarrow{E} P$$

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

Michaelis&Menten (1913)

Leonor Michaelis (1875 –1949)

Maud Leonora Menten (1879 –1960)

Briggs, G.E.; Haldane, J.B.S. (1925). "A note on the kinematics of enzyme action". *Biochem J* **19** (2): 338–339.

L. A NOTE ON THE KINETICS OF ENZYME ACTION.

By GEORGE EDWARD BRIGGS and JOHN BURDON SANDERSON HALDANE.

(*From the Botanical and Biochemical Laboratories, Cambridge.*)

(*Received March 9th, 1925.*)

The equation of Michaelis and Menten [1913] has been applied with success by Kuhn [1924] and others to numerous cases of enzyme action. It is therefore desirable to examine its theoretical basis. Consider the irreversible reaction $A \rightarrow B$, unimolecular as regards A, and catalysed by an enzyme. Suppose one molecule of A to combine reversibly with one of enzyme, the compound then changing irreversibly into free enzyme and B, where B may represent several molecules. We may represent this as:

$$\begin{matrix} A + E & \rightleftharpoons & AE & \rightarrow & B + E. \\ (a-x)(e-p) & & p & & x \end{matrix}$$

Now let a be the initial concentration of A, e the total concentration of enzyme, x the concentration of B produced after time t, and p the concentration of enzyme combined with substrate at time t. We suppose e and p to be negligibly small compared with a and x. Then by the laws of mass action

$$\frac{dp}{dt} = k_1 (a-x)(e-p) - k_2 p - k_3 p,$$

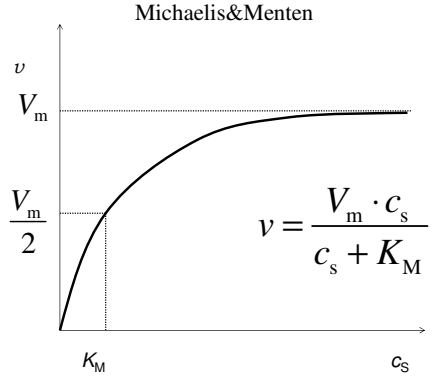
where k_1, k_2, k_3 are the velocity constants of the reactions

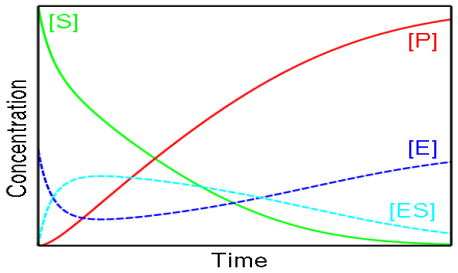
$$A + E \rightarrow AE, \quad AE \rightarrow A + E, \quad \text{and} \quad AE \rightarrow B + E,$$

respectively. Now since p is always negligible compared with x and $a-x$, its rate of change must, except during the first instant of the reaction, be negligible compared with theirs.

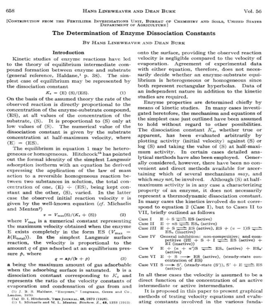
For during the remainder of the reaction p diminishes from a value not exceeding e to zero, whilst x increases from zero to a. Thus the average value of $-\frac{dp}{dt}$ or $\frac{dx}{dt}$ is less than $\frac{e}{t}$. And provided $\frac{e}{t}$ is small it is clear that if the amount of combined enzyme decreased for a measurable time at a rate comparable with that of its substrate the reaction would come to an end. To take a concrete example Kuhn [1924] calculates that a yeast saccharase molecule at 15° and p_H 4·6 can invert 100 or more molecules of sucrose per second. Even if the enzyme concentration is so unusually large that the inversion of a strong sucrose solution is half completed in 10 minutes, $\frac{p}{e}$ cannot be less than 120,000, and if $-\frac{dp}{dt}$ attained 1 % of the value of $\frac{dx}{dt}$ for 1 second the

Michaelis&Menten





Lineweaver, H and Burk, D. (1934). "The Determination of Enzyme Dissociation Constants", *Journal of the American Chemical Society* **56** (3): 658–666.



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[CONTRIBUTION FROM THE FERTILIZER INVESTIGATIONS UNIT, BUREAU OF CHEMISTRY AND SOILS, UNITED STATES DEPARTMENT OF AGRICULTURE]

The Determination of Enzyme Dissociation Constants

BY HANS LINEWEAVER AND DEAN BURK

Introduction

Kinetic studies of enzyme reactions have led to the theory of equilibrium intermediate compound formation between enzyme and substrate (general reference, Haldane,[1] p. 38). The simplest case of equilibrium may be represented by the dissociation constant

$$K_s = (E)(S)/(ES). \quad (1)$$

On the basis of the assumed theory the rate of the observed reaction is directly proportional to the concentration of the enzyme-substrate compound, (ES), at all values of the concentration of the substrate, (S). It is proportional to (S) only at low values of (S). The numerical value of the dissociation constant is given by the substrate concentration at half-maximum velocity, where (E) = (ES).

