Bio-Systems Modeling and Control

Lecture 20

Enzymes

Simple Enzyme Kinetics

Simplest Enzyme-catalyzed Reaction: $S+E \rightleftharpoons C$; $C \rightarrow P+E$

- An enzyme E acts on a single substrate S.
- First, a complex C (enzyme + substrate together) is created.
- This is typically a reversible reaction, with a forward rate constant k_1 and a reverse rate constant k_{-1}.
- C decomposes into a product P and enzyme E. Assume irreversible reaction with rate constant k_2.

Comments regarding the above simple enzymatic reaction:

- In general, the units of k_1 are different than those of k_{-1} and k_2. Reason: S + E is typically bi-molecular, whereas C is uni-molecular.
- Decomposition of C to P+E may too be a reversible process. Often, P is continually removed, which prevents the reverse reaction. This is frequently not the case *in-vivo* (i.e. inside the living organism)!

Rate Equations (using the Law of Mass Action): [S]=s,[E]=e,[C]=c,[P]=p

$$\frac{dc}{dt} = k_1 es - k_{-1} c - k_2 c$$

$$\frac{ds}{dt} = -k_1 es + k_{-1} c$$

$$\frac{de}{dt} = -k_1 es + k_{-1} c + k_2 c$$

$$\frac{dp}{dt} = k_2 c$$

First three rates are independent of p; Variables c,s,e are not independent

$$\frac{dc}{dt} = k_1 es - k_{-1} c - k_2 c$$

$$\frac{ds}{dt} = -k_1 es + k_{-1} c$$

$$\frac{de}{dt} = -k_1 es + k_{-1} c + k_2 c$$

$$\frac{dp}{dt} = k_2 c$$

What happens if we add the first and third equations?

Combining the rate of c with the rate of e:

$$\frac{dc}{dt} + \frac{de}{dt} = 0 \Rightarrow e = e_0 - c$$

- We assume that at t=0: $e(0)=e_0$, $s(0)=s_0$, $c(0)=0$, $p(0)=0$.
- Often $e_0 << s_0$: A small quantity of an enzyme controls the reaction rate for a large quantity of s. We'll also look at cases involving large s_0.

Use $e=e_0-c$ to eliminate the equation of e:

$$\frac{dc}{dt} = k_1 s(e_0 - c) - (k_{-1} + k_2)c$$

$$\frac{ds}{dt} = -k_1 s(e_0 - c) + k_{-1} c$$

- Initial conditions: $c(0)=0, s(0)=s_0$.
- Total amount of available enzyme is e_0.
- Equilibrium ($t \to \infty$): $c(t) \to 0$ and $s(t) \to 0$.
- Obviously, c(t) rises to a certain maximum c_{max} before falling again to 0.

As c(t) rises and falls, e(t) falls and rises:

$$e = e_0 - c$$

The active enzyme molecules concentration starts at $e(0)=e_0$. It falls to some minimum value e_{min} (possibly zero, if all enzyme molecules are occupied), and eventually rises back again to $e(\infty)=e_0$.

Expected product p(t) behavior:

$$\frac{dp}{dt} = k_2 c \Rightarrow p(t) = k_2 \int_0^t c(\tau) d\tau$$

- As the curve c(t) has a finite area, the product p(t) $\rightarrow p_\infty$=constant.
- Typically the larger c_{max} the larger is p_∞.

Let's verify the expected solutions by simulation. First let's normalize:

$$\frac{dc}{dt} = k_1 s(e_0 - c) - (k_{-1} + k_2)c$$

$$\frac{ds}{dt} = -k_1 s(e_0 - c) + k_{-1} c$$

$$\tau = k_1 e_0 t _ u = \frac{s}{s_0} _ v = \frac{c}{e_0}$$

$$\lambda = \frac{k_2}{k_1 s_0} _ K = \frac{k_{-1} + k_2}{k_1 s_0} _ \varepsilon = \frac{e_0}{s_0}$$

Here we only non-dimensionalize every variable and coefficient. Total number of parameters is reduced only by 1.

