Kinetics study of proteins

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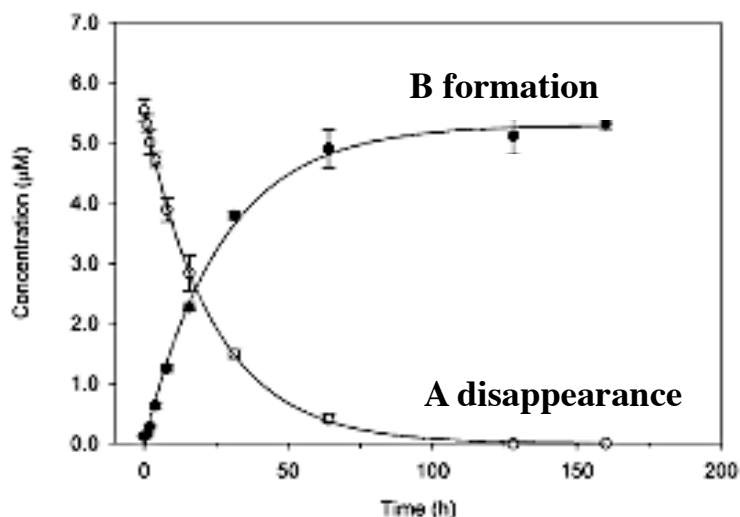**Department Of Applied Chemistry**

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Why do we study kinetics for the biological system

- We study the speed of the proteins-protein interactions.

- Proteins as molecules have kinetics and different reaction rates.

- So, therefore, we study the kinetics of the proteins by measuring the speed of the reactions during the interactions.

- How? What are based on?

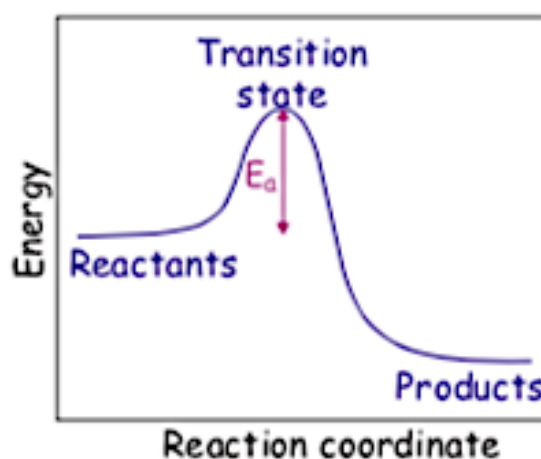- It is based on the formation of Product or disappearance of reactants.

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What happened with reaction progress?
Formation of Product or disappearance of reactants

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What information do we get from kinetics?

- Uni-molecular - dissociation or isomerisation

- Bi-molecular - reactive collisions between two atoms, molecules or ions·

- All involve a transition between two atomic or molecular states separated by a potential energy barrier.

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What is Rate (speed)?

- **What is rate, rate constant?**

- In physics:

- Rate= distance/ time. So, the rate can be:

- Instantaneous rate: a change of concentration at any particular time.

- Average rate: a change in measured concentration in any particular time.

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Rate law

- The rate is proportional to concentrations.

- Example: The rate law is v = k[A][B] where each reactant is raised to the first power.

- The coefficient k is called the rate constant.

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Rate of reaction

Consider the reaction

$$N_2 + 3H_2 \rightleftharpoons 2NH_3$$

According to our current definition, we could define the rate of reaction in terms of the rate of change of concentration of N_2, H_2 or NH_3.

If the rate of change of $[N_2]$ is x, then we have

$$-\frac{d[N_2]}{dt} = x \text{ mol dm}^{-3} \text{ s}^{-1}.$$
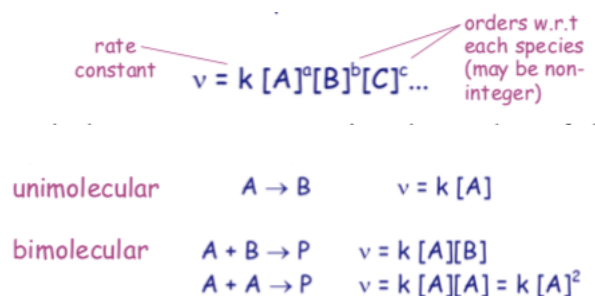
Three different rates for the same reaction!

