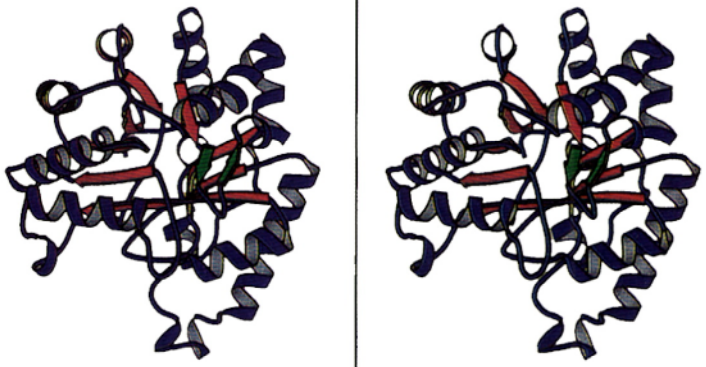
Here, ENZ = enzyme = OPH (a.k.a. phosphotriesterase)



M.M. Benning, J.M. Kuo, F.M. Raushel and H.M. Holden, Biochemistry, Vol. 33 pp. 15001-15007 (1994).

The kinetics can be monitored by following the production of p-nitrophenol, a yellow colored chemical, spectroscopically.

Enzyme kinetics can be used to determine the efficiency of OPH when it is integrated into a polymeric foam compared to OPH's efficiency in solution.

- **evaluate K_M and V_{max} and compare**

Let's review how these parameters are derived in enzyme kinetics:

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

$$V = \text{rate} = \frac{dP}{dt} = k_2[ES]$$

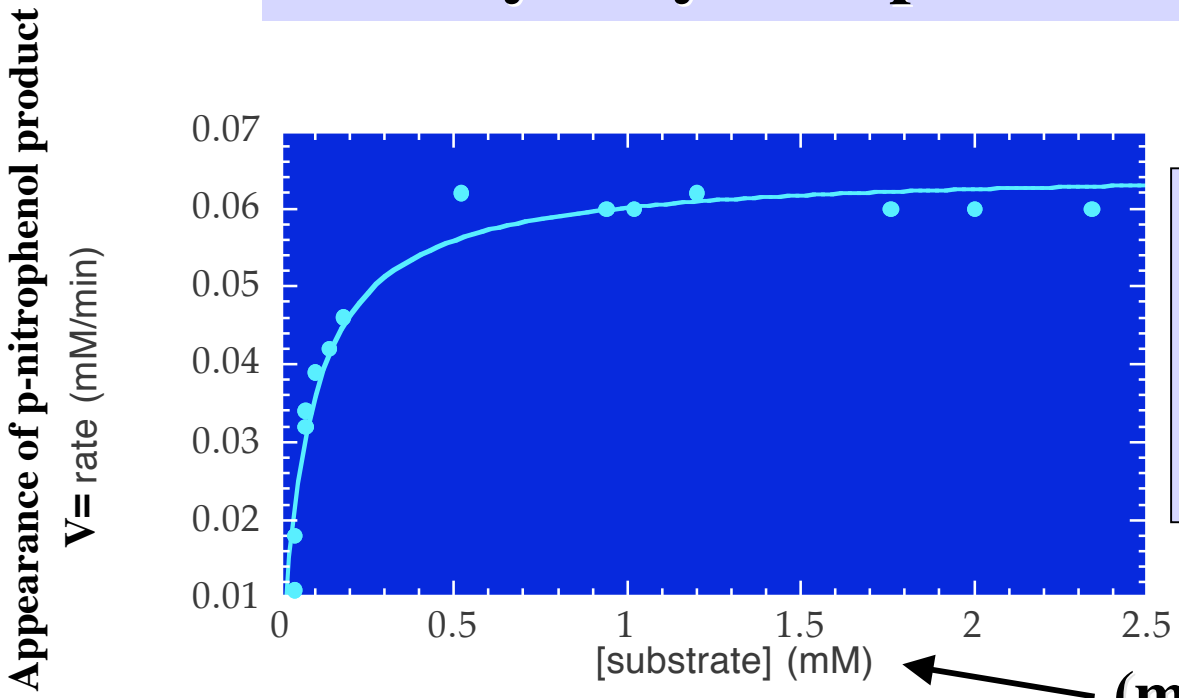
Apply steady-state approximation:

