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Enzyme Kinetics

Experimental Kinetics

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Outline: Experimental kinetics

- rate *vs* rate constant
- rate laws and molecularity
- practical kinetics
- kinetic analyses (simple equations)
    - first order
    - second order
    - pseudo-first order
- kinetic analyses (complex reactions)
    - consecutive first order reactions
    - steady state
    - changes in kinetic order
    - saturation kinetics
    - rapid pre-equilibrium

see A&D sections 7.4-7.5 for review

Rate *vs* rate constant

- the **reaction rate** depends on the activation barrier of the global reaction and the concentration of reactants, according to **rate law** for the reaction
  - e.g. $v = k[A]$
- the proportionality constant, k, is called the **rate constant**

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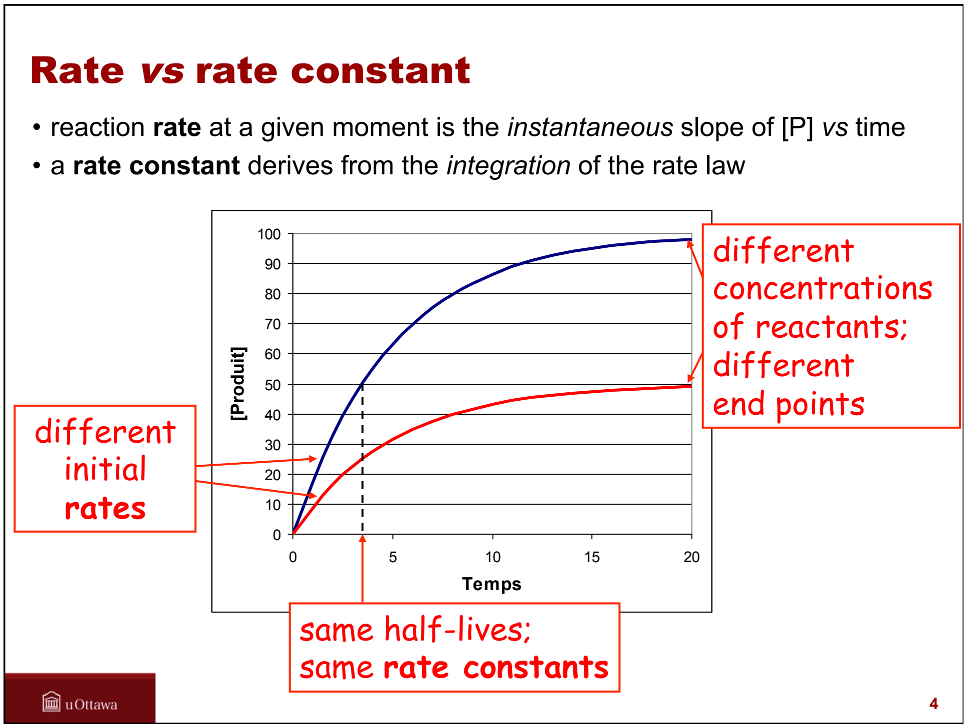

Rate *vs* rate constant

- reaction **rate** at a given moment is the *instantaneous* slope of [P] *vs* time
- a **rate constant** derives from the *integration* of the rate law

different concentrations of reactants; different end points

different initial **rates**

same half-lives; same **rate constants**

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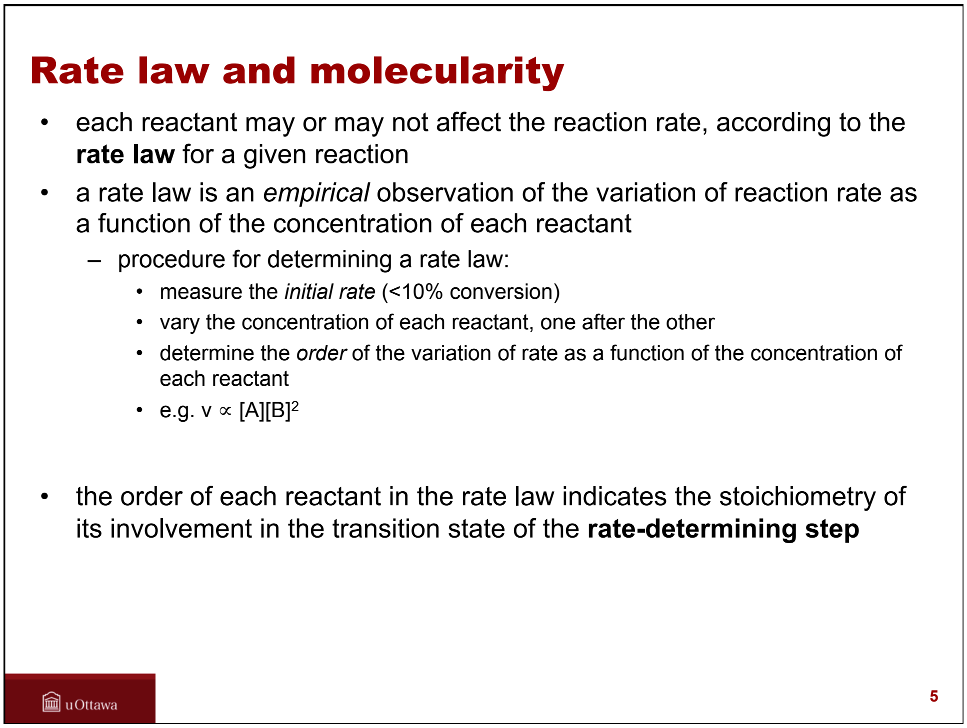
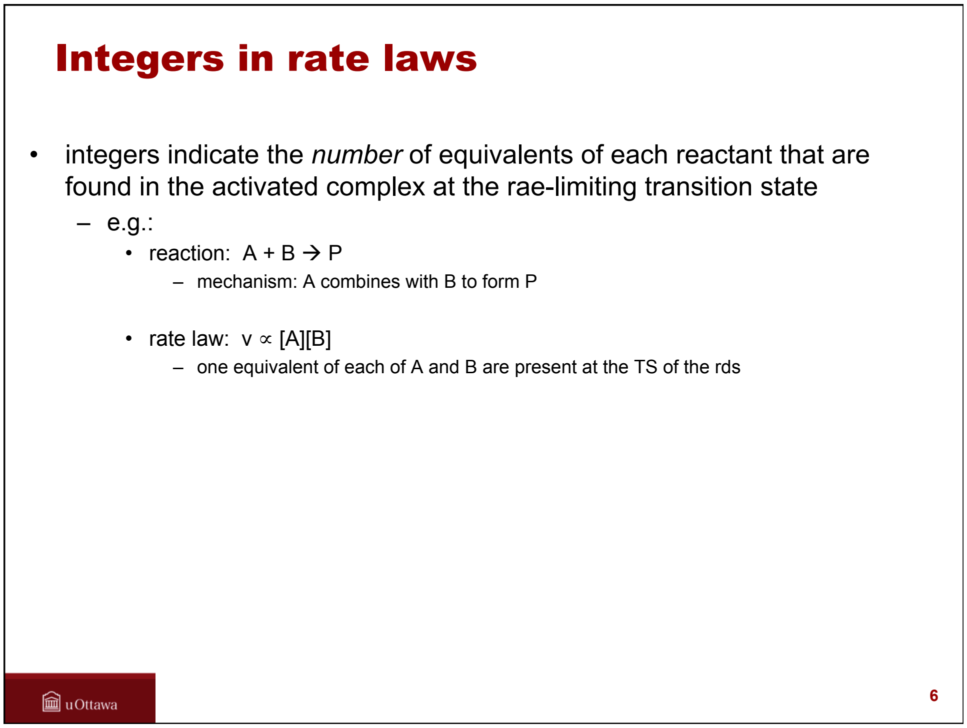


Rate law and molecularity

- each reactant may or may not affect the reaction rate, according to the **rate law** for a given reaction
- a rate law is an *empirical* observation of the variation of reaction rate as a function of the concentration of each reactant
  - procedure for determining a rate law:
    - measure the *initial rate* (<10% conversion)
    - vary the concentration of each reactant, one after the other
    - determine the *order* of the variation of rate as a function of the concentration of each reactant
    - e.g. $v \propto [A][B]^2$
- the order of each reactant in the rate law indicates the stoichiometry of its involvement in the transition state of the **rate-determining step**



Integers in rate laws

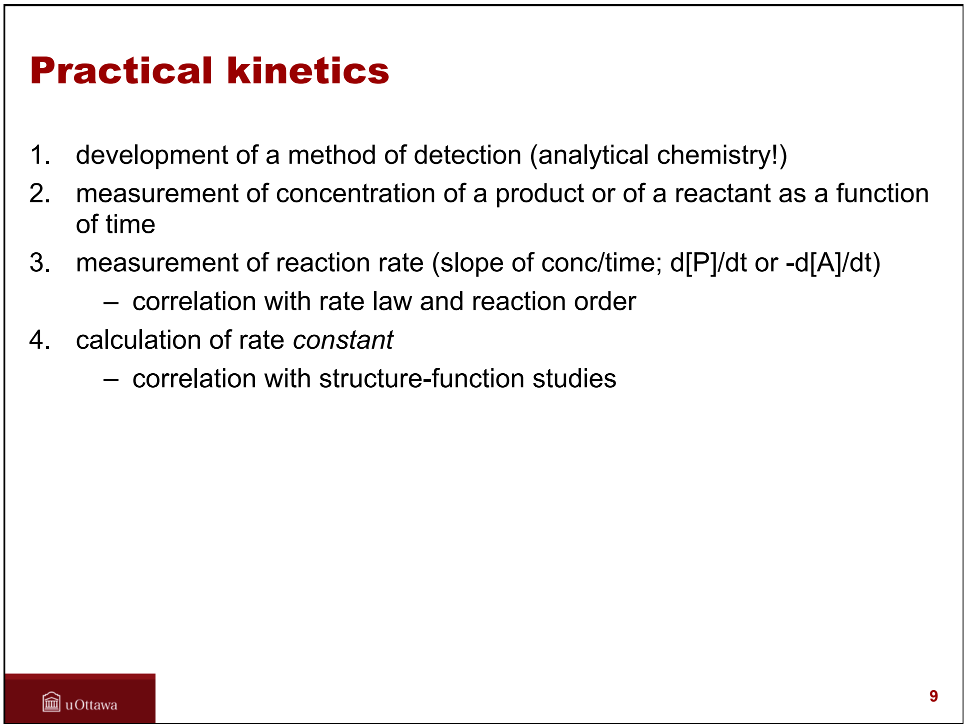
- integers indicate the *number* of equivalents of each reactant that are found in the activated complex at the rae-limiting transition state
  - e.g.:
    - reaction: $A + B \rightarrow P$
      - mechanism: A combines with B to form P
    - rate law: $v \propto [A][B]$
      - one equivalent of each of A and B are present at the TS of the rds