To solve this problem, we define the reaction rate as the rate of change of a reactant or product concentration divided by its stoichiometric coefficient.

$$-\frac{d[H_2]}{dt} = 3x \text{ mol dm}^{-3} \text{ s}^{-1}$$

$$\frac{d[NH_3]}{dt} = 2x \text{ mol dm}^{-3} \text{ s}^{-1}$$

$$v = -\frac{d[N_2]}{dt} = -\frac{1}{3}\frac{d[H_2]}{dt} = \frac{1}{2}\frac{d[NH_3]}{dt}$$

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Many reactions follow a simple rate law

- Many reactions follow a simple rate law...
- ''in which the rate is proportional to the product of the concentrations raised to some power''.
- The overall order is the sum of the individual orders.

rate constant

orders w.r.t each species (may be non-integer)

$$v = k\,[A]^a[B]^b[C]^c...$$

unimolecular	$A \rightarrow B$	$v = k\,[A]$
bimolecular	$A + B \rightarrow P$	$v = k\,[A][B]$
	$A + A \rightarrow P$	$v = k\,[A][A] = k\,[A]^2$

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Reaction velocities

- The law of mass action: the rate is proportional to concentration.

- **k is the rate of constant.**

- $\frac{\Delta[A]}{\Delta t} = \frac{d[A]}{dt}$.   Therefore, rate of a change A= -k [A].

- $\frac{d[P]}{dt} = -\frac{d[A]}{dt} = kt$.

- To drive the equation of the first order:

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Units of the rate constant

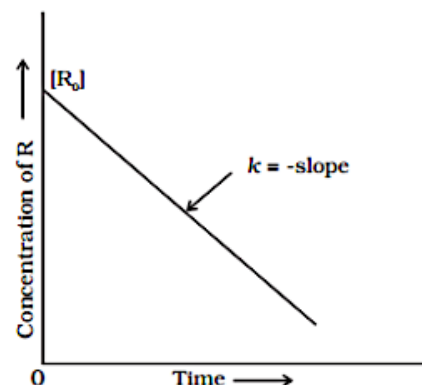- The units of rate constant are always such that they convert into a rate expressed as concentration divided by time.

$$Rate = k [A] [B]$$
$$(mol\ L^{-1}\ s^{-1}) = (L\ mol^{-1}\ s^{-1})(mol\ L^{-1})(mol\ L^{-1})$$

$$Rate = k [A]^2 [B]$$
$$(mol\ L^{-1}\ s^{-1}) = (L^2\ mol^{-2}\ s^{-1})(mol^2\ L^{-2})(mol\ L^{-1})$$
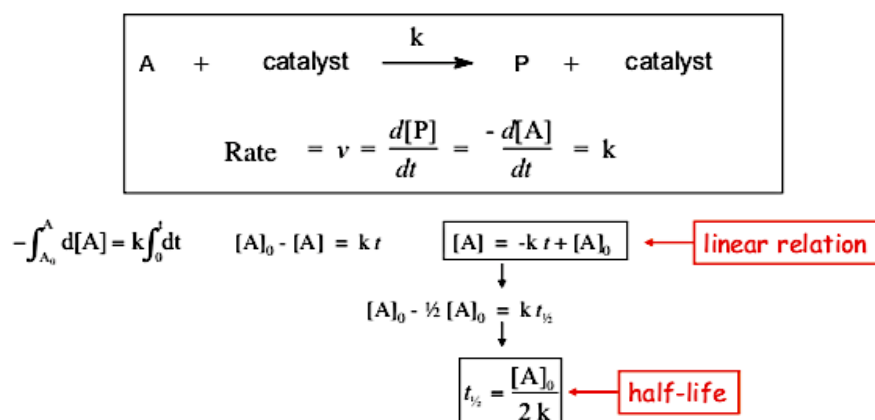
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Describe the Reaction orders?

- There are different orders of the reactions depending on the number of the reactants and their concentrations:

- What is the **Zero order reaction?**

- The rate is entirely independent of concentration of reactants.

- Thus, rate is constant.

- Rate $= k \, [A]$

- Rate $= k$.

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Example of 0 order?

- In the presence of a catalyst and a large excess of reactant, the rate of a reaction can appear to be constant

$$A \quad + \quad catalyst \xrightarrow{\ k\ } P \quad + \quad catalyst$$

$$Rate \quad = v = \frac{d[P]}{dt} = \frac{-d[A]}{dt} = k$$

$$-\int_{A_0}^{A} d[A] = k \int_0^t dt \qquad [A]_0 - [A] = k\,t \qquad \boxed{[A] = -k\,t + [A]_0} \longleftarrow \boxed{\text{linear relation}}$$

$$[A]_0 - \tfrac{1}{2}[A]_0 = k\,t_{\frac{1}{2}}$$

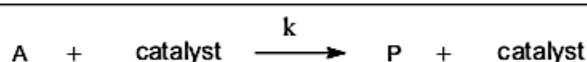$$\boxed{t_{\frac{1}{2}} = \frac{[A]_0}{2k}} \longleftarrow \boxed{\text{half-life}}$$

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Describe the First order reaction?

