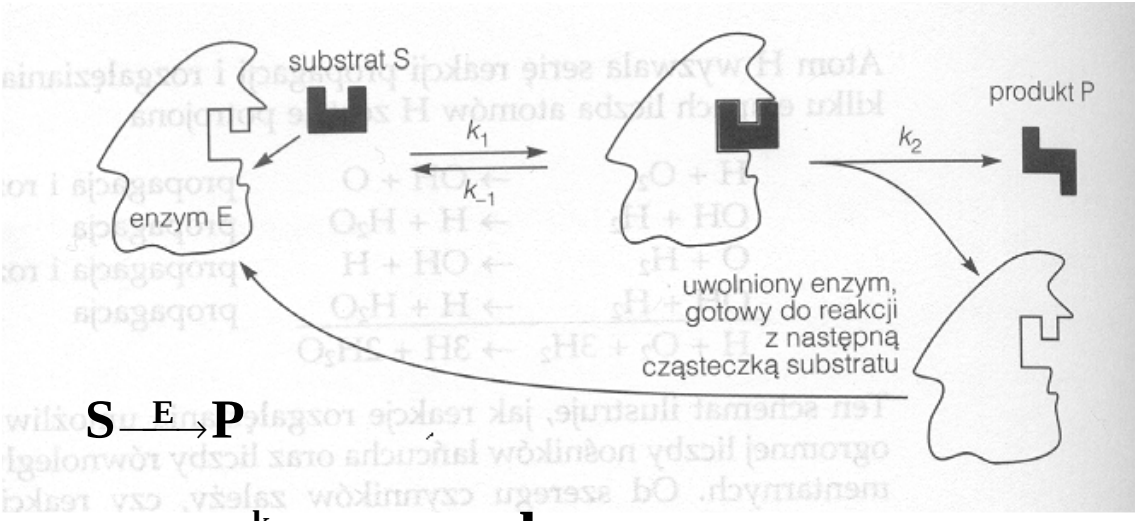
Department of Physical Pharmacy and Pharmacokinetics, PUMS.

Pharmacokinetics for Pharmacy students, IV year. **Laboratory nr 5.**

Michaelis-Menten model in interpretation of nonlinear pharmacokinetics of fenytoine in human plasma

Aim of the laboratory: Determination of the maximum velocity (V_{max}) and Michaelis constant (K_M), to interpret nonlinear elimination of fenytoine in human using difference methods of calculations.

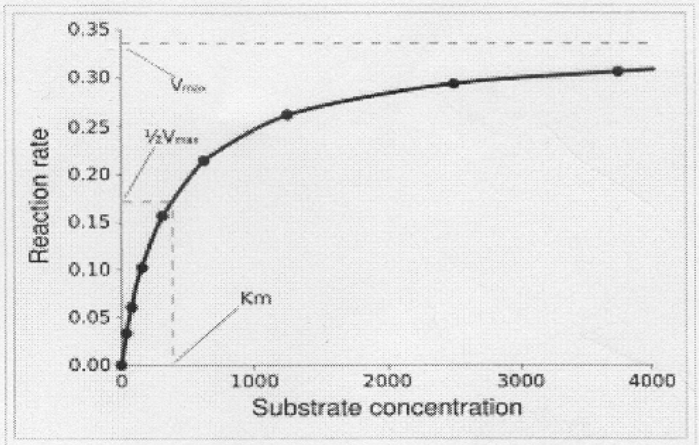
The modern relationship between substrate, it can be medicines and enzyme concentration was proposed in 1903 by Victor Henri. A microscopic interpretation was thereafter proposed my Leonor **Michelis** and Maud **Menten,** following earlier work by Arcchibald Vivian Hill. It postulated that that enzyme (catalyst) and substrate (reactant) are in fast equilibrium with their complex, which then dissociates to yield product and free enzyme



$$S \xrightarrow{E} P$$

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

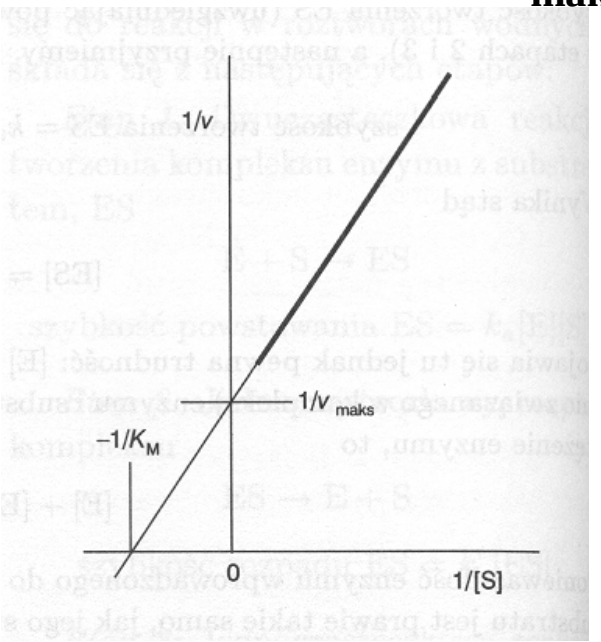
$$v = -\frac{d[S]}{dt} = \frac{k_2 \cdot [E]_0 \cdot [S]}{K_M + [S]} = \frac{v_{maks}[S]}{K_M + [S]}$$