Fractions in rate laws

- fractions signify the *dissociation* of a complex of reactants, leading up to the rds:
  - e.g. :
    - elementary reactions: $A \rightarrow B + B$; $B + C \rightarrow P$
      - mechanism: reactant A exists in the form of a dimer that must dissociate before reacting with C to form P
    - rate law: $v \propto [A]^{1/2}[C]$
      - true rate law is $v \propto [B][C]$, but B comes from the dissociation of dimer A
      - observed rate law, written in terms of reactants A and C, reflects the dissociation of A
      - *it is therefore very important to know the nature of reactants in solution!*

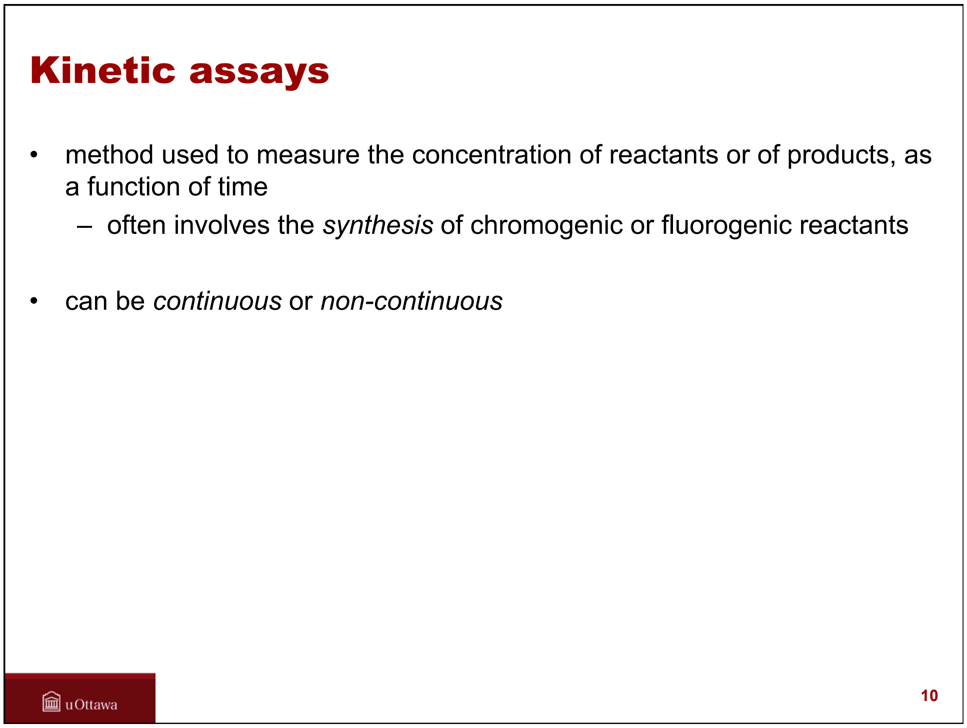
Negative integers in rate laws

- negative integers indicate the presence of an equilibrium that provides a reactive species:
  - e.g. :
    - elementary reactions: $A \rightleftharpoons B + C$; $B + D \rightarrow P$
      - mechanism: A dissociates to give B and C, before B reacts with D to give P
    - rate law: $v \propto [A][C]^{-1}[D]$
      - true rate law is $v \propto [B][D]$, but B comes from the dissociation of A
      - observed rate law, written in terms of reactants A and D, reflects the dissociation of A
      - apparent inhibition by C reflects the displacement of the initial equilibrium



Practical kinetics

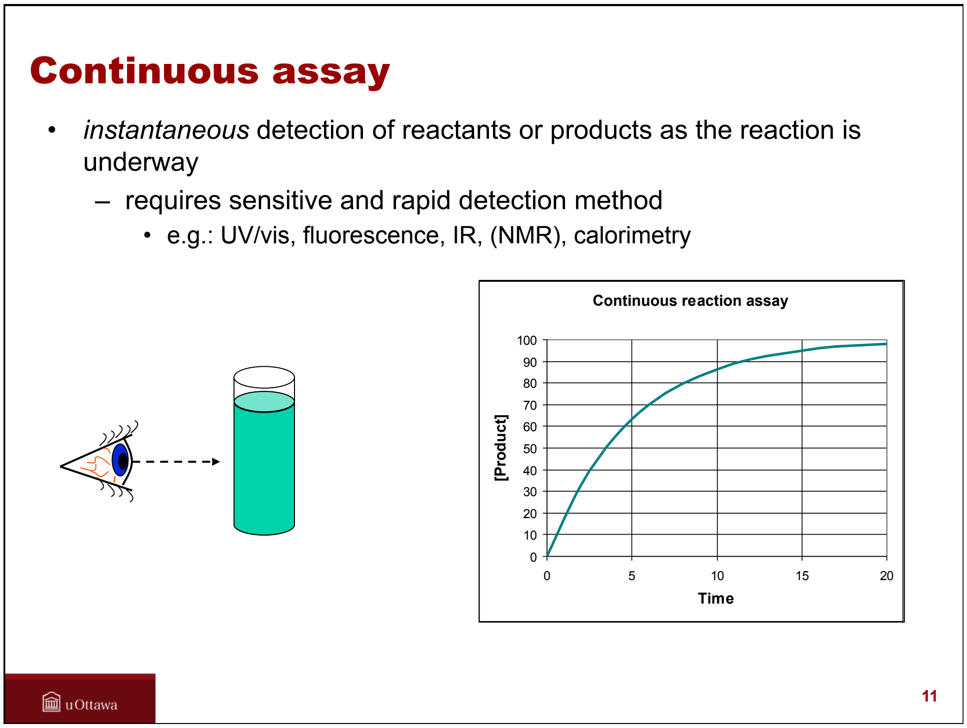
1. development of a method of detection (analytical chemistry!)
2. measurement of concentration of a product or of a reactant as a function of time
3. measurement of reaction rate (slope of conc/time; d[P]/dt or -d[A]/dt)
    - correlation with rate law and reaction order
4. calculation of rate *constant*
    - correlation with structure-function studies



Kinetic assays

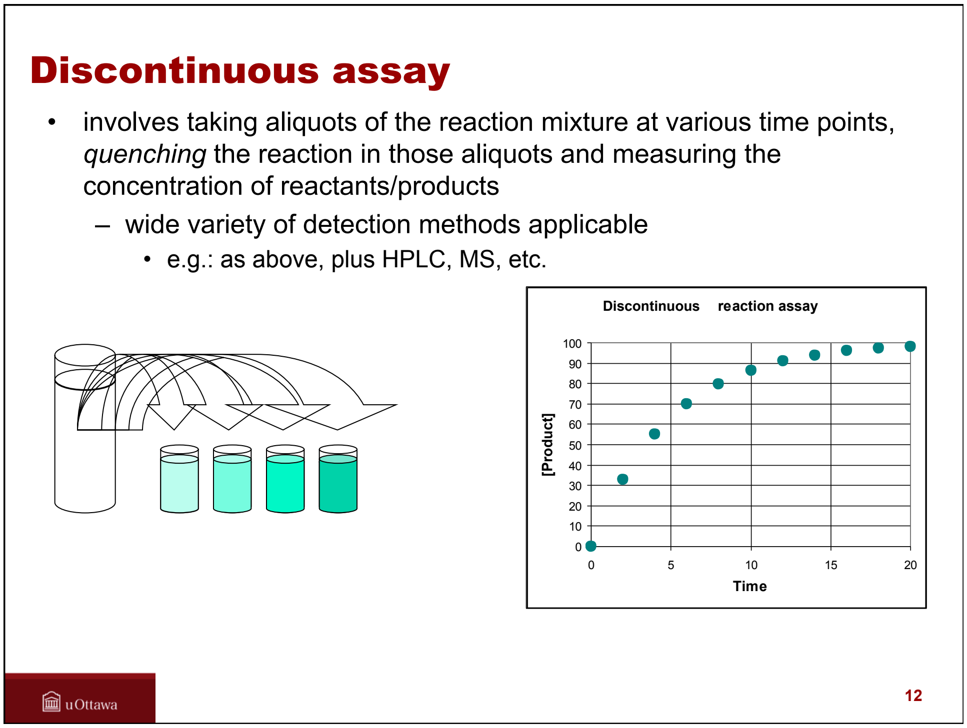
- method used to measure the concentration of reactants or of products, as a function of time
    - often involves the *synthesis* of chromogenic or fluorogenic reactants

- can be *continuous* or *non-continuous*



Continuous assay

- *instantaneous* detection of reactants or products as the reaction is underway
  - – requires sensitive and rapid detection method
    - e.g.: UV/vis, fluorescence, IR, (NMR), calorimetry

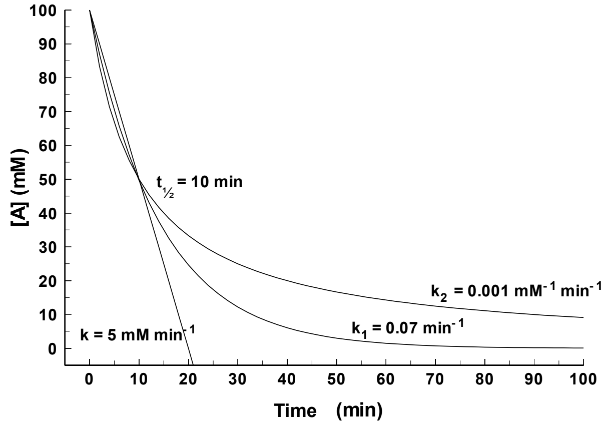


Discontinuous assay

- involves taking aliquots of the reaction mixture at various time points, *quenching* the reaction in those aliquots and measuring the concentration of reactants/products
  - – wide variety of detection methods applicable
    - e.g.: as above, plus HPLC, MS, etc.