Define $E_0 = E + ES$

$$\mathbf{V = rate = k_2[ES]} \longrightarrow V = \text{rate} = \frac{k_2[E_0]}{1 + \frac{1}{[S]}\frac{k_{-1}+k_2}{k_1}}$$

$$V = \text{rate} = \frac{k_2[E_0]}{1 + \frac{1}{[S]}K_M}$$

OPH hydrolysis of paraoxon



First, let's look at the activity of OPH in solution

(mM = millimolar)

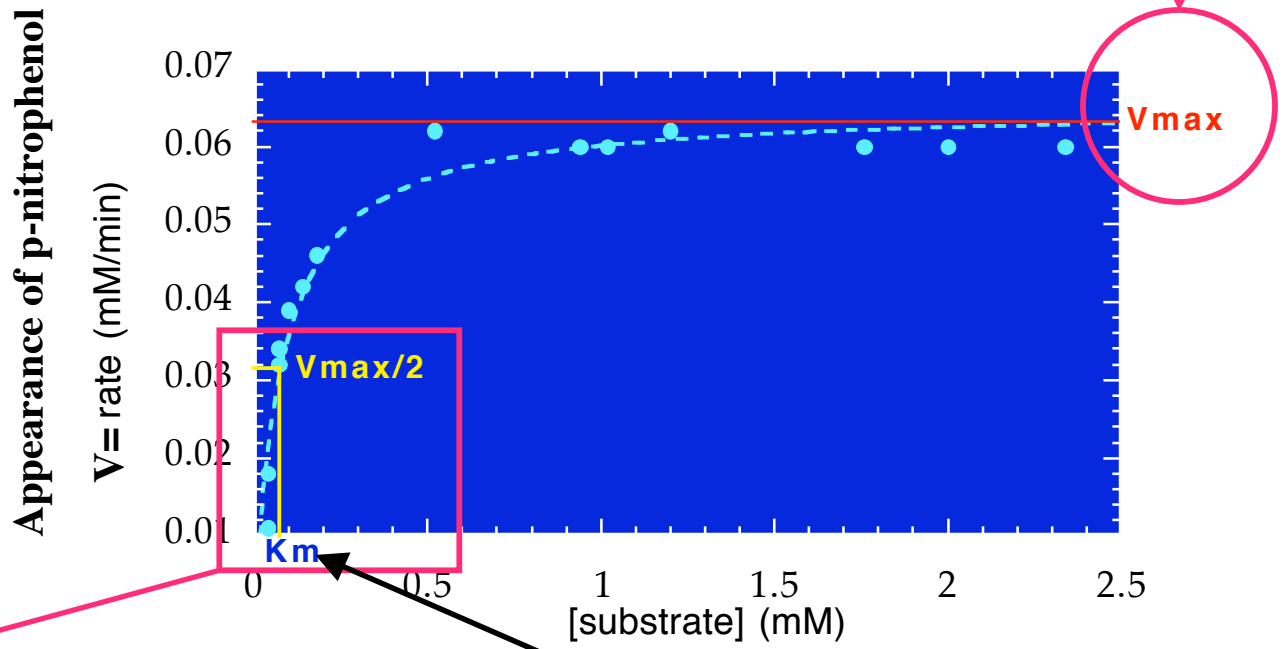
To obtain V_{max} and K_M from this data:

Method I

- **extrapolate plot above to find V_{max}**
- **find $V_{max}/2$**
- **find [substrate] corresponding to the value at $V_{max}/2$**
- **This [substrate] is K_M**

