Development of Complex Curricula for Molecular Bionics and Infobionics Programs within a consortial* framework**

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**Molekuláris bionika és Infobionika Szakok tananyagának komplex fejlesztése konzorciumi keretben

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INTRODUCTION TO BIOPHYSICS

(Bevezetés a biofizikába)

REACTION KINETICS

(Reakciókinetika)

GYÖRFFY DÁNIEL, ZÁVODSZKY PÉTER

Introduction

- Rate equation mathematically describes the velocities of reactions
- Advanced experimental techniques exist to measure the rate of fast reactions
- Reaction order of reactions can be determined by investigating the relation between initial concentrations of reactants and the rate of reaction
- Arrhenius equation reveals an exponential dependence of rate constant on the reciprocal of temperature

Equation of reaction

- The general equation of chemical reactions is:

$$\sum_j \nu_j^r A_j \rightarrow \sum_j \nu_j^p A_j$$

where ν_j^r and ν_j^p are the stoichiometrical coefficients for A_j substance as a reactant or a product, respectively

- Let us consider a simple reaction:

$$A+2B\rightarrow 3C+4D$$

the velocity of the reaction is:

$$v=-\frac{d[A]}{dt}=-\frac{1}{2}\frac{d[B]}{dt}=\frac{1}{3}\frac{d[C]}{dt}=\frac{1}{4}\frac{d[D]}{dt}$$

or generally

$$v=\frac{1}{\left(\nu_j^p-\nu_j^r\right)}\frac{d\left[A_j\right]}{dt}$$

where ν_j^p and ν_j^r are the stoichiometric coefficient of substance A_j as a product or a reactant, respectively, [ ] refers to concentration

Rate law

- In any reaction the rate can depend on the concentration of reactants
- This relationship is mathematically described by the rate law

- The most general form of the rate law is:

$$v = k \prod_j [A_j]^{\alpha_j}$$

where k is the rate constant and α_j is the order of reaction with respect to the substance A_j

Elementary reactions

- The order of a reaction regarding a given participant is not necessarily equal to the stoichiometric coefficient of it
- In the case of elementary reactions, however, these two measures are equal
- **DEF** elementary reaction: reaction where the order is equal to the stoichiometric coefficient for each reactant and product
- Complex reactions can be divided into elementary reactions

- A well known reaction is the chain reaction of hydrogen with bromine:

$$H_2(g)+Br_2(g)\rightarrow 2\mathrm{HBr}(g)$$

the rate law for this reaction is:

$$v=k\frac{[H_2][Br_2]^{3/2}}{[Br_2]+k'[HBr]}$$

- An assumed mechanism of reaction is:
  - Initiation

$$Br_2 \xrightarrow{h\nu} 2\mathrm{Br}\cdot \qquad v = k_1[Br_2]$$

  - elongation

$$Br\cdot + H_2 \rightarrow HBr + H\cdot \qquad v = k_2[Br][H_2]$$
$$H\cdot + Br_2 \rightarrow HBr + Br\cdot \qquad v = k_3[H][Br_2]$$

- inhibition

$$H\cdot + HBr \rightarrow H_2 + Br\cdot \qquad v = k_4[H][HBr]$$

- termination

$$Br\cdot + Br\cdot \rightarrow Br_2 \qquad v = k_5[Br]^2$$

where

$$k = 2k_2\sqrt{k_1/k_5} \qquad k' = k_4/k_3$$

- It can be seen that the orders regarding different reactants in the overall reaction are not equal to stoichiometric coefficients
- On the other hand, in elementary reactions, orders are equal to stoichiometric coefficients
- Order needs not to be an integer value as it can be seen in the rate law for the overall reaction

Concentration change during the reaction

- Rate law for elementary reactions is a simple linear differential equation
- We study the concentration of reactants and products as they change in time
- To obtain this relationship, the differential equations of the rate law must be solved

First-order reactions

- First, let us consider a simple first-order elementary reaction

$$A \rightarrow B$$

- The rate law for this reaction is:

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

- Let us begin with the form

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

- To solve this equation, we should separate the variables

$$\frac{1}{[A]}d[A] = -k\,dt$$

- Integrate both site of the equation from *0* to *t*

$$\int_{[A_0]}^{[A]} \frac{1}{[A]} d[A] = -k \int_0^t dt$$

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

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

$$\frac{[A]}{[A]_0} = e^{-kt}$$

- So the concentration of substance *A* against time

$$[A]=[A]_0 e^{-kt}$$

where [A] and $[A]_0$ are the concentration of substance *A* at time *t* and at the beginning of reaction, respectively, *k* is the rate constant

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Example of first order reaction

- Perhaps the best known first order reaction is the radioactive decay
- In mathematical form, such decays are described by

$$I = I_0 e^{-t/\tau}$$

where I and I_0 are the number of atoms of a radioactive isotope at time t and at the beginning of the reaction, respectively, and $\tau = 1/\lambda$ is the lifetime, where $\lambda = k$ is the *decay constant*

- The rate of decay is often characterized by the *half life* of reaction
- **DEF** half life: the time needed the number of atoms of a radioactive isotope to drop to the half of the initial value, in mathematical form

$$\frac{I_0}{2} = I_0 e^{-\tau_{1/2}/\tau}$$

- To get the relation between half life and decay constant, divide both sides of the equation above by I_0

$$\frac{1}{2} = e^{-\tau_{1/2}/\tau}$$

$$2 = e^{\tau_{1/2}/\tau}$$

$$\ln 2 = \tau_{1/2}/\tau$$

$$\tau_{1/2} = \tau \ln 2$$

Second-order reaction

- Now let us consider an association reaction

$$2A \rightarrow B$$

- The rate law for this reaction is:

$$v = -\frac{d[A]}{dt} = \frac{d[B]}{dt} = k[A]^2$$

- Let us begin with the form

$$\frac{d[A]}{dt} = -k[A]^2$$

- To solve this equation, we should separate the variables

$$\frac{1}{[A]^2} d[A] = -k\,dt$$

- Integrate both sides of the equation from *0* to *t*

$$\int_{[A_0]}^{[A]} \frac{1}{[A]^2}\, d[A] = -k \int_0^t dt$$

$$\frac{1}{[A]} - \frac{1}{[A]_0} = kt$$

- So the concentration of substance *A* against time

$$[A]=\frac{1}{kt+\frac{1}{[A]_0}}$$

where [A] and $[A]_0$ are the concentration of substance *A* at time *t* and at the beginning of reaction, respectively, *k* is the rate constant

Determination of the order of a reaction

- The order of a reaction with respect to any participant can be determined by experiments
- In the experiment, we measure the amount of the participant for which we would like to know the order of reaction

Problem 1

- We investigate the oxidation of formic acid to carbon dioxide and hydrogen at 800 K and would like to know whether this reaction is first order
- If it is then the concentration of formic acid is:

$$[HCOOH]=[HCOOH]_0 e^{-kt}$$

- The following values were measured

t / min	0	30	60	90	120	150
[HCOOH] / 10^{-3} M	2.000	1.219	0.743	0.453	0.276	0.168

Table 1.