Initial rates

- the first ~10 % of a reaction is almost linear, regardless of the order of a reaction
- ΔC *vs* Δt gives the rate, but the rate constant depends on the order of the reaction



Calculation of a rate constant

- now, how can a *rate constant*, *k*, be determined quantitatively?
- the mathematical equation to use to determine the value of *k* differs according to the order of the reaction in question
  - the equation must be **derived from a kinetic scheme**
  - next, the data can be "fitted" to the resulting equation using a computer (linear, or more likely, non-linear regression)

Outline: Kinetic analyses (simple eqns)

- first order reactions
- second order reactions
- pseudo-first order
- third order reactions
- zeroth order

The language of Nature

- *"Nul ne saurait comprendre la nature si celui-ci ne connaît son langage qui est le langage mathématique"*

  - Blaise Pascal
  - ('None can understand Nature if one does not know its language, which is the language of mathematics')
- Natural order is revealed through special mathematical relationships
- mathematics are *our attempt to understand Nature*
  - exponential increase: the value of e
  - volume of spherical forms: the value of π

Blaise Pascal, French mathematician and philosopher, 1623-1662

First order (simple)

$$A \xrightarrow{k_1} P$$

$$\text{Rate} = v = \frac{d[P]}{dt} = -\frac{d[A]}{dt} = k_1[A]$$

$$\frac{d[P]}{dt} = k_1([A]_0 - [P]) \qquad \frac{d[P]}{dt} = k_1([P]_\infty - [P]) \qquad \frac{d[P]}{[P]_\infty - [P]} = k_1\, dt$$

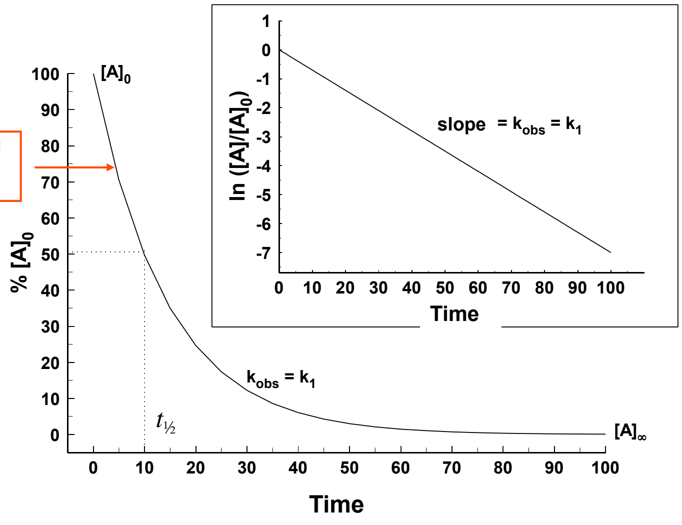
$$\int_0^P \frac{d[P]}{[P]_\infty - [P]} = k_1 \int_0^t dt \qquad ln\,([P]_\infty - [P]_0) - ln\,([P]_\infty - [P]) = k_1 t$$

linear relation → $\boxed{ln\frac{[A]_0}{[A]} = k_1 t}$ → $ln\frac{[A]_0}{\frac{1}{2}[A]_0} = k_1 t_{\frac{1}{2}}$

mono-exponential decrease → $\boxed{[A] = [A]_0 e^{-k_1 t}}$

half-life → $\boxed{t_{\frac{1}{2}} = \frac{ln\,2}{k_1}}$

First order (simple)



mono-exponential decrease

First order (reversible)

$$A \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} P$$

$$\text{Rate} = v = \frac{d[P]}{dt} = -\frac{d[A]}{dt} = k_1 [A] - k_{-1} [P]$$

$$-\frac{d[A]}{dt} = \frac{d[P]}{dt} = k_1\left([A]_{éq} + [P]_{éq} - [P]\right) - k_{-1}[P]$$
$$= k_1[A]_{éq} + k_1[P]_{éq} - \left(k_1 + k_{-1}\right)[P]$$

At equilibrium, $k_1 [A]_{éq} = k_{-1} [P]_{éq}$

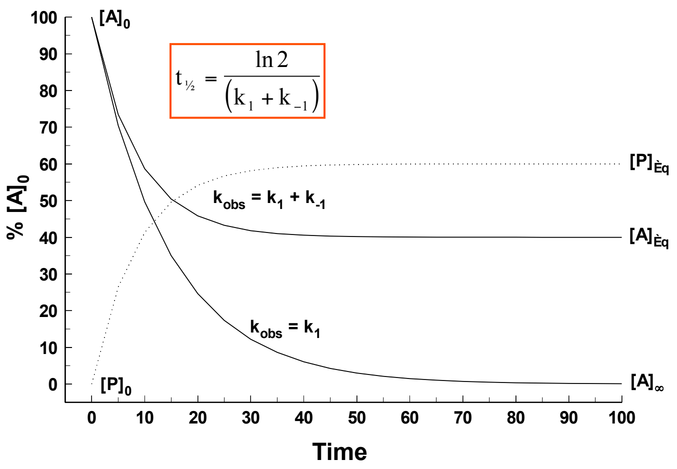
$$-\frac{d[A]}{dt} = \frac{d[P]}{dt} = k_{-1}[P]_{éq} + k_1[P]_{éq} - \left(k_1 + k_{-1}\right)[P]$$
$$= \left(k_1 + k_{-1}\right)\left([P]_{éq} - [P]\right)$$

$$\int_0^P \frac{d[P]}{[P]_{éq} - [P]} = \left(k_1 + k_{-1}\right)\int_0^t dt$$

linear relation ⟶ $$\ln\frac{\left([P]_{éq} - [P]_0\right)}{\left([P]_{éq} - [P]\right)} = \left(k_1 + k_{-1}\right)t$$

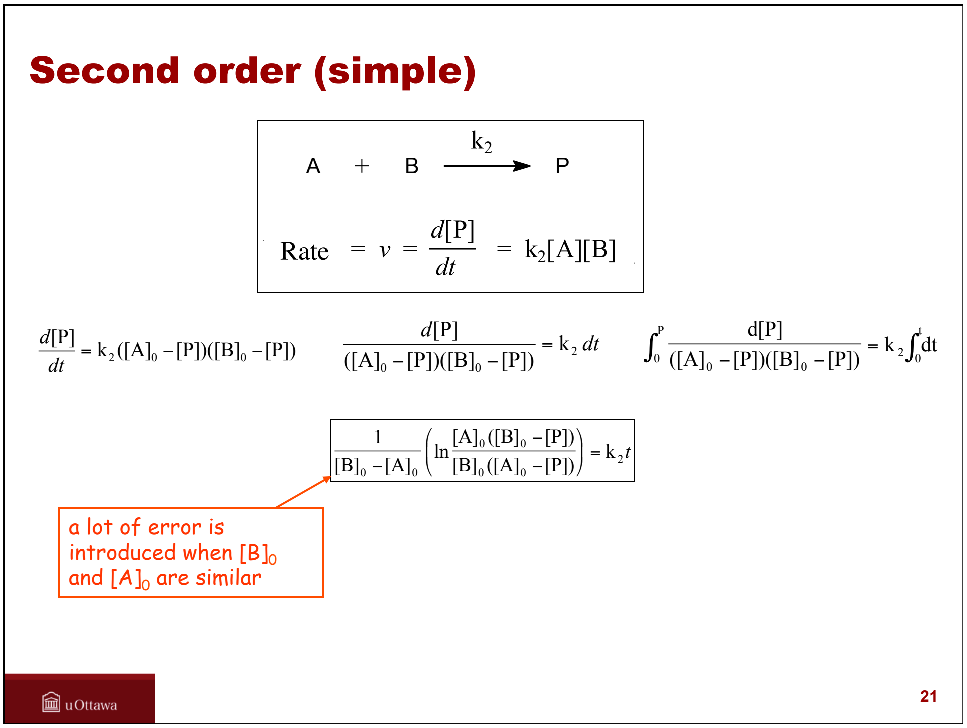
$$\boxed{k_{obs} = (k_1 + k_{-1})}$$

First order (reversible)