OPH hydrolysis of paraoxon

- Extrapolate plot to find V_{max}

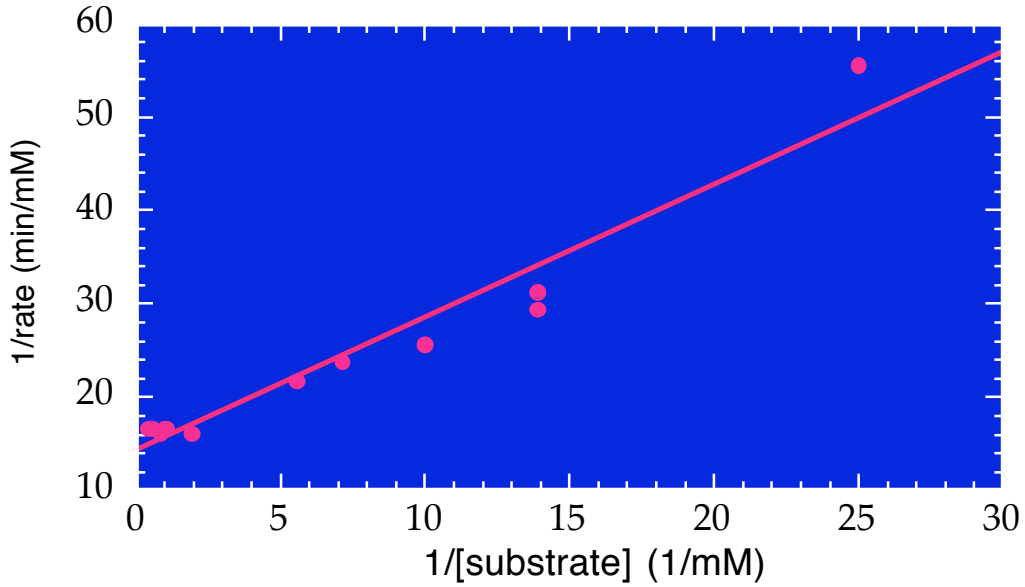


- find $V_{max}/2$
- find [substrate] corresponding to the value at $V_{max}/2$
- This [substrate] is K_M

Note: $K_M = K_m$

Here, $V_{max} = 0.063$ mM/min and $K_M = 0.08$ mM

Method II: plot 1/V versus 1/[substrate] (Lineweaver-Burke Plot)



The line that fits the data has the following form:
$y = 14.3\ \text{min/mM} + 1.4\ \text{min} \cdot x$

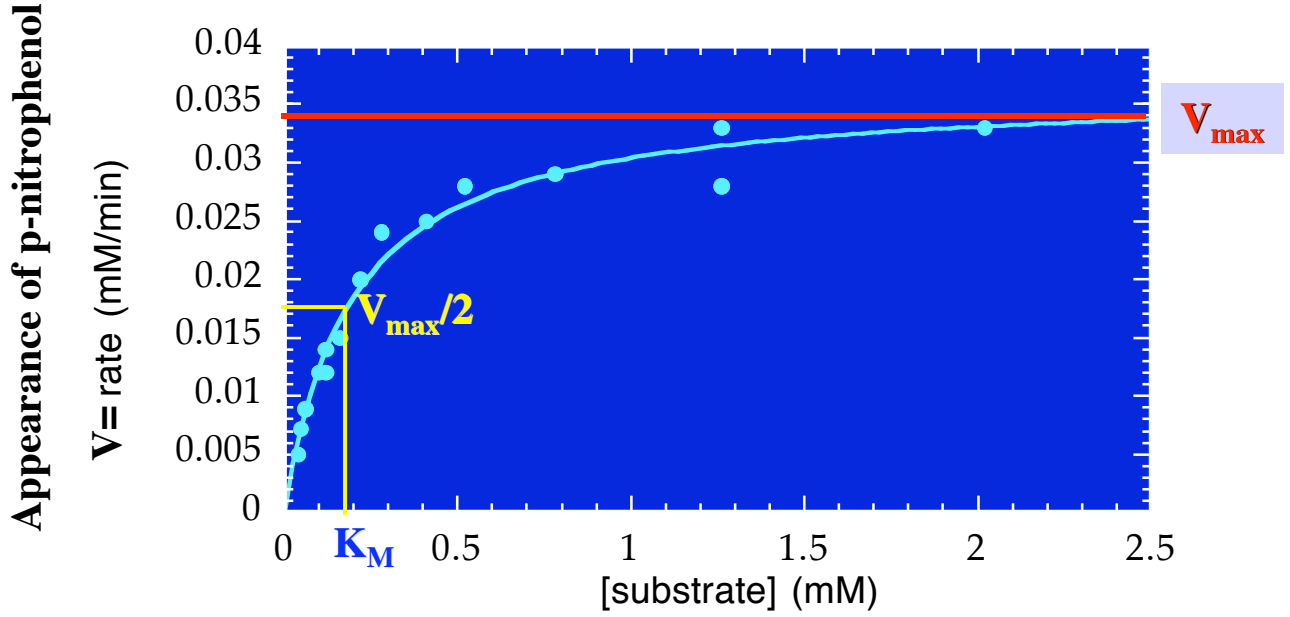
Recall that $y = \frac{1}{V}$ and $x = \frac{1}{[\text{substrate}]}$