- Rate of reaction depends on the concentration of reactants (not linear).

- As the reaction processed, the concentration changes, and the reaction becomes slower.

- The reaction it is irreversible.

- We can monitor the disappearance of the [A] or the formation of [B] as a function of time.

- But, practically, we want to determine the rate constant of the product formation.

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Integration of rate of reaction

$$A \quad + \quad catalyst \quad \xrightarrow{k} \quad P \quad + \quad catalyst$$

$$Rate \quad = v = \frac{d[P]}{dt} = \frac{-d[A]}{dt} = k$$

$$Rate = \frac{-d[A]}{dt} = k[A]$$

$$\frac{-d[A]}{dt} = k[A]$$

$$\frac{-d[A]}{[A]} = kdt$$

$$\int_{A_0}^{A_t} \frac{-d[A]}{[A]} = \int_0^t kdt$$

$$\int_{A_0}^{A_t} -d\ln[A] = kt$$

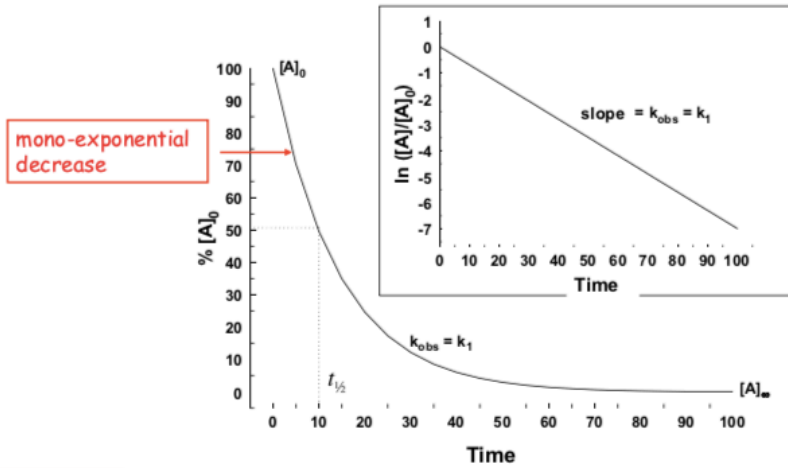$$\ln[A_0] - \ln[A_t] = kt$$

$$\ln[A_t] = -kt + \ln[A_0]$$

For exponential fitting we can use:

$$A = A_0\, e^{-k1t}$$

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The

From curve to linear

First order (simple)

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What is Half-life?

Half of life is a time scale, on which the initial population that is decreased by half ½ of its original value.

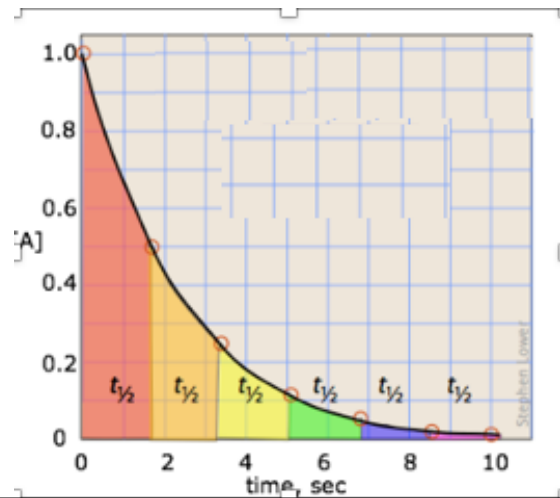$$\ln[A] = -kt + \ln[A]_0$$

$$\ln\frac{[A]_0}{2} = -kt + \ln[A]_0$$

$$\ln[A]_0 - \ln 2 = -kt + \ln[A]_0$$

$$\ln[A]_0 - \ln[A]_0 - \ln 2 = -kt$$

$$-\ln 2 = -kt$$

$$\ln 2 = kt$$
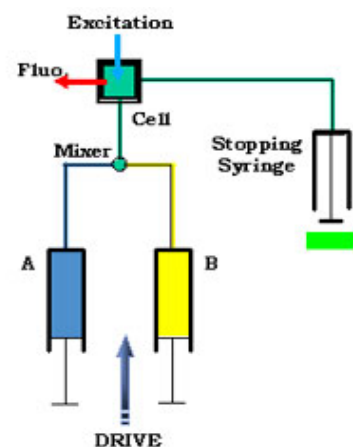
$$t_{1/2} = \frac{\ln 2}{k}$$

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Which reaction order we do use for biochemical reactions?: Pseudo-first order

• **Pseudo-first order: or Semi-second order.**

• In the case, one of the reactants is much larger than other. It is irreversible.