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

$$K_{éq} = \frac{k_1}{k_{-1}}$$



Second order (simple)

$$\boxed{\begin{array}{c} A + B \xrightarrow{k_2} P \\ \text{Rate} = v = \dfrac{d[P]}{dt} = k_2[A][B] \end{array}}$$

$$\frac{d[P]}{dt} = k_2([A]_0 - [P])([B]_0 - [P]) \qquad \frac{d[P]}{([A]_0 - [P])([B]_0 - [P])} = k_2\, dt \qquad \int_0^P \frac{d[P]}{([A]_0 - [P])([B]_0 - [P])} = k_2 \int_0^t dt$$

$$\boxed{\frac{1}{[B]_0 - [A]_0}\left(\ln \frac{[A]_0([B]_0 - [P])}{[B]_0([A]_0 - [P])}\right) = k_2 t}$$

a lot of error is introduced when $[B]_0$ and $[A]_0$ are similar

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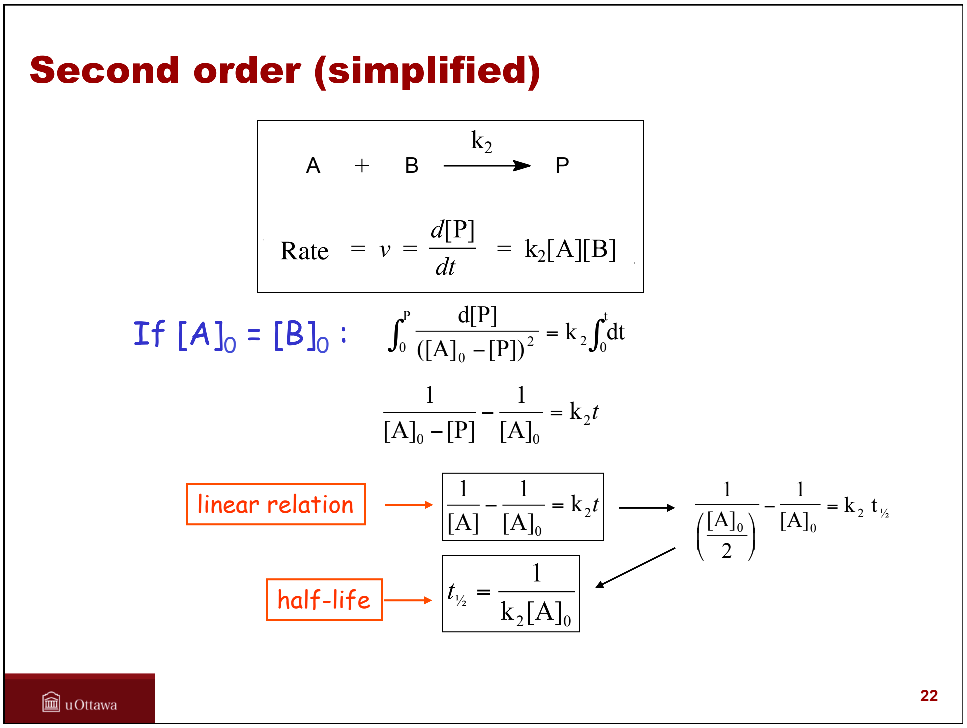

Second order (simplified)

$$\boxed{\begin{array}{c} A + B \xrightarrow{k_2} P \\ \text{Rate} = v = \dfrac{d[P]}{dt} = k_2[A][B] \end{array}}$$

If $[A]_0 = [B]_0$:

$$\int_0^P \frac{d[P]}{([A]_0 - [P])^2} = k_2 \int_0^t dt$$

$$\frac{1}{[A]_0 - [P]} - \frac{1}{[A]_0} = k_2 t$$

linear relation →

$$\boxed{\frac{1}{[A]} - \frac{1}{[A]_0} = k_2 t} \longrightarrow \frac{1}{\left(\frac{[A]_0}{2}\right)} - \frac{1}{[A]_0} = k_2\, t_{1/2}$$

half-life →

$$\boxed{t_{1/2} = \frac{1}{k_2[A]_0}}$$

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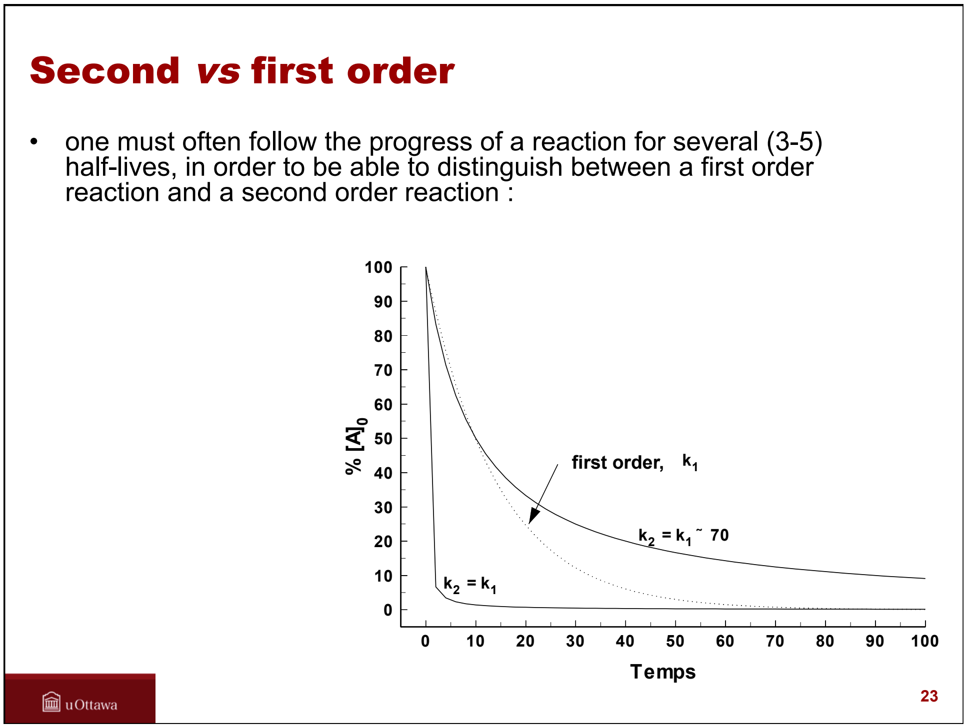
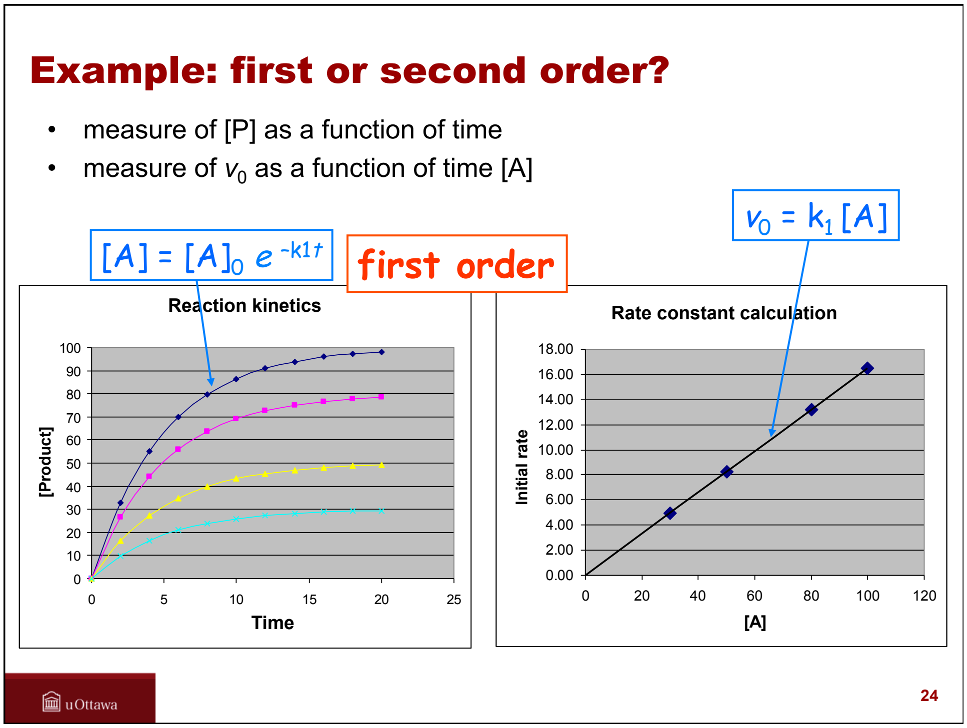


Second *vs* first order

- one must often follow the progress of a reaction for several (3-5) half-lives, in order to be able to distinguish between a first order reaction and a second order reaction :



Example: first or second order?

- measure of [P] as a function of time
- measure of v_0 as a function of time [A]