Such that $\frac{1}{V} = \frac{1}{V_{max}} + \left(\frac{K_M}{V_{max}}\right)\frac{1}{[\text{substrate}]}$

Obtained from V vs [S] plot

This yields $V_{max} = 1/[14.3(\text{min/mM})] = 0.07$ mM/min, vs (0.063)
and $K_M = [1.4\ (\text{min})] \cdot V_{max} = 0.098$ mM vs (0.08)

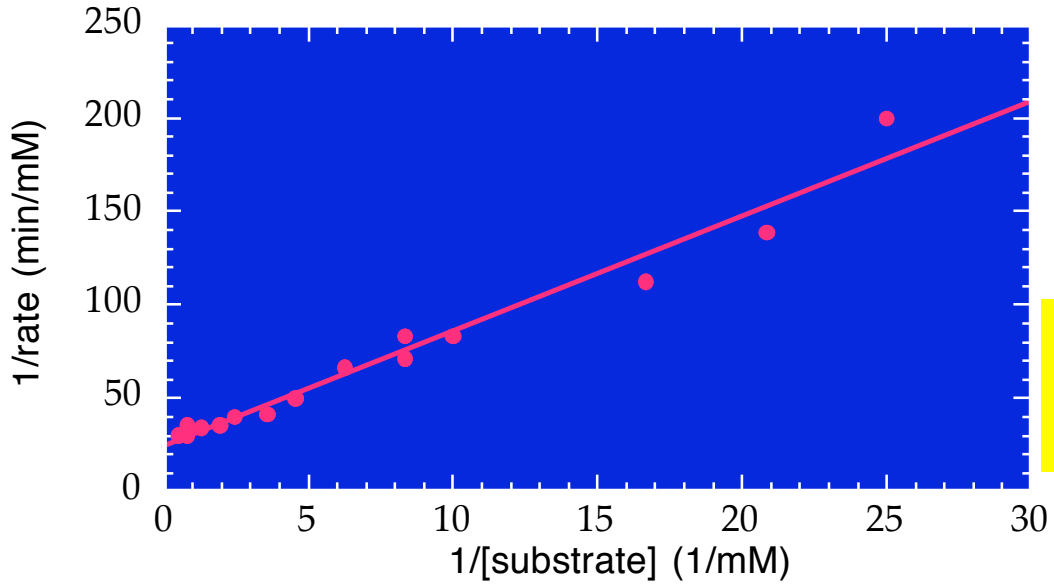
Now, let's see how well OPH hydrolyzes paraoxon when it is encapsulated in foam.



Again, V_{max} can be found from the asymptote of the curve, and K_M is that substrate concentration which corresponds to $V_{max}/2$.