The equilibrium in equation 1 may be heterogeneous or homogeneous. Hitchcock[1a] has pointed out the formal identity of the simplest Langmuir adsorption isotherm with an equation he derived expressing the application of the law of mass action to a reversible homogeneous reaction between two substances in solution, the total concentration of one, (E) + (ES), being kept constant and the other, (S), varied. In the latter case the observed initial reaction velocity v is given by the well-known equation (*cf.* Michaelis and Menten[2])

$$v = V_{max}(S)/(K_s + (S)) \quad (2)$$

where V_{max} is a numerical constant representing the maximum velocity obtained when the enzyme E exists completely in the form ES ($V_{max} = k(E_{total})$). In the simplest Langmuir surface reaction, the velocity is proportional to the amount q of gas adsorbed at an equilibrium pressure p, where

$$q = \mathbf{a}p/(\mathbf{b} + p) \quad (3)$$

a being the maximum amount of gas adsorbable when the adsorbing surface is saturated. **b** is a dissociation constant corresponding to K_s and represents the ratio of the velocity constants of evaporation and condensation of gas from and onto the surface, providing the observed reaction velocity is negligible compared to the velocity of evaporation. Agreement of experimental data with either equation, therefore, does not necessarily decide whether an enzyme-substrate equilibrium is heterogeneous or homogeneous since both represent rectangular hyperbolas. Data of an independent nature in addition to the kinetic studies are required.

Enzyme properties are determined chiefly by means of kinetic studies. In many cases investigated heretofore, the mechanism and equations of the simplest case just outlined have been assumed to hold without regard to other possibilities. The dissociation constant K_s, whether true or apparent, has been evaluated arbitrarily by plotting activity (initial velocity) against (S) or log (S) and taking the value of (S) at half-maximum activity. In certain cases detailed analytical methods have also been employed. Generally considered, however, there have been no convenient and direct methods available for ascertaining which of several mechanisms *may*, and which *may not*, be involved. Although (S) at half-maximum activity is in any case a characterizing property of an enzyme, it does not necessarily represent a thermodynamic dissociation constant. In many cases the kinetics involved do not correspond to equation 2 (Case I), but to Cases II to VII, briefly outlined as follows

Case I $E + S \rightleftharpoons ES$ (active)
Case II $E + nS \rightleftharpoons ES_n$ (active)
Case III $E + S \rightleftharpoons ES$ (active), $ES + (n-1)S \rightleftharpoons ES_n$ (inactive)
Case IV General inhibition: non-competitive; and competitive ($2E + S + I \rightleftharpoons ES$ (active) + EI (inactive))
Case V $E + (n + n')S \rightleftharpoons ES_n$ (active) + $ES_{n'}$ (active)
Case VI $E + S \longrightarrow ES$ (active), (steady-state concentration of ES)
Case VII $S \longrightarrow S'$, (steady-state (S')), $S' + E \rightleftharpoons ES$ (active)

In all these cases the velocity is assumed to be a direct function of the concentration of an active intermediate or active intermediates.

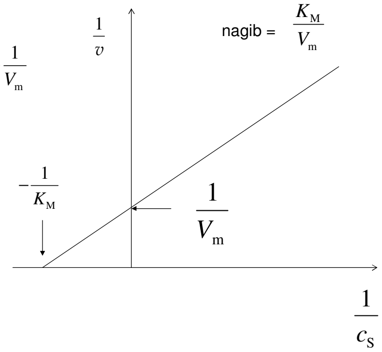
It is proposed in this paper to present graphical methods of testing velocity equations and evaluating constants involved in the various funda-

(1) J. B. S. Haldane, "Enzymes," Longmans, Green and Co., London, 1930.
(1a) D. I. Hitchcock, THIS JOURNAL, **48**, 2870 (1926).
(2) L. Michaelis and M. L. Menten, *Biochem. Z.*, **49**, 1333 (1913).

H. Lineweaver i D. Burk

$$\frac{1}{v} = \frac{K_M}{V_m c_S} + \frac{1}{V_m}$$

nagib = $\frac{K_M}{V_m}$



G. S. Eadie

$$\frac{v}{c_S} = -\frac{v}{K_M} + \frac{V_m}{K_M}$$

nagib = $-\frac{1}{K_M}$