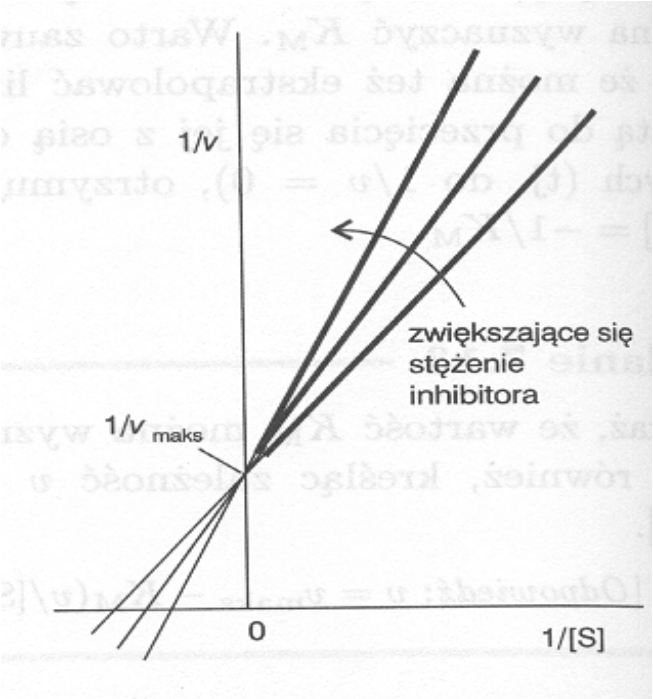
Saturation curve for an enzyme showing the relation between the concentration of substrate and rate.

Michaelis-Menten $$v = \frac{v_{maks} \cdot [S]}{K_M + [S]}$$

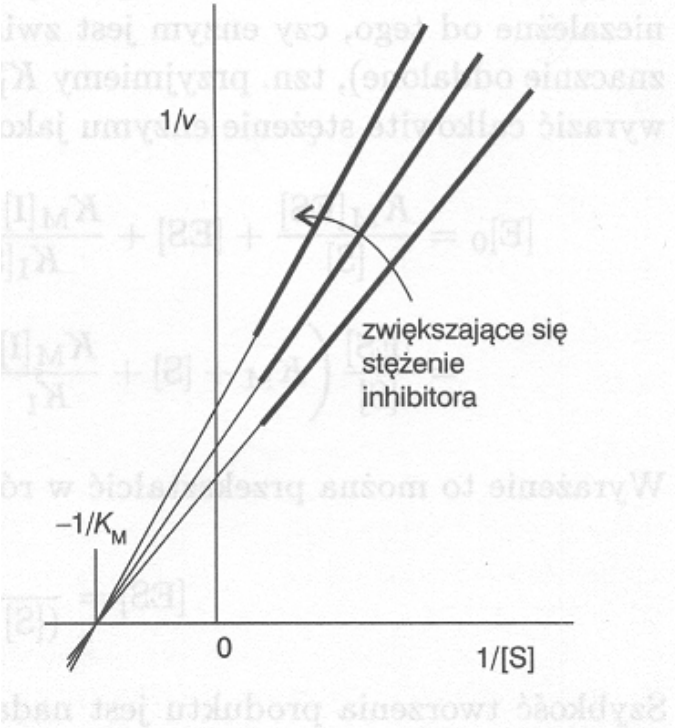
Lineweaver-Burk equation $$\frac{1}{v} = \frac{K_M}{v_{maks}} \cdot \frac{1}{[S]} + \frac{1}{v_{maks}}$$



1. Competitive inhibition



2. Noncompetitive inhibition



If **[S]>> K_M** the equation become the the zero order equation on account of substrate

$$v = -\frac{d[S]}{dt} = v_{max}$$

$$-dS = v_{max} \cdot dt$$

$$-\int_{S_0}^{S} dS = v_{max} \int_{t=0}^{t} dt$$

$$-\left[[S]-[S]_0\right] = v_{max}\left[t - t_{=0}\right]$$

$$-[S]+[S]_0 = v_{max} \cdot t$$

$$[S] = [S]_0 - v_{max} \cdot t \qquad \textbf{(zero order equation)}$$

If **K_M >>[S]** the equation become the the first order equation on account of substrate