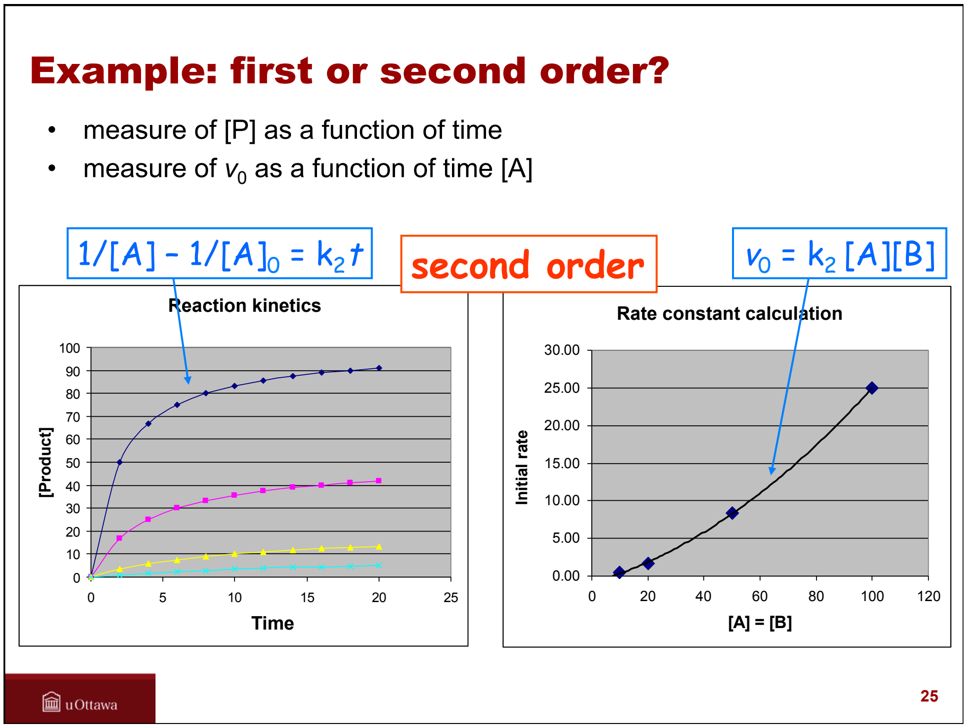
$[A] = [A]_0\, e^{-k1t}$

first order

$v_0 = k_1\,[A]$

Reaction kinetics

Rate constant calculation



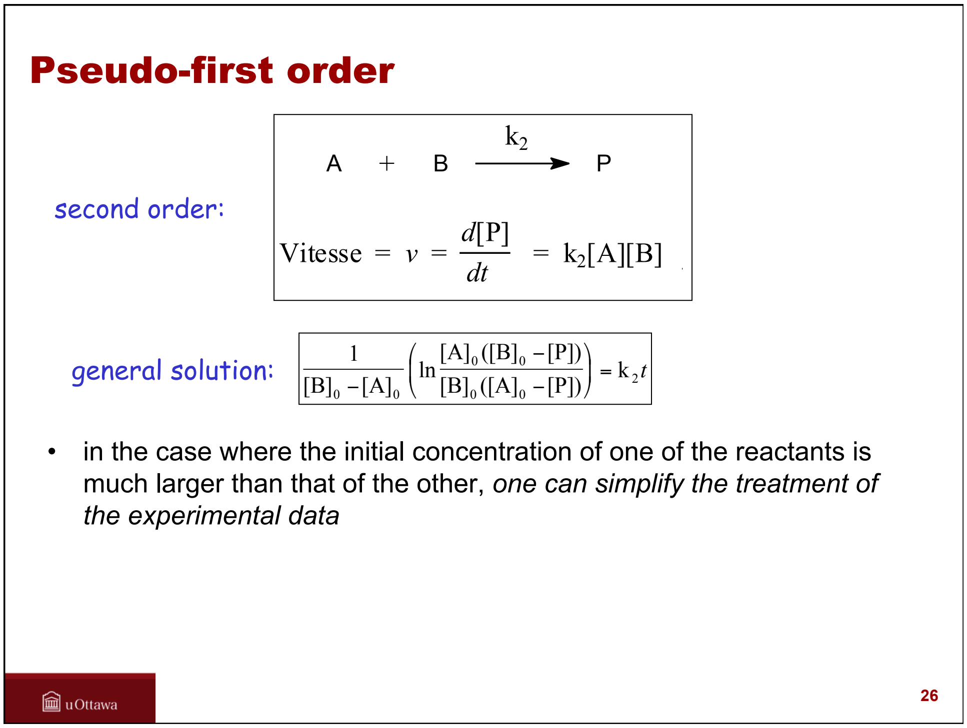
Example: first or second order?

- measure of [P] as a function of time
- measure of v_0 as a function of time [A]

$1/[A] - 1/[A]_0 = k_2 t$

second order

$v_0 = k_2 [A][B]$



Pseudo-first order

second order:

$$A + B \xrightarrow{k_2} P$$

$$\text{Vitesse} = v = \frac{d[P]}{dt} = k_2[A][B]$$

general solution:

$$\frac{1}{[B]_0-[A]_0}\left(\ln\frac{[A]_0([B]_0-[P])}{[B]_0([A]_0-[P])}\right) = k_2 t$$

- in the case where the initial concentration of one of the reactants is much larger than that of the other, *one can simplify the treatment of the experimental data*

Pseudo-first order

- consider the case where $[B]_0 >> [A]_0$ (>10 times larger)
  - the concentration of B will not change much over the course of the reaction; $[B] = [B]_0$ (quasi-constant)

$$\boxed{\frac{1}{[B]_0-[A]_0}\left(\ln\frac{[A]_0([B]_0-[P])}{[B]_0([A]_0-[P])}\right)=k_2t} \longrightarrow \frac{1}{[B]_0}\left(\ln\frac{[A]_0[B]_0}{[B]_0([A]_0-[P])}\right)=k_2t$$

$$ln\frac{[A]_0}{([A]_0-[P])}=k_2[B]_0t$$

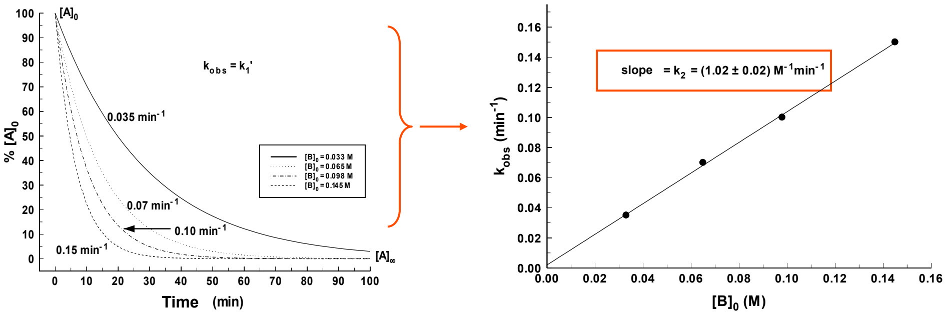
$$ln\frac{[A]_0}{([A]_0-[P])}=k_1^{'}t \quad \text{where} \quad k_1^{'}=k_2[B]_0$$

$$ln\frac{[A]_0}{[A]}=k_1^{'}t$$

mono-exponential decrease → $\boxed{[A]=[A]_0e^{-k_1^{'}t}}$

Pseudo-first order

- from a practical point of view, it is more reliable to determine a second order rate constant by measuring a series of first order rate constants as a function of the concentration of B (provided that $[B]_0 >> [A]_0$)



Third order

$$A + B + C \xrightarrow{k_3} P$$

$$\text{Rate} = v = \frac{d[P]}{dt} = k_3[A][B][C]$$

- reactions that take place in <u>one termolecular step</u> are rare in the gas phase and *do not exist in solution*
  - the entropic barrier associated with the simultaneous collision of three molecules is too high

Third order, revisited

- however, a reaction that takes place in <u>two consecutive bimolecular steps</u> (where the second step is rate-limiting) would have a third order rate law!

$$A + B \xrightarrow{k_1} AB$$

$$AB + C \xrightarrow{k_2} P$$

$$\text{Rate} = v = \frac{d[P]}{dt} = k_3[A][B][C]$$

Zeroth order

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

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

- in the presence of a catalyst (organo-metallic or enzyme, for example) and a large excess of reactant, the rate of a reaction can appear to be constant

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

linear relation

$$\downarrow$$

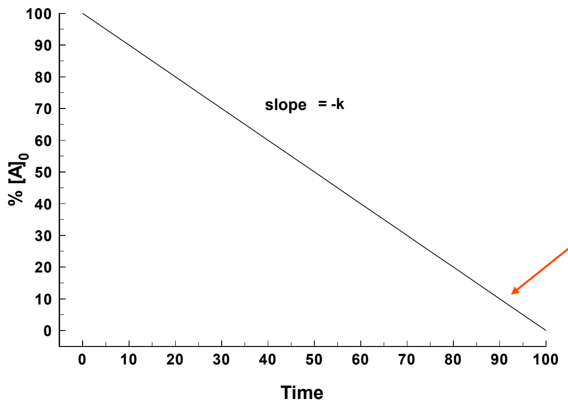
$$[A]_0 - \tfrac{1}{2}[A]_0 = k\,t_{1/2}$$

$$\downarrow$$

$$\boxed{t_{1/2} = \frac{[A]_0}{2\,k}}$$

half-life

Zeroth order



not realistic that rate would be constant all the way to end of reaction...

Summary of observed parameters

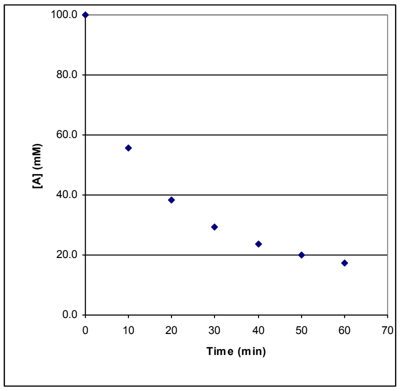
Reaction order	Rate law	Explicit equation	Linear equation	Half-life
zero	$\frac{d[P]}{dt} = k$	$[A] = -k\,t + [A]_0$	$[A]_0 - [A] = k\,t$	$t_{1/2} = \frac{[A]_0}{2\,k}$
first	$\frac{d[P]}{dt} = k_1[A]$	$[A] = [A]_0 e^{-k_1 t}$	$ln\frac{[A]_0}{[A]} = k_1\,t$	$t_{1/2} = \frac{ln 2}{k_1}$
second	$\frac{d[P]}{dt} = k_2[A]^2$	$[A] = \frac{1}{k_2 t + \frac{1}{[A]_0}}$	$\frac{1}{[A]} - \frac{1}{[A]_0} = k_2 t$	$t_{1/2} = \frac{1}{k_2[A]_0}$
	$\frac{d[P]}{dt} = k_2[A][B]$	complex	$\frac{1}{[B]_0 - [A]_0}\left(\ln\frac{[A]_0([B]_0 - [P])}{[B]_0([A]_0 - [P])}\right) = k_2 t$	complex