Normalized enzyme kinetics model

$$\frac{du}{d\tau} = -u + (u + K - \lambda)v$$

$$\varepsilon \frac{dv}{d\tau} = u - (u + K)v$$

Normalized substrate rate

Normalized complex rate

$$\tau = k_1 e_0 t _ u = \frac{s}{s_0} _ v = \frac{c}{e_0}$$

$$\lambda = \frac{k_2}{k_1 s_0} _ K = \frac{k_{-1} + k_2}{k_1 s_0} _ \varepsilon = \frac{e_0}{s_0}$$

Initial conditions: u(0)=1,v(0)=0

Note: K-λ>0

Let's add normalized enzyme and product concentrations to the model

$$\frac{du}{d\tau} = -u + (u + K - \lambda)v _ z = 1 - v$$

$$\varepsilon \frac{dv}{d\tau} = u - (u + K)v _ \frac{dq}{d\tau} = \lambda v$$

u=substrate
v=complex,
z=enzyme
q=product

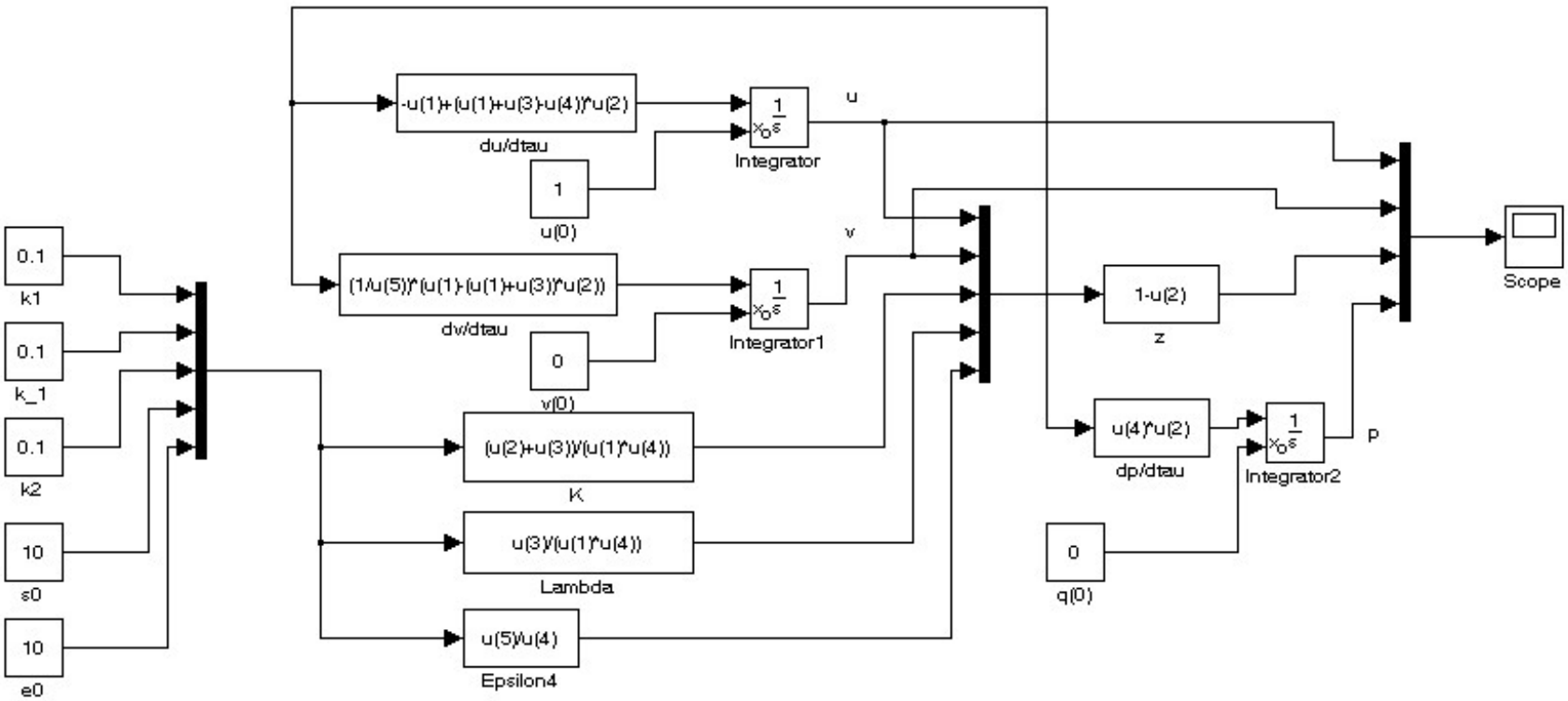
$$\tau = k_1 e_0 t _ u = \frac{s}{s_0} _ v = \frac{c}{e_0} _ z = \frac{e}{e_0} _ q = \frac{p}{s_0}$$

$$\lambda = \frac{k_2}{k_1 s_0} _ K = \frac{k_{-1} + k_2}{k_1 s_0} _ \varepsilon = \frac{e_0}{s_0}$$