$$\mathbf{v} = -\frac{\mathbf{d[S]}}{\mathbf{dt}} = \frac{\mathbf{k_2 \cdot [E]_0}}{\mathbf{K_M}} \cdot \mathbf{[S]} = \frac{\mathbf{v_{maks}}}{\mathbf{K_M}}[S]$$

$$-\frac{d[S]}{dt} = \frac{v_{max}}{K_M}[S]$$

$$-\frac{d[S]}{[S]} = \frac{v_{max}}{K_M} \cdot dt$$

$$-\int_{S_0}^{S} \frac{1}{[S]} d[S] = \frac{v_{max}}{K_M} \cdot \int_{t=0}^{t} dt$$

$$-\left[\ln[S]-\ln[S]_0\right] = \frac{v_{max}}{K_M} \cdot (t - t_0)$$

$$-\ln[S]+\ln[S]_0 = \frac{v_{max}}{K_M} \cdot t$$

$$\ln[S] = \ln[S]_0 - \frac{v_{max}}{K_M} \cdot t$$

Performance:
The elimination of fenytoine in humans following administration of overdose represents example of nonlinear pharmacokinetics. Main way of elimination of fenytoine is, limited of capacity, metabolism of the medicine in liver. As a result of biotransformation of fenytoine, first epoxide and then hydroxyfenytoine is eliminated as glucuronide with urine. Unchanged fenytoine is eliminated by kidney in smal percent (1-5%).
Case: Patient, after 5hrs after administration of fenytoine as single dose, was taken to hospital with signs of intolerance. In laboratory the concentrations of fenytoine in plasma of the patient were determined. Results of the determination was presentend in table 1.

Tab.1. Concentration of fenytoine in plasma samples of the patient

Time, **in hours** elapsed, from taken of the patient to hospital	Concentration of fenytoine **[mg/l]**
0	**29.7**
10	**27.1**
20	**24.5**
30	**21.9**
40	**19.4**
50	**17.0**
70	**12.5**
80	**10.5**
100	**7.4**
110	**5.3**
120	**4.0**
130	**3.0**

1. From the results inserted in table 1 calculate velocity (**v)** of elimination of fenytoine for individual time ranges.

Example:
$$v_1 = \frac{29.7 - 24.5}{20 - 0} = 0.26; \quad C_1 = 27.1$$
$$v_2 = \frac{27.1 - 21.9}{30 - 10} = 0.26; \quad C_2 = 24.5; \text{etc}$$

2. **Calculate K_M (Michaelis constant) and V_{max} from Lineweaver-Burk equation, using last square method, where b= $1/v_{max}$ and a= K_M/V_{max}.** The K_M it can be find from plot 1/V=f (1/S)., because $\frac{1}{[S]} = -\frac{1}{K_M}$ .Take into consideration that at great v, $1/v \rightarrow 0$,

$$\frac{1}{v} = \frac{K_M}{v_{maks}} \cdot \frac{1}{[S]} + \frac{1}{v_{maks}} \quad \text{Lineweaver-Burk equation}$$

3. Draw plot as a relationship velocity (v) of elimination of fenytoine and its concentration. (v=f(c)). Determine from the plot V_{max} and K_M.
4. Draw, on milineter paper, relationship of concentration [mg/l] of fenytoine as a function of time [h]. Additionally, the points from terminal phase of elimination of fenytoine put on semilogarithmic paper. **Using last square method** determine parameters of linear equations for concentrations to involve first phase of elimination of fenytoine, representing kinetics of **zero order** agree with equation: $\mathbf{C=C_0 - V_{max}\, t}$; and involving the last phase of elimination , representing kinetics of **first order** agree with equation: $\ln C = \ln C_0 - \frac{V_{\max}}{K_M} C$. Determine V_{max} i K_M.
5. Example. Phenytoin was administered to patient every day in two different doses. Concentrations of the drug were determined in plasma at steady state (Table2). Question: what dose of the medicine should be administered to the patient to receive concentration of 12 mg/l?

Table 2. Concentration of fenytoine at steady state in patient after administration of tow different doses.

Dose [mg/day]	Concentration of fenytoine at steady state [mg/l]
200	3.7
250	8.7
?	**12**

Solution:

In linear pharmacokinetics of the dose is calculated from equation : $D_2 = D_1 \frac{C_{SS}^2}{C_{SS}^1}$

in nonlinear pharmacokinetics the dose id calculated that way :

$$D_2 = D_1 \frac{\left(K_M + C_{SS}^1\right) C_{SS}^2}{\left(K_M + C_{SS}^2\right) C_{SS}^1}$$

$$V_{\max} = \frac{D_2 (K_M + C_{SS}^2)}{C_{SS}^2}$$

The dose of phenytoin to receive concentration at steady state equal C^3_{SS} =12 mg/l is calculated from the equation:

$$D_3 = \frac{V_{\max} \cdot C_{SS}^3}{K_M + C_{SS}^3}$$