non-linear regression

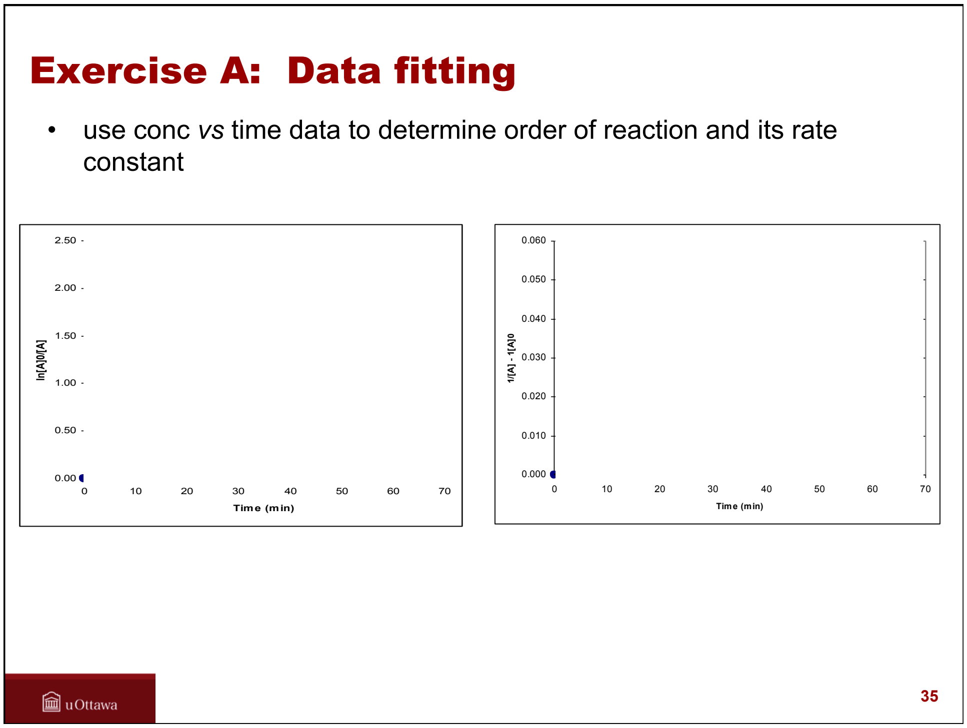
linear regression

Exercise A: Data fitting

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

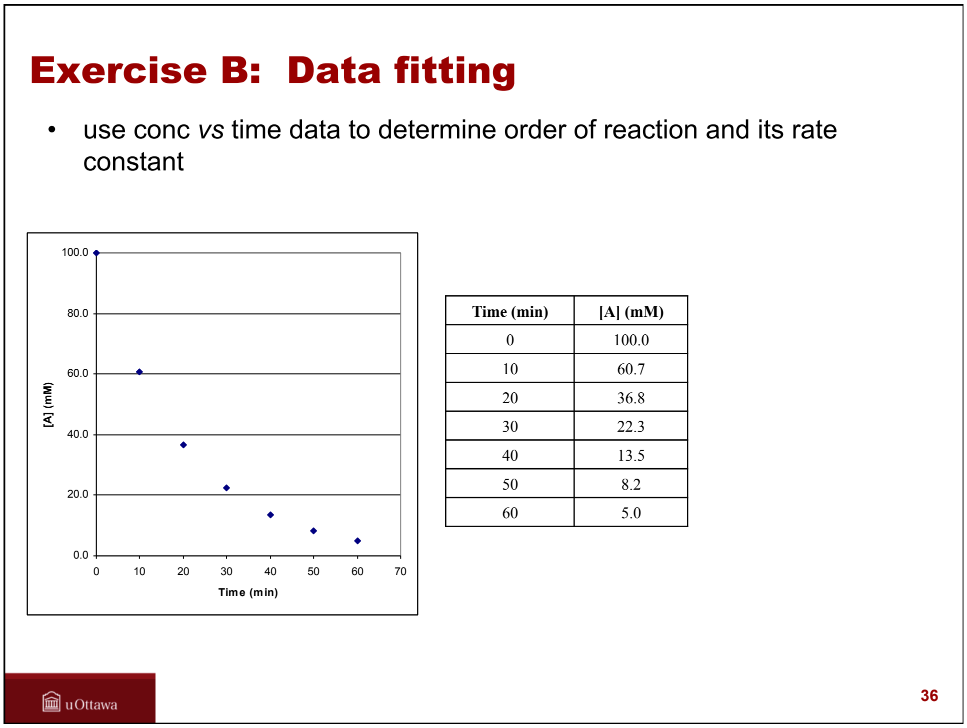


Time (min)	[A] (mM)
0	100.0
10	55.6
20	38.5
30	29.4
40	23.8
50	20.0
60	17.2



Exercise A: Data fitting

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



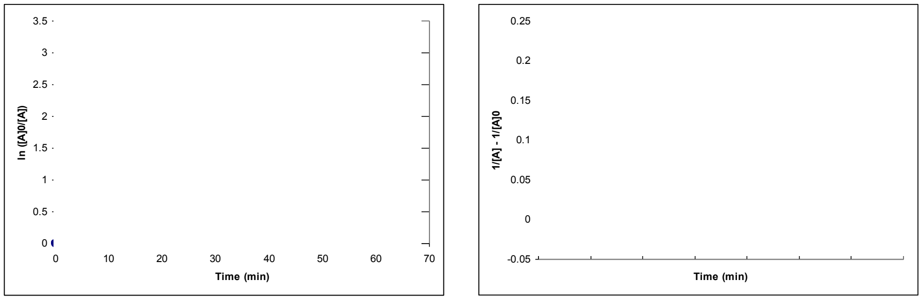
Exercise B: Data fitting

- 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

Exercise B: Data fitting

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



Outline: Kinetic analyses (complex rxns)

- consecutive first order reactions
- steady state
- changes in kinetic order
- saturation kinetics
- rapid pre-equilibrium

First order (consecutive)

$$\boxed{\begin{array}{c} A \xrightarrow{k_1} B \xrightarrow{k_2} P \\ \text{Rate} = v = \frac{d[P]}{dt} = k_2[B] \neq -\frac{d[A]}{dt} \end{array}}$$

$$-\frac{d[A]}{dt} = k_1[A] \qquad \boxed{[A] = [A]_0 e^{-k_1 t}}$$

$$\frac{d[B]}{dt} = k_1[A] - k_2[B] \qquad \frac{d[B]}{dt} = k_1[A]_0 e^{-k_1 t} - k_2[B] \qquad \frac{d[B]}{dt} + k_2[B] = k_1[A]_0 e^{-k_1 t}$$

solved by using the technique of partial derivatives

$$\boxed{[B] = \frac{k_1[A]_0}{(k_2 - k_1)}(e^{-k_1 t} - e^{-k_2 t})}$$

$$[P] = [A]_0 - [A]_0 e^{-k_1 t} - \frac{k_1[A]_0}{(k_2 - k_1)}(e^{-k_1 t} - e^{-k_2 t}) \qquad \boxed{[P] = [A]_0\left(1 - e^{-k_1 t} - \frac{k_1}{(k_2 - k_1)}(e^{-k_1 t} - e^{-k_2 t})\right)}$$

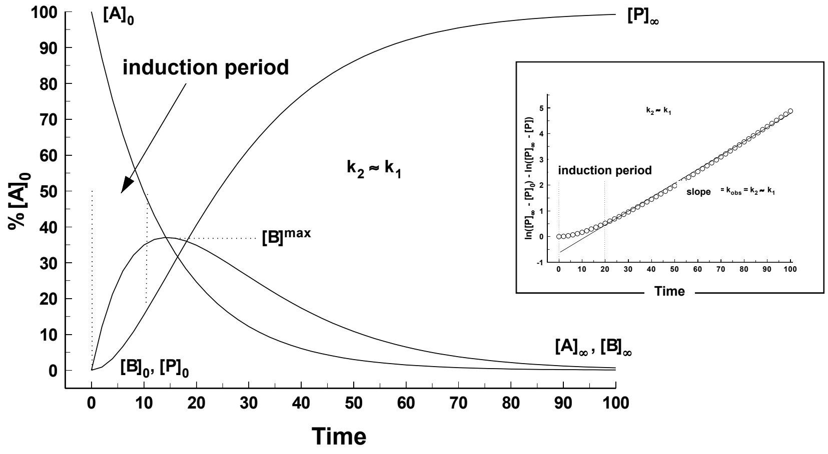
Induction period

- in consecutive reactions, there is an induction period in the production of P, during which the concentration of B increases to its maximum before decreasing

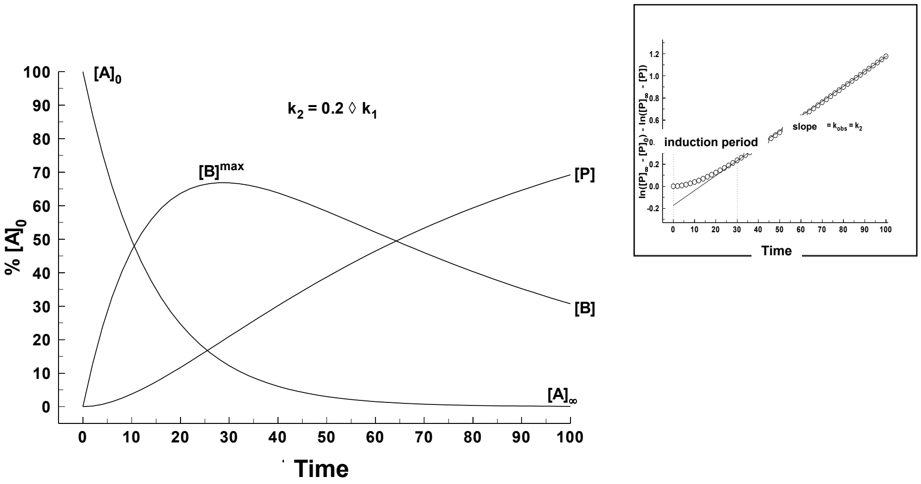
$$A \rightarrow B \rightarrow P$$

- the length of this period and the maximal concentration of B varies as a function of the relative values k_1 and k_2
  - consider three representative cases :
    - $k_1 = k_2$
    - $k_2 < k_1$
    - $k_2 > k_1$

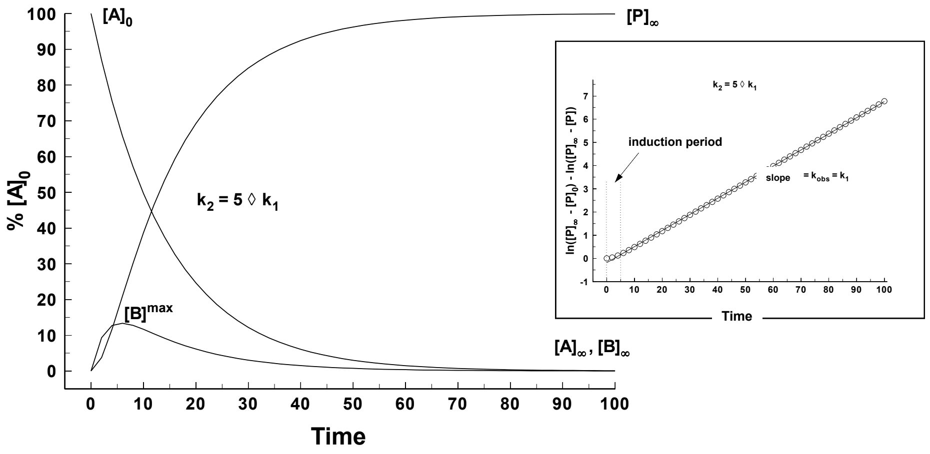
Consecutive reactions, $k_1 = k_2$



Consecutive reactions, $k_2 = 0.2 \times k_1$



Consecutive reactions, $k_2 = 5 \times k_1$



Steady state

- often multi-step reactions involve the formation of a reactive intermediate that does not accumulate but reacts as rapidly as it is formed
- the concentration of this intermediate can be treated as though it is constant
- this is called the *steady state approximation (SSA)*

Steady State Approximation

- consider a typical example (in bio-org and organometallic chem) of a two step reaction:
  - kinetic scheme: $\boxed{\mathrm{A} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \mathrm{I} \xrightarrow{k_2[\mathrm{B}]} \mathrm{P}}$
  - rate law: $\boxed{\frac{d[\mathrm{P}]}{dt} = k_2[\mathrm{I}][\mathrm{B}]}$
  - SSA : $\boxed{\frac{d[\mathrm{I}]}{dt} = k_1[\mathrm{A}] - k_{-1}[\mathrm{I}] - k_2[\mathrm{I}][\mathrm{B}] = 0}$
    - expression of [I] : $[\mathrm{I}] = \left(\frac{k_1[\mathrm{A}]}{k_{-1} + k_2[\mathrm{B}]}\right)$
  - rate equation: $\boxed{\frac{d[\mathrm{P}]}{dt} = \left(\frac{k_1 k_2[\mathrm{A}][\mathrm{B}]}{k_{-1} + k_2[\mathrm{B}]}\right)}$ ←

first order in A;
less than first order in B

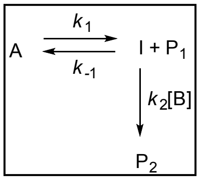
Steady State Approximation

- consider another example of a two-step reaction:
  - kinetic scheme: $\boxed{\mathrm{A} + \mathrm{B} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \mathrm{I} \xrightarrow{k_2} \mathrm{P}}$
  - rate law: $\boxed{\frac{d[\mathrm{P}]}{dt} = k_2[\mathrm{I}]}$
  - SSA: $\boxed{\frac{d[\mathrm{I}]}{dt} = k_1[\mathrm{A}][\mathrm{B}] - k_{-1}[\mathrm{I}] - k_2[\mathrm{I}] = 0}$
    - expression of [I] : $[\mathrm{I}] = \left(\frac{k_1[\mathrm{A}][\mathrm{B}]}{k_{-1} + k_2}\right)$
  - rate equation: $\boxed{\frac{d[\mathrm{P}]}{dt} = \left(\frac{k_1 k_2[\mathrm{A}][\mathrm{B}]}{k_{-1} + k_2}\right) = k_{\mathrm{obs}}[\mathrm{A}][\mathrm{B}]}$ ←

kinetically
indistinguishable
from the mechanism
with no intermediate!

Steady State Approximation

- consider a third example of a two-step reaction:
  - kinetic scheme:

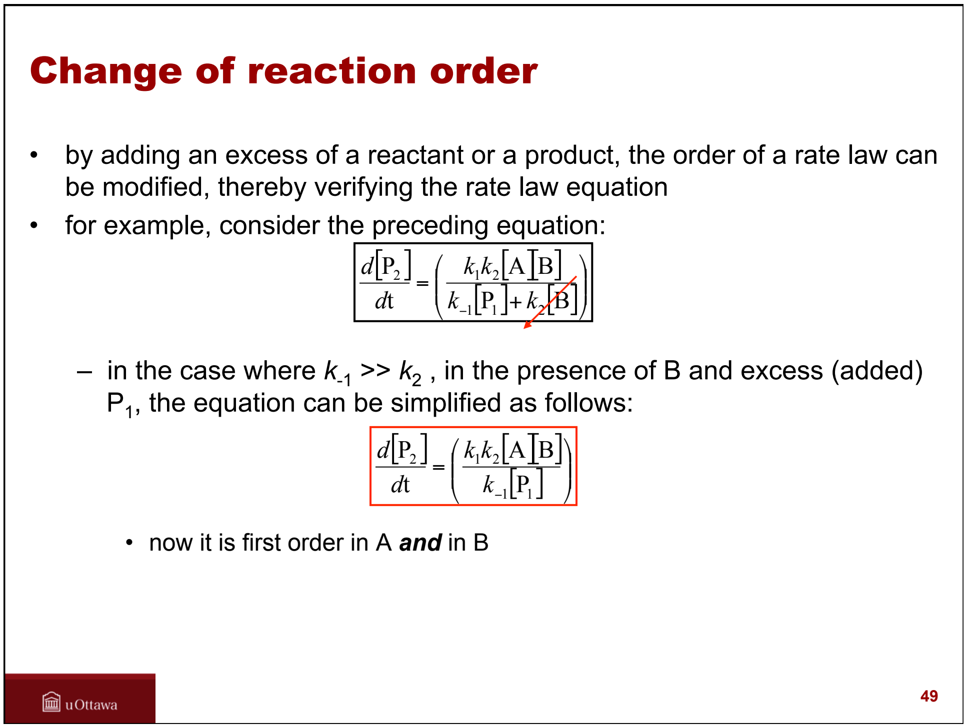


  - rate law: $\boxed{\frac{d[P_2]}{dt} = k_2[I][B]}$
  - SSA: $\boxed{\frac{d[I]}{dt} = k_1[A] - k_{-1}[I][P_1] - k_2[I][B] = 0}$
    - expression of [I] : $[I] = \left(\frac{k_1[A]}{k_{-1}[P_1] + k_2[B]}\right)$
  - rate equation: $\boxed{\frac{d[P_2]}{dt} = \left(\frac{k_1 k_2[A][B]}{k_{-1}[P_1] + k_2[B]}\right)}$ ←

first order in A,
less than first order in B;
slowed by P_1

SSA Rate equations

- useful generalisations:
  1. the numerator is the *product* of the rate constants and concentrations necessary to form the product; the denominator is the *sum* of the rates of the different reaction pathways of the intermediate
  2. terms involving concentrations can be controlled by varying reaction conditions
  3. reaction conditions can be modified to make one term in the denominator much larger than another, thereby simplifying the equation as zero order in a given reactant



Change of reaction order

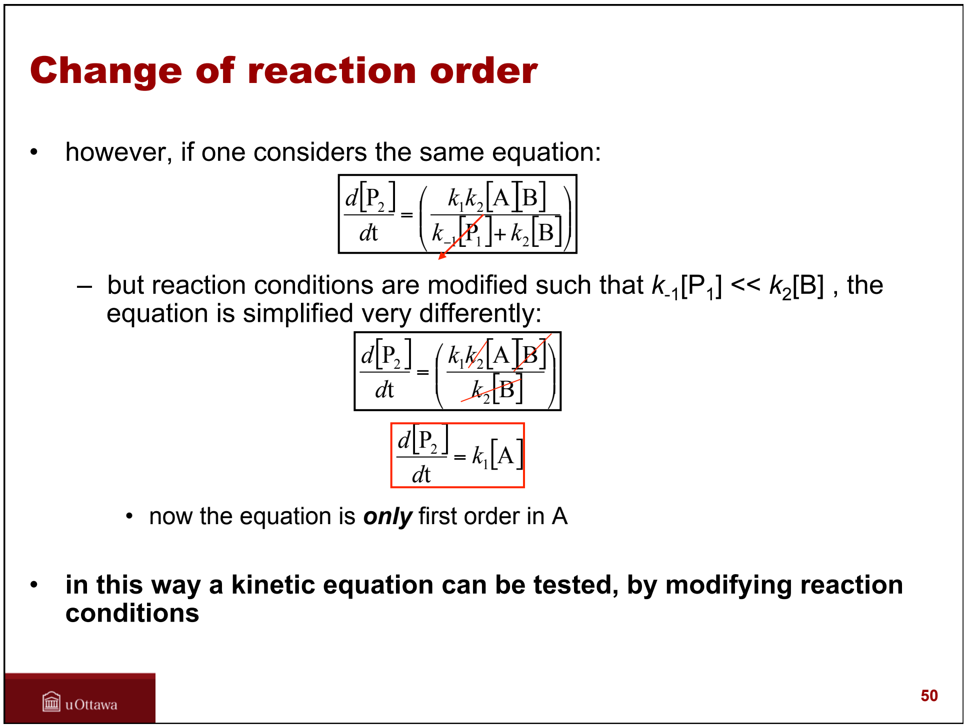
- by adding an excess of a reactant or a product, the order of a rate law can be modified, thereby verifying the rate law equation
- for example, consider the preceding equation:

$$\boxed{\frac{d[\mathrm{P}_2]}{dt} = \left(\frac{k_1k_2[\mathrm{A}][\mathrm{B}]}{k_{-1}[\mathrm{P}_1]+\cancel{k_2[\mathrm{B}]}}\right)}$$

  - in the case where $k_{-1} >> k_2$, in the presence of B and excess (added) P_1, the equation can be simplified as follows:

$$\boxed{\frac{d[\mathrm{P}_2]}{dt} = \left(\frac{k_1k_2[\mathrm{A}][\mathrm{B}]}{k_{-1}[\mathrm{P}_1]}\right)}$$

    - now it is first order in A ***and*** in B



Change of reaction order

- however, if one considers the same equation:

$$\boxed{\frac{d[\mathrm{P}_2]}{dt} = \left(\frac{k_1k_2[\mathrm{A}][\mathrm{B}]}{\cancel{k_{-1}[\mathrm{P}_1]}+k_2[\mathrm{B}]}\right)}$$

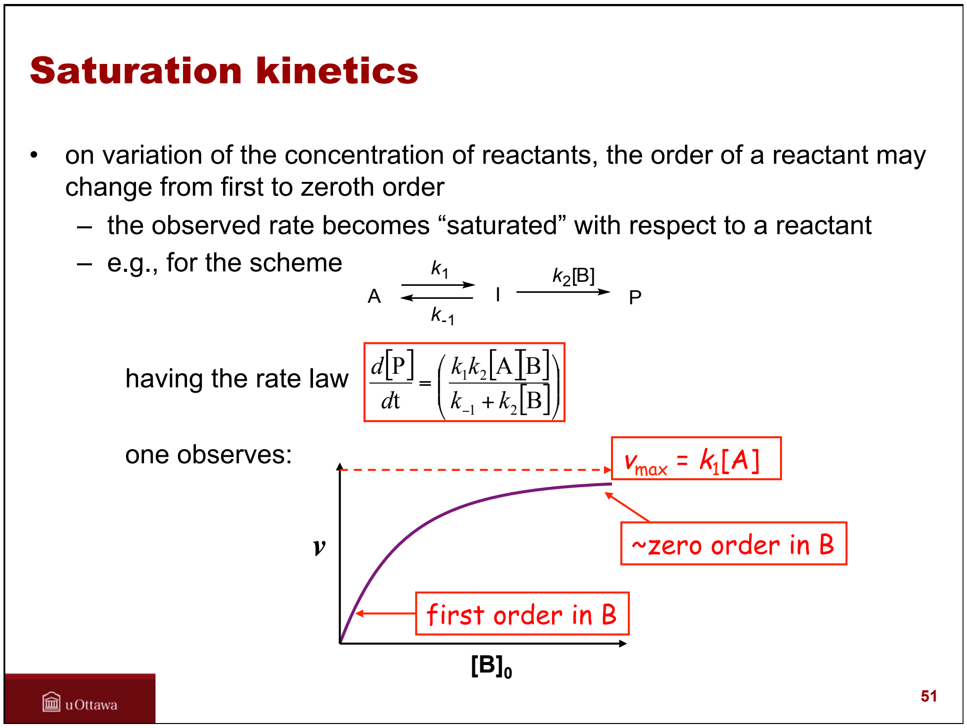
  - but reaction conditions are modified such that $k_{-1}[P_1] << k_2[B]$, the equation is simplified very differently:

$$\boxed{\frac{d[\mathrm{P}_2]}{dt} = \left(\frac{k_1\cancel{k_2}[\mathrm{A}]\cancel{[\mathrm{B}]}}{\cancel{k_2}\cancel{[\mathrm{B}]}}\right)}$$

$$\boxed{\frac{d[\mathrm{P}_2]}{dt} = k_1[\mathrm{A}]}$$

    - now the equation is ***only*** first order in A

- **in this way a kinetic equation can be tested, by modifying reaction conditions**



Saturation kinetics

- on variation of the concentration of reactants, the order of a reactant may change from first to zeroth order
  - the observed rate becomes “saturated” with respect to a reactant
  - e.g., for the scheme $A \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} I \xrightarrow{k_2[B]} P$

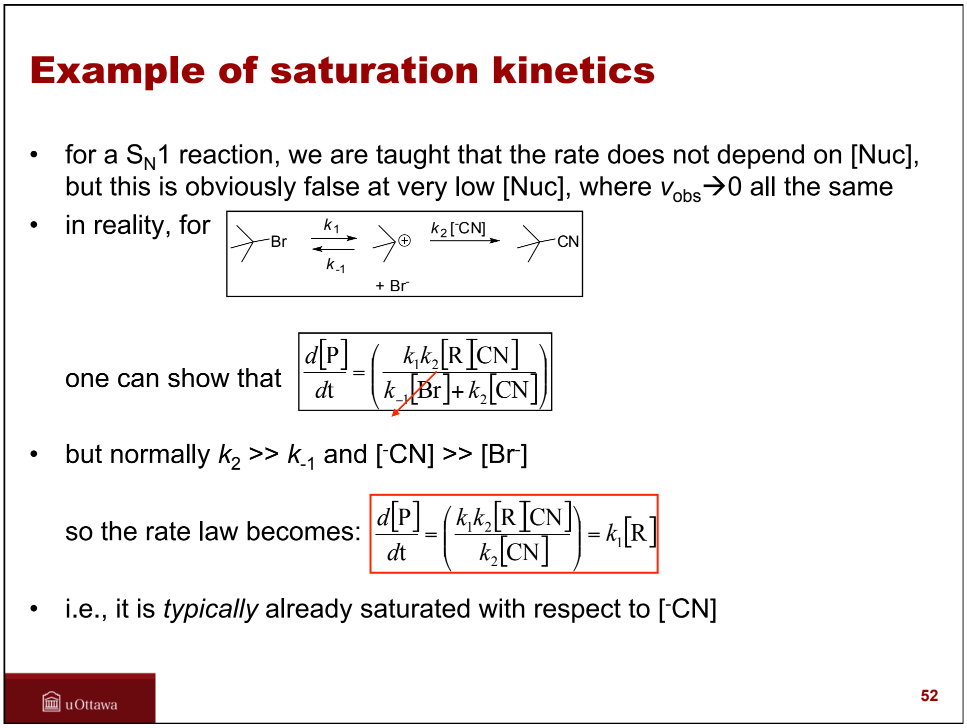
having the rate law $\boxed{\dfrac{d[P]}{dt} = \left(\dfrac{k_1 k_2 [A][B]}{k_{-1} + k_2 [B]}\right)}$

one observes:

$\boxed{v_{max} = k_1[A]}$

~zero order in B

first order in B



Example of saturation kinetics

- for a S_N1 reaction, we are taught that the rate does not depend on [Nuc], but this is obviously false at very low [Nuc], where $v_{obs} \rightarrow 0$ all the same
- in reality, for

one can show that $\boxed{\dfrac{d[P]}{dt} = \left(\dfrac{k_1 k_2 [R][CN]}{\cancel{k_{-1}[Br]} + k_2[CN]}\right)}$

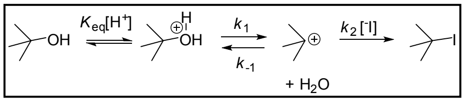
- but normally $k_2 \gg k_{-1}$ and $[^-CN] \gg [Br^-]$

so the rate law becomes: $\boxed{\dfrac{d[P]}{dt} = \left(\dfrac{k_1 k_2 [R][CN]}{k_2[CN]}\right) = k_1[R]}$

- i.e., it is *typically* already saturated with respect to $[^-CN]$

Rapid pre-equilibrium

- in the case where a reactant is in rapid equilibrium before its reaction, one can replace its concentration by that given by the equilibrium constant
- for example, for



the protonated alcohol is always in rapid eq'm with the alcohol and therefore

$$\boxed{\frac{d[P]}{dt} = \left(\frac{k_1 k_2 K_{eq}[H^+][tBuOH][I]}{k_{-1}[H_2O] + k_2[I]}\right)}$$

- normally, $k_2[I^-] >> k_{-1}[H_2O]$ and therefore

$$\boxed{\frac{d[P]}{dt} = \left(\frac{k_1 k_2 K_{eq}[H^+][tBuOH][I]}{k_2[I]}\right) = k_1 K_{eq}[H^+][tBuOH]}$$

first order in *t*BuOH,
zero order in I^-,
pH dependent

Summary: Kinetic approach to mechanisms

- kinetic measurements provide rate laws
  - 'molecularity' of a reaction
- rate laws limit what mechanisms are consistent with reaction order
  - several hypothetical mechanisms may be proposed
- detailed studies (of substituent effects, etc) are then necessary in order to eliminate all mechanisms – except one!
  - one mechanism is retained that is consistent with all data
  - in this way, the scientific method is used to <u>refute</u> inconsistent mechanisms (and support consistent mechanisms)