• Therefore: **A + B** $\longrightarrow$ **P**

• **where B is very large.**

• And therefore: **$K_{obs} = k_1$**

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What is Stopped flow machine ?

• Stopped flow instrument is a very rapid mixing device used to study chemical kinetics of fast reactions in solution.

• It computes the initial rate of reaction when two reactants held in separate cylinders which are prevented from freely flowing by syringe pump.

• The reactants are monitored by observing the change in absorbance or (Fluorescence) of the reaction as a function of time (it measures UV/vis in the same time).
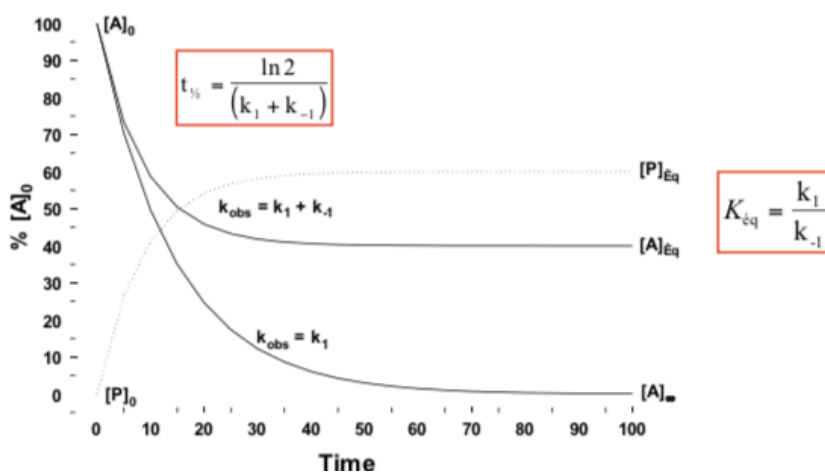

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Association and the dissociation from rate constant

- **Also,**

- **K_{+1} is k Forward k_f or k_{on}**

- **K_{-1} is k reverse k_r or k_{off}.**

- **$K_d = K_{off}/ k_{on}$ ....dissociation of the product.**

- **$K_a = K_{on}/ k_{off}$ ...... association of the product.**

- **NOTES:**

- Measuring the association and the dissociation is very informative.
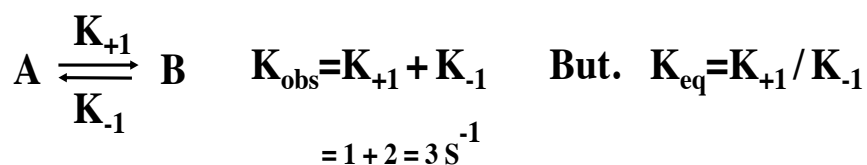
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Kinetics and equilibrium

First order (reversible)

$$t_{\frac{1}{2}} = \frac{\ln 2}{\left(k_1 + k_{-1}\right)}$$

$$k_{obs} = k_1 + k_{-1}$$

$$k_{obs} = k_1$$

$$K_{eq} = \frac{k_1}{k_{-1}}$$

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What do we measure ?

- We measure the amounts of product over time and then we plot and fit the data with equations: **$A=A° * e^{-kt}$ which comes from an exponential model: $y=A*e^{Bx}$.**

- Thus, for **$A=A° * e^{-kt}$, k_{obs} will be used; $A=A° * e^{-kobs * t}$**

$$A \underset{K_{-1}}{\overset{K_{+1}}{\rightleftharpoons}} B \qquad K_{obs}=K_{+1}+K_{-1} \qquad But. \quad K_{eq}=K_{+1}/K_{-1}$$

$$= 1 + 2 = 3 \, S^{-1}$$

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So, we measure first K1 and K-1

- We use Pseudo-first order or called semi second order

- Since the 2nd order reaction depends on both reactants, however, if the concentration of one reactant is in large excess over the other one (the reaction is considered as pseudo 1st or semi 2nd):

- **when [B] >> [A], therefore [B] is almost constant.**

- **A + B $\longrightarrow$ AB**

- **$A=A° * e^{-kobs \, t} = A=A° * e^{(-K+1 *[B] *t)}$**

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Approach to equilibrium

- **Equilibrium:** It is a condition in which all influences actions cancel each other. $aA + bB \rightleftharpoons cC + dD$

- Also, it is a state in which both reactants and products are present in concentrations which have no further tendency to change with time.