V_{max} for OPH in foam = 0.035 mM/min, while K_M = 0.19 mM.

We get similar results from a Lineweaver-Burke plot:



Obtained from V vs [S] plot

from the y-intercept, V_{max} = 0.040 mM/min; (vs 0.035)

from the slope, K_M = 0.24 mM (vs 0.19)

This comes from: $$\frac{1}{V} = \frac{1}{V_{max}} + \left(\frac{K_M}{V_{max}}\right)\frac{1}{[substrate]}$$

Now, we can compare these parameters for the cases where OPH is in solution and integrated into foam:

	in solution	in foam
$V_{max}(^{mM}/_{min})$	0.063	0.035
K_M(mM)	0.08	0.19

V_{max} decreases by a factor of 2 when the enzyme OPH is encapsulated in foam and K_M increases by approximately the same factor.

What does this mean????

$$\text{OPH} + \text{paraoxon} \underset{k_{-1}}{\overset{k_1}{<\!\!>}} [\text{OPH}...\text{paraoxon}] \xrightarrow[H_2O]{k_2} \text{OPH} + \text{p–nitrophenol} + \text{product}$$

$$V = \text{rate} = \frac{k_2[OPH_0]}{1 + \frac{k_{-1} + k_2}{k_1}\frac{1}{[\text{paraoxon}]}}$$

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

The lower the value for K_M, the better the enzyme works (tighter binding) as a catalyst for the hydrolysis of paraoxon.

	in solution	in foam
K_M(mM)	0.08	0.19

OPH is a tighter binding (more efficient) catalyst in solution than in foam!!!

Why is OPH less efficient as a catalyst (has smaller V_{max}) when used in a foam?

Let's look at V_{max} for some clues:

Recall

$$(V)_{max} = \lim_{[\text{paraoxon}] \to \infty} \left(\frac{k_2[OPH_0]}{1 + \frac{K_M}{[\text{paraoxon}]}} \right) = k_2[OPH_0]$$

where $[OPH_0]$ is the amount of enzyme present.

There are 2 possible effects at work here:

1. "Inhibition" effect:

In order to get the enzyme into foam, certain groups of atoms (called functional groups) within the enzyme (e.g. NH_2 and OH) react with the foam, immobilizing a percentage of the total enzyme.

That is, the foam is NOT inert! $[OPH_0]$ is reduced.

2. Change in the rate constant k_2.

A decrease in k_2 may occur when OPH is used in foam, leading to a smaller V_{max} as compared to that parameter measured when OPH is in solution.

Is the decrease in catalytic efficiency when OPH is used in a foam a negative result?

Not exactly ...

- for use on pesticides, delivery of catalysts like OPH via foams is likely not the best means to degrade organo-phosphates -- reduced efficiency

- for use on other toxins (e.g. nerve agents) delivery in foams is extremely beneficial

  OPH is still highly effective and delivery via foams is more convenient

References

K.E. LeJeune and A. J. Russell, Biotechnology and Bioengineering, Vol. 51, pp. 450-457 (1996)

K.E. LeJeune and J.R. Wild, Nature, Vol. 395, pp. 27-28 (1998)

K.E. LeJeune, A.J. Mesiano, S.B. Bower, J.K. Grimsley, J.R. Wild and A.J. Russell, Biotechnology and Bioengineering, Vol. 54 pp. 105-114 (1997)

S.E. Manahan, Environmental Chemistry (5th ed), Lewis Publishers: Chelsea, MI (1991)

D.J. Hanson, Chemical and Engineering News, pp. 20-22 (Sept. 28,1998)

M.M. Benning, J.M. Kuo, F.M. Raushel and H.M. Holden, Biochemistry, Vol. 33 pp. 15001-15007 (1994).

Remarks by Arrhenius

The April issue of the ACS Chicago Section's Chemical Bulletin carries excerpts from a talk by Svente Arrhenius on May 11, 1912, when he received the section's first Willard Gibbs Award. The full address appears in J. Am. Chem. Soc., 36, 353 (1912). A few lines follow:

"I came to my professor, Cleve, and I said, 'I have a new theory of electrical conductivity as a cause of chemical reactions.' He said, 'That is very interesting,' and then he said 'Goodbye'. He explained to me later, when he had to pronounce the reason for my receiving the Nobel Prize for that work, that he knew very well that there are so many different theories formed, and that they are all almost certain to be wrong, for after a short time they disappear; and therefore, by using the statistical manner of forming his ideas, he concluded that my theory also would not exist very long."

"I was not very content with that opinion, and then I thought, in foreign countries there are such prominent scientist they might look at it differently; it might appeal to them. Then I wrote to Clausius, and said, 'What do you think of that?' I wrote to Ostwald – he worked on the same line. I wrote to Thomsen. I received friendly answers … they were very glad to make my acquaintance, and so on, but it was not very much more. The only exception was Ostwald, and he describes how it was that he got on the same day this dissertation, a toothache, and a nice daughter, and that was too much for one day, and the worst was the dissertation."

Wag (9) a humorous person; joker (Am. College Dictionary, Random House, NY, 1961)

Temperature Dependence of k_{rate}

Arrhenius first guessed the form of the kinetic rate constant in the late 1800's:

In the "standard" Arrhenius form of the rate constant, A, and E_A are constants independent of temperature.

A is called the "pre-exponential" factor and E_A the activation energy.

Using this form and taking the natural log of k_{rate} gives

Taking the derivative with respect to T:

$$d\{\ln[k_{rate}]\}/dT = d\{-E_A/RT\}/dT$$

(Because A is assumed Constant in this model.)

$$d\{\ln[k_{rate}]\} = [-E_A/R]\, d(1/T)$$

This expression predicts that a plot of $\ln[k_{rate}]$ vs 1/T will be a a straight line with a slope of $-E_A/R$

We saw using gas kinetic theory collision rate arguments (binary collision model) that a form for k_{rate} like

could be obtained from a reaction cross section of the form

$\sigma_R^2 = 0$ $\quad E < E_A$

$\sigma_R^2 = \sigma_{AB}^2 (1 - E_A / E)$ $\quad E \geq E_A$

Where σ_{AB} is the sum of the radii of molecules A and B

$\langle u_{rel} \rangle = (8kT/\pi\mu)^{1/2}$

But notice A here scales like $T^{1/2}$. It is NOT independent of T as Arrhenius assumed!

If we write $A=(\text{const})\ T^{1/2}$, then k_{rate} becomes

$$\ln[k_{rate}] = \ln(\text{const}) + \ln[T^{1/2}] - E_A/RT$$

$$d\{\ln[k_{rate}]\}/dT = [(1/(2T)] + E_A/RT^2$$

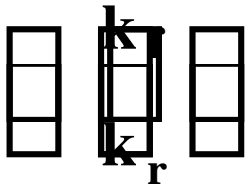
Define $[(1/2)RT + E_A] = \square_A$

Since the slope is not a constant, the plot of $\ln k_{rate}$ vs 1/T is not a straight line.

$\square_A = [(1/2)RT + E_A]$ provides a more strict definition of the activation energy for a reaction since it includes the T dependence of relative speed. Note that this activation energy is T dependent.

Kinetics and Equilibria

By definition, kinetic processes are not equilibrium processes. In fact, we may think of kinetic processes as the mechanism that nature uses to reach the equlibrium state.

If we realize A + B  **C + D**

Binary Collision rate in forward direction.

Binary Collision rate in reverse direction.

has 2 rate constants, we can write, assuming these are ELEMENTARY reaction steps:

$k_f[A]_e[B]_e = k_r[C]_e[D]_e$ **(Equilibrium condition)**

Where $[A]_e$ etc. are the equilibrium concentrations of [A] etc.

$k_f/k_r = [C]_e[D]_e / [A]_e[B]_e = K_{equilibrium}$