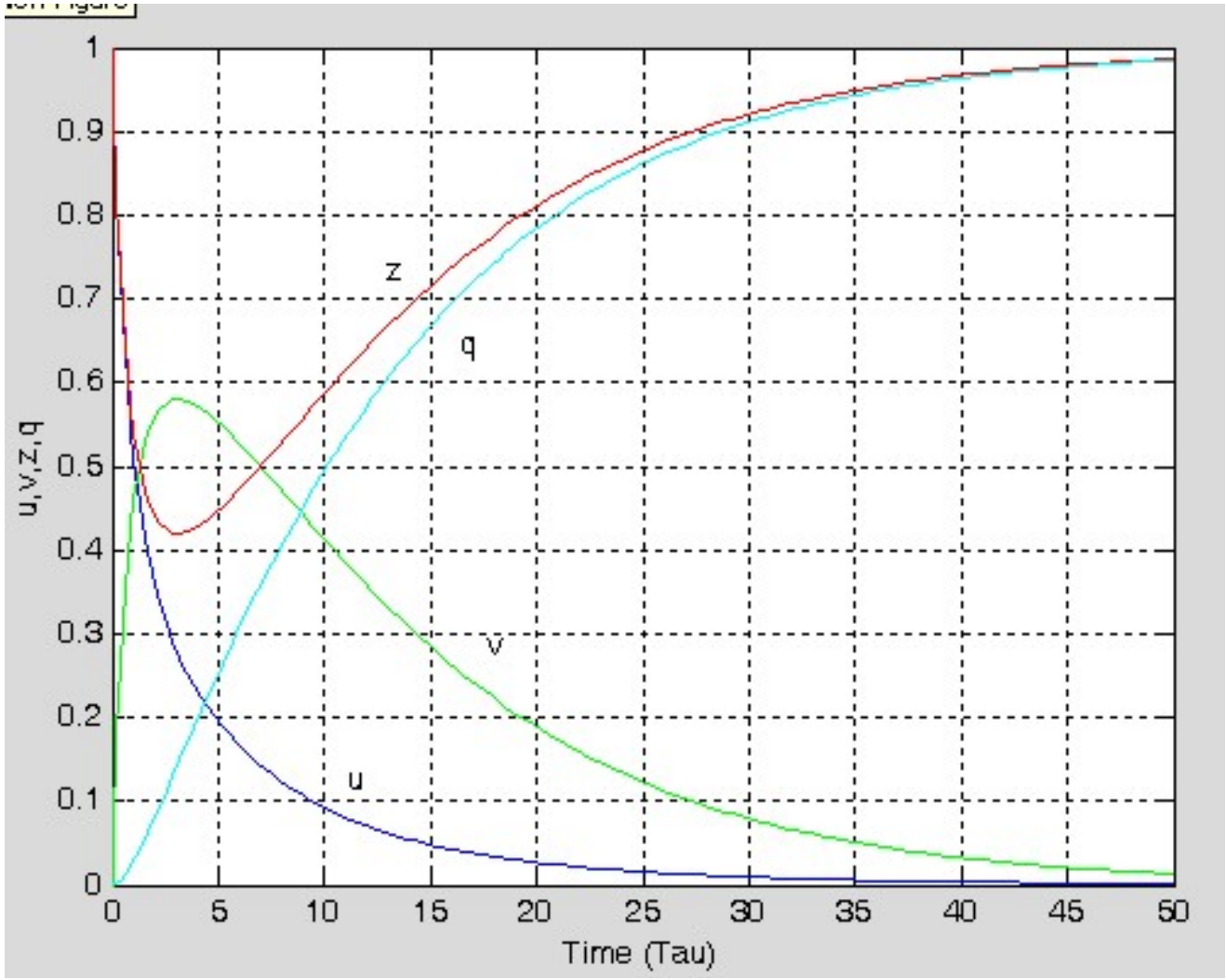
Initial conditions:
u(0)=1,v(0)=0
z(0)=1,q(0)=0

Discussion of theory – later;
First let's simulate

Enzyme kinetics: Same amount of substrate and enzyme



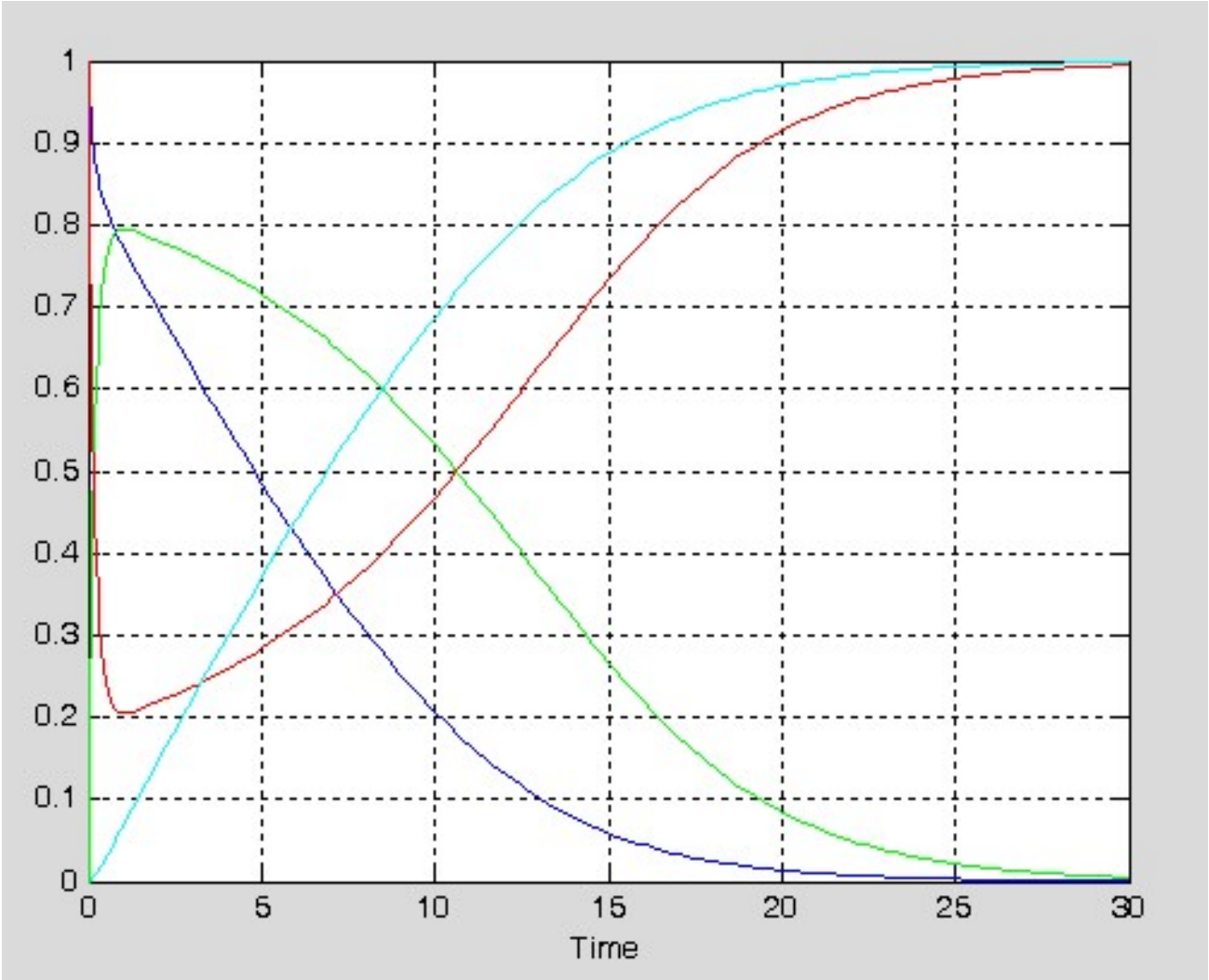
Enzyme kinetics: Same amount of substrate and enzyme (Results)



Enzyme kinetics: More substrate than enzyme (10:2) (Results)

Note: Complex reaches peak faster.

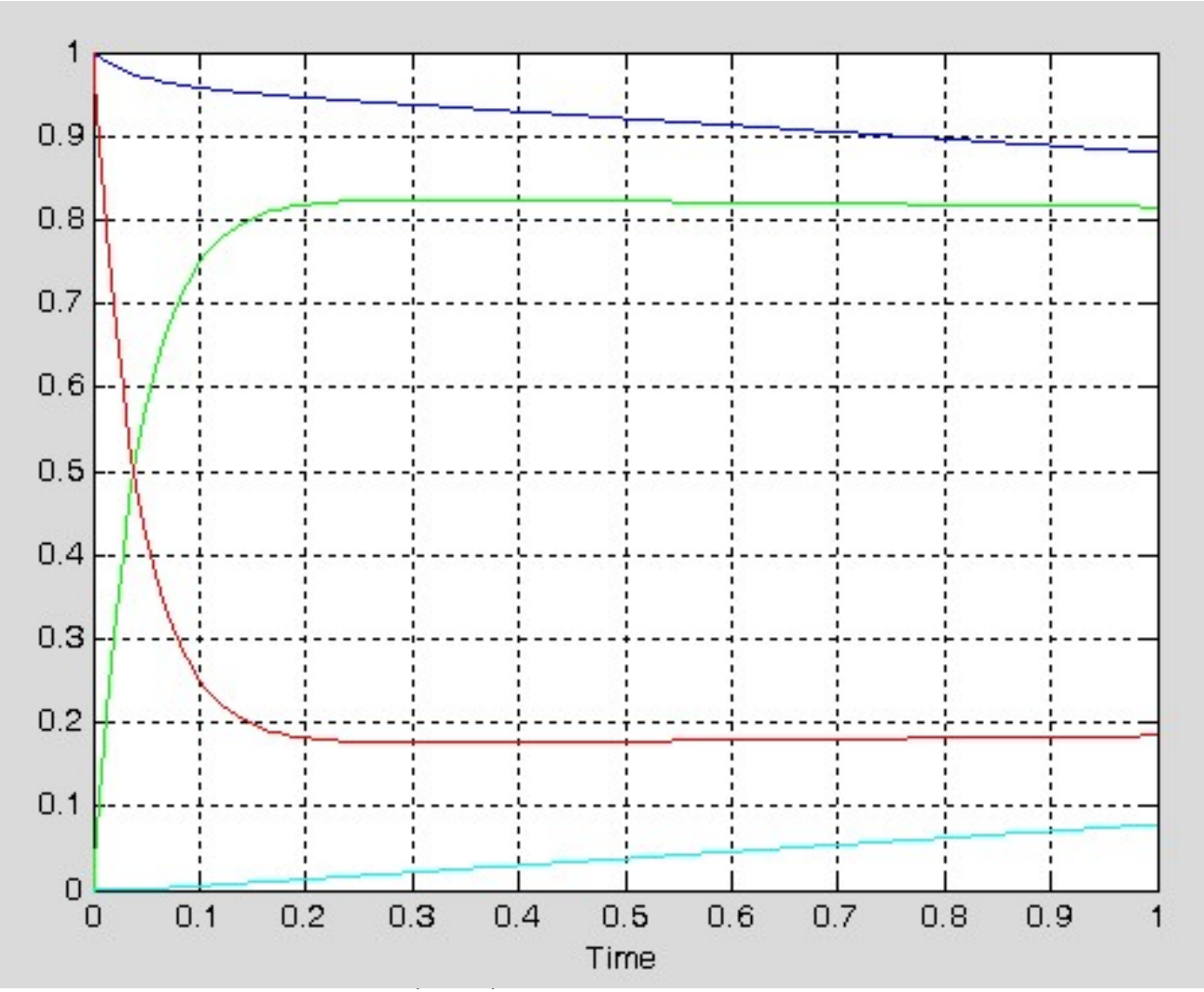
Faster reaching steady state.



Enzyme kinetics: Much more substrate than enzyme (10:0.5) (Results)

Note: Complex reaches peak very fast.

Then – slow convergence to steady state.



Michaelis-Menten Theory (1913):

$$\frac{dc}{dt} = k_1 es - (k_{-1} + k_2)c$$

$$\frac{ds}{dt} = -k_1 es + k_{-1} c$$

$$e = e_0 - c$$

$$\frac{dp}{dt} = k_2 c$$

- Assume that $\varepsilon = e_0/s_0$ is small (10^{-2}-10^{-7}).
- Then: substrate and complex reach equilibrium almost instantly, and from there on maintain a quasi-steady-state relationship $c \approx e_0 s/(s+K_m)$, where $K_m = (k_{-1}+k_2)/k_1$

Historical comments

- Leonor Michaelis and Maud Menten, in their original 1913 set-up, assumed $dc/dt \approx 0$ at the quasi-steady-state.
- In 1925, Briggs and Haldane assumed (more correctly mathematically) that $\varepsilon \cdot dc/dt \approx 0$, where $\varepsilon = e_0/s_0$. Result ended up being the same.
- Nowadays, we use the theory even if s_0 is small and e_0 is large.

Michaelis-Menten Theory : s & c equilibrium derivation and observations

$$\frac{dc}{dt} \approx 0 = k_1 es - (k_{-1} + k_2)c$$

$$e = e_0 - c$$

$$\Rightarrow c \approx \frac{e_0 s}{K_m + s}$$

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

- K_m=Michaelis-Menten constant.
- Recall that $dp/dt=k_2c$. Therefore, the rate of product generation equals approximately to $k_2e_0s/(K_m+s)$.
- When $s>>K_m$ we have $dp/dt\approx k_2e_0=V_{max}$

Michaelis-Menten Theory : Product Reaction Speed

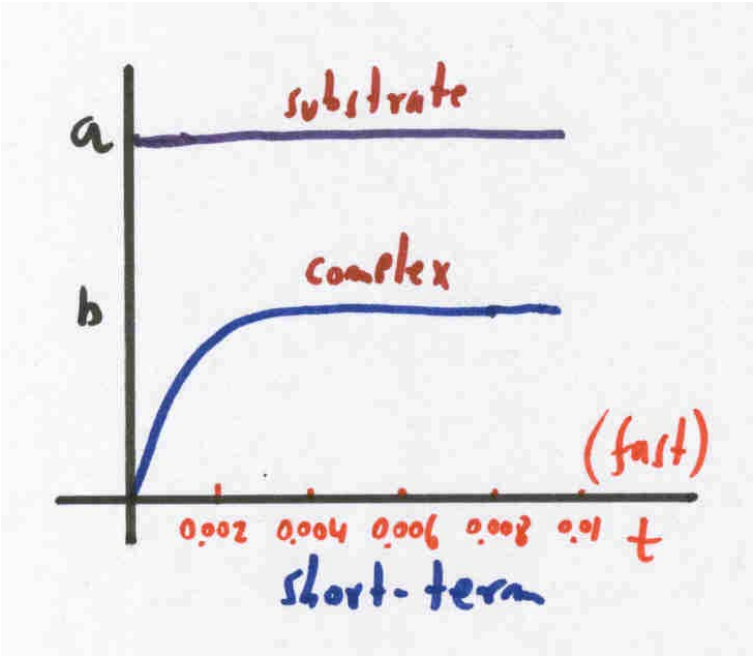
$$c \approx \frac{e_0 s}{K_m + s}$$

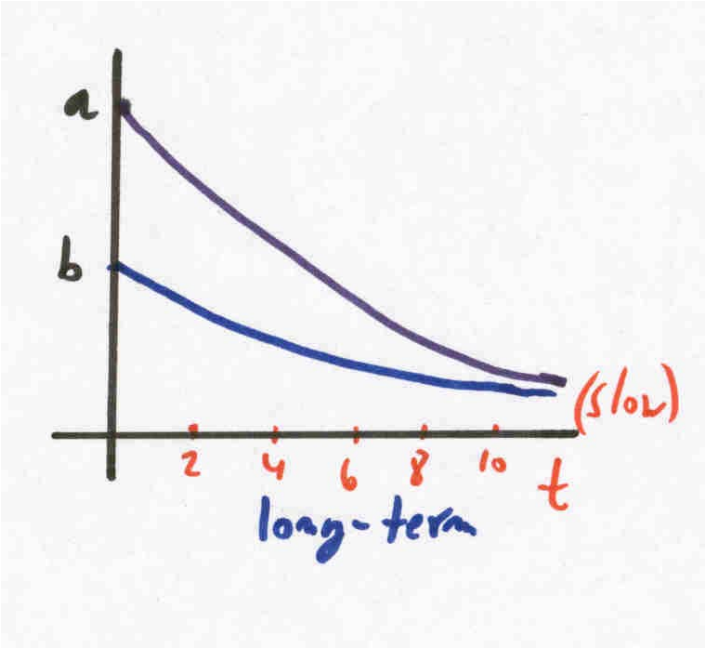
$$K_m = \frac{k_{-1} + k_2}{k_1}$$

$$V(t) = \frac{dp}{dt} = k_2 c = \frac{k_2 e_0 s}{K_m + s}$$

- When $s >> K_m$ we have $dp/dt \approx k_2 e_0 = V_{max}$.
- This maximum reaction speed is proportional to e_0.
- Initial reaction speed: $V_0 \approx V_{max} s_0/(K_m + s_0)$

Michaelis-Menten two time scales

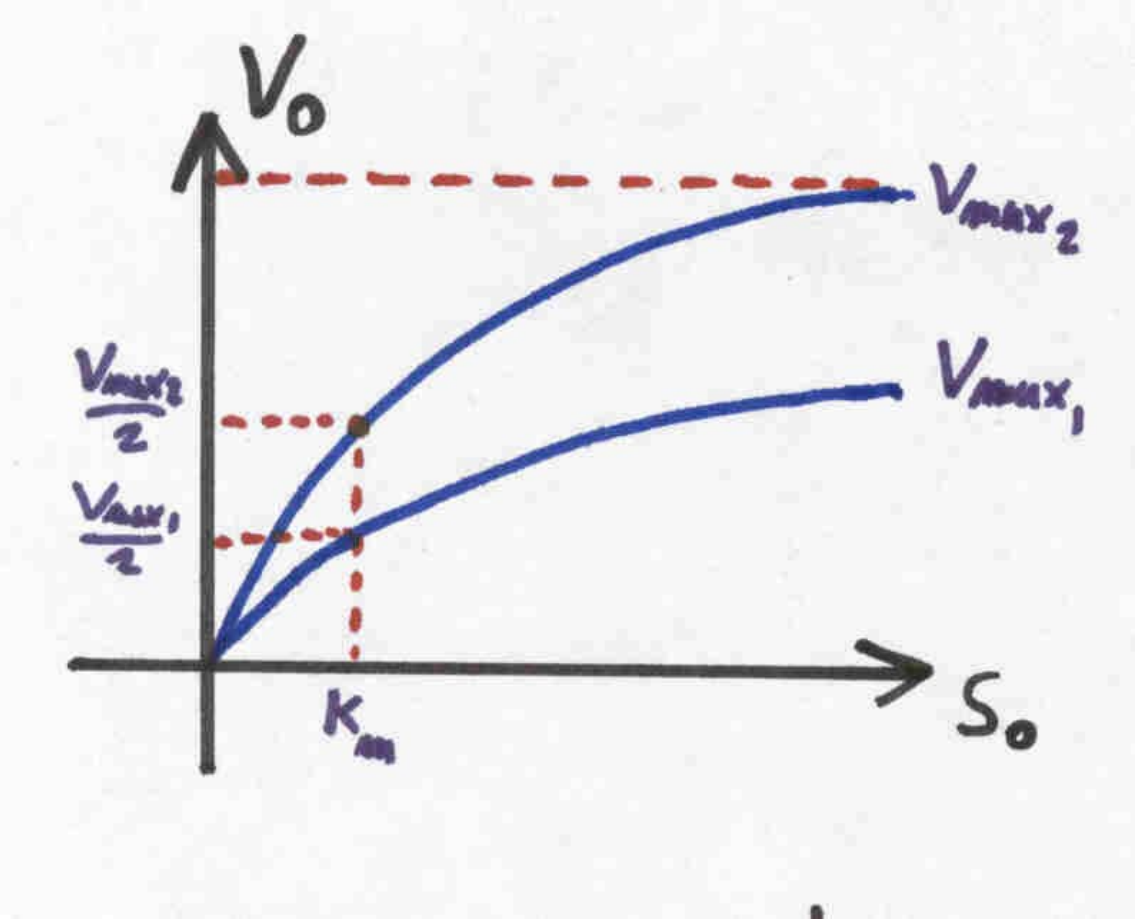




- Short term: Very fast rise of complex's concentration. Both substrate and complex appear constant, after a while.
- Long term: Initial rise of complex is now invisible. Both substrate and complex decay together.

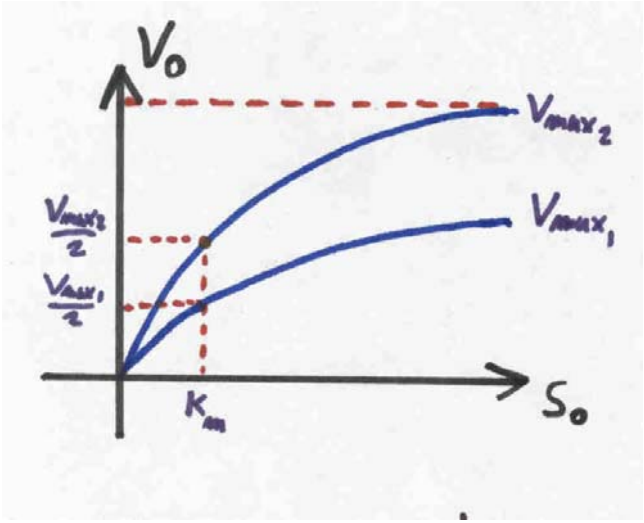
Experiment: Initial reaction rate V_0 vs. initial substrate concentration s_0

$$V_0 = \frac{V_{\max} s_0}{K_m + s_0}$$



Initial reaction rate V_0 vs. initial substrate concentration s_0 - observations

$$V_0 = \frac{V_{\max} s_0}{K_m + s_0}$$

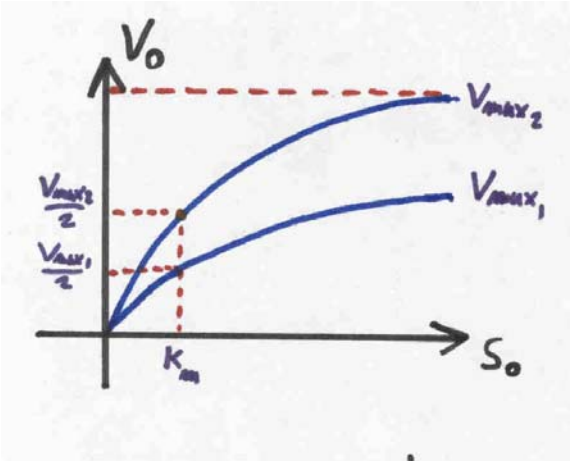


- Assuming "saturated enzyme" (limited amount of e_0), V_{max} is proportional to e_0.
- The concentration s_0 at which the initial reaction rate V_0 equals $0.5V_{max}$ is independent of V_{max}. K_m does not depend on e_0.

Meaning of the Michaelis-Menten constant K_m:

$$V_0 = \frac{V_{\max}}{2} = \frac{V_{\max} s_0}{K_m + s_0}$$

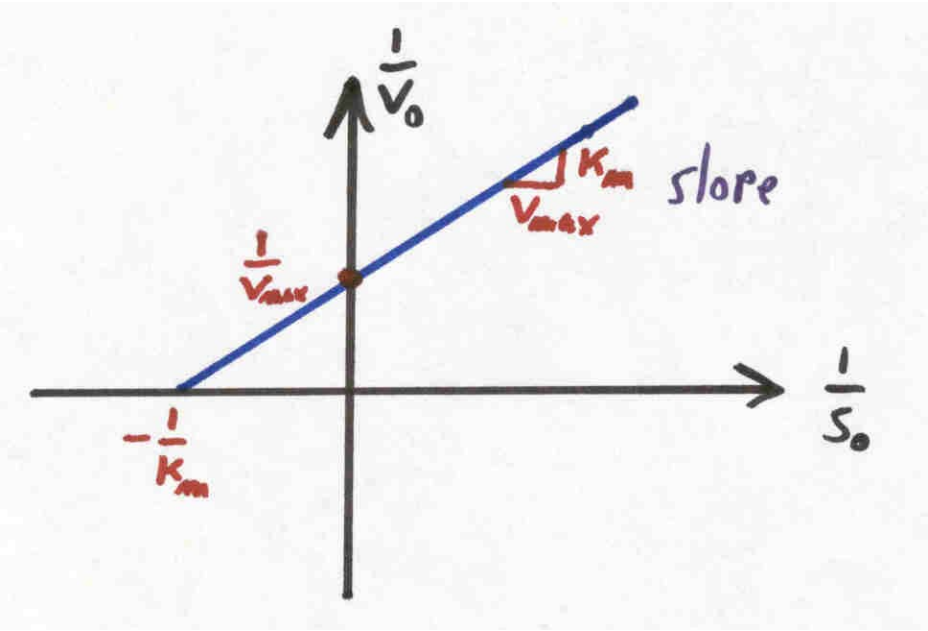
$$\Rightarrow K_m = s_0$$



- The concentration s_0 at which the initial reaction rate V_0 equals $0.5V_{max}$ is independent of V_{max}.
- K_m equals s_0 at which initial rate is half of V_{max}.
- The greater the affinity between an enzyme and its substrate, the lower K_m.

The Lineweaver-Burk Plot

$$\frac{1}{V_0} = \frac{K_m}{V_{\max}} \cdot \frac{1}{s_0} + \frac{1}{V_{\max}}$$



- K_m and V_{max} are the unknowns.
- Only two experiments (with two different values of s_0) are needed – we then find the unknowns from the x-axis and y-axis intercepts of the line.

Bottom Line: Practical Model of Simple Enzyme Kinetics

$$V_{\max} = k_2 e_0$$

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

$$\boxed{\frac{dp}{dt} \approx \frac{V_{\max} s}{K_m + s}}$$

- In practice, we may neglect Phase 1 (complex formation)
- The process nonlinear model comes from Michaelis-Menten hyperbolic relationship

Practical Model of Simple Enzyme Kinetics with Two Substrates

$$V_{\max} = k_2 e_0$$

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

$$\boxed{\frac{dp}{dt} \approx \frac{V_{\max} s_1 s_2}{K_m + s_1 s_2}}$$

- Repeating the derivation for a simple enzyme reaction involving two substrates:

$$S_1 + S_2 + E \underset{k_{-1}}{\overset{k_1}{\rightleftarrows}} C \xrightarrow{k_2} P + E$$