- The rate of forward process= rate of reverse process.

- **The equilibrium constant Keq** $K = \dfrac{[C]^c[D]^d}{[A]^a[B]^b}$ $\dfrac{products}{reactants}$

- It is the ratio between amounts of reactants and the products for particular chemical reaction used to calculate behaver.
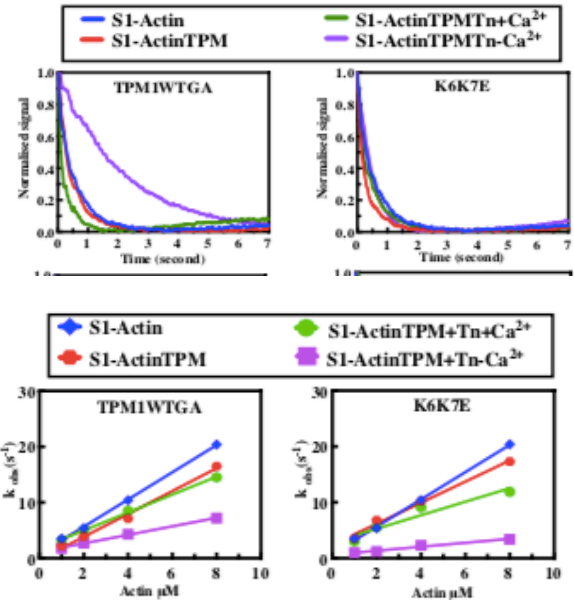
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What is the relationship between Keq and kobs?

- $Keq = \dfrac{kf}{kr}$ **or** $k\dfrac{forward}{kreverse}$ **or** $= k_{+1}/k_{-1}$.

- **We have measured K_B between blocked and closed states.**

- If KB > 1 that means the product is dominant.

- If KB < 1 that means the reactant is dominant.

- if KB =1 then 50 % of the reactant is dominant.

- **50% = 50%**

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KB and k obs :Muscle system as example

- Actin + myosin +

- Tropomyosin TPM +Troponin Tn

- We calculate k obs from curves.

- Then we calculate KB from kobs.

Table 4.5 Effect of TPM mutants on K_B parameter.

The table shows the summary of the calculation of the K_B parameter which represents the equilibrium between blocked and closed stated in the filament regulation in both ($\pm Ca^{2+}$).

TPM	K_B (+Ca^{2+})	K_B (-Ca^{2+})	K_B (TPM)
TPM1WTGA	1.92 ± 0.08	0.59 ± 0.04	3.88 ± 0.15
K6K7E	1.73 ± 0.26	0.21 ± 0.05	2.73 ± 0.14

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Calculate ?

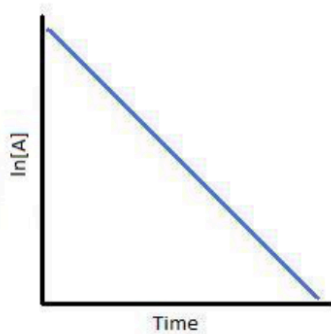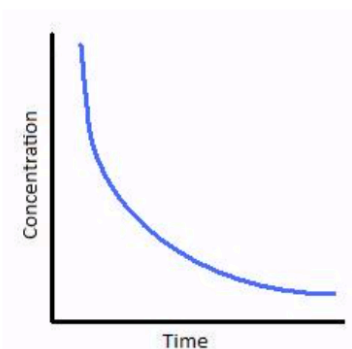- The half-life for 238U is 4.5 x 10^9 years. What is the rate constant for radioactive decay of 238U?.

  A. 1.5 x 10-10 year^{-1}

- B. 7.5 x 10-9 year^{-1}

- C. 2.25 x 109 year^{-1}
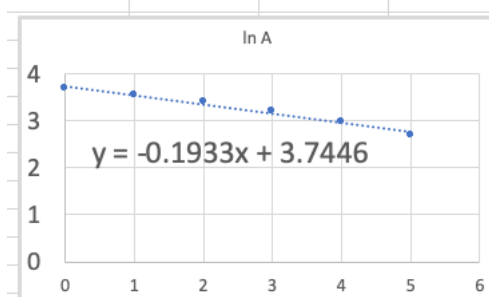
- D. 1.5 x 10-9 year^{-1}

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Graphing First-order Reactions from curve to linear

Plotting $\ln[A]$ with respect to time for a first-order reaction gives a straight line with the slope of the line equal to $-k$.

Time	First order	ln A
0	40	3.688879454
1	35	3.555348061
2	30	3.401197382
3	25	3.218875825
4	20	2.995732274
5	15	2.708050201

$y = -0.1933x + 3.7446$

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Example for pseudo order

- If a 2nd order reaction has the rate equation $R = k[A][B]$, and the rate constant, k, is $3.67M^{-1}s^{-1}$, [A] is 4.5M and [B] is 99M, what is the rate constant of its pseudo-1st-order reaction?

- Because [B] is in excess we multiply 99M with $3.67M^{-1}s^{-1}$

- $(99M)(3.67M^{-1}s^{-1}) = 363.33s^{-1}$

- https://chem.libretexts.org/Bookshelves/Physical_and_Theoretical_Chemistry_Textbook_Maps/Supplemental_Modules_(Physical_and_Theoretical_Chemistry)/Kinetics/Reaction_Rates/Second-Order_Reactions/Pseudo-1st-order_reactions

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units ?

- What are the units of k in vA = k[A]? A. mol L^{-1} s^{-1}

- B. L mol-1 s^{-1}

- C. mol L^{-1}

- D. s^{-1}

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Example

The following data were obtained for the reaction 3 A → 2 B:
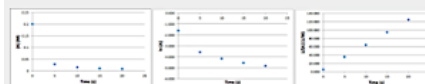
Time, s	0	5	10	15	20
[A], M	0.200	0.0282	0.0156	0.0106	0.008

Determine the order of the reaction.

Solution

We can plot the characteristic kinetic plots of zero-, first-, and second-order reactions to determine which will give a straight line.

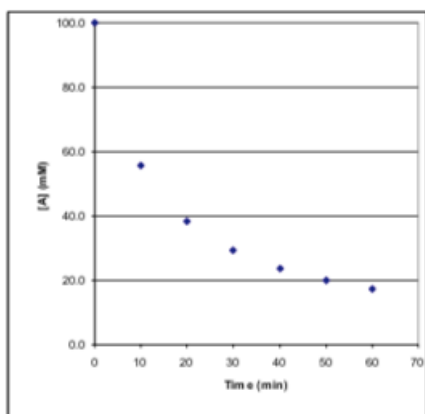| Time, s	[A], mol L^{-1}	ln [A]	1/[A], L mol^{-1}
0	0.200	-1.61	5.00
5	0.0282	-3.57	35.5
10	0.0156	-4.16	64.1
15	0.0106	-4.55	94.3
20	0.008	-4.83	125

Example Kinetics Plots

The reaction is second order since 1/[A]$_t$ versus t gives a straight line.

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Exercises

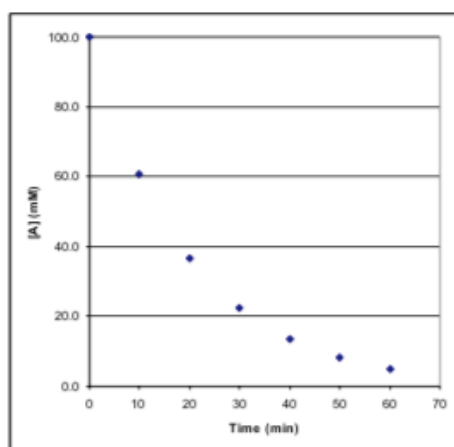- use conc *vs* time data to determine order of reaction and its rate constant

Time (min)	[A] (mM)
0	100.0
10	55.6
20	38.5
30	29.4
40	23.8
50	20.0
60	17.2

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Exercises

- use conc *vs* time data to determine order of reaction and its rate constant

Time (min)	[A] (mM)
0	100.0
10	60.7
20	36.8
30	22.3
40	13.5
50	8.2
60	5.0

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Question ?

- A first order process occurs with a rate constant of 0.1 s^{-1}. If the initial concentration $[A]_0 = 1$ M what is the concentration after 30 seconds?

  A. 0.5 M

- <mark>B. 0.05 M</mark>

- C. 0.005 M

- D. 0.0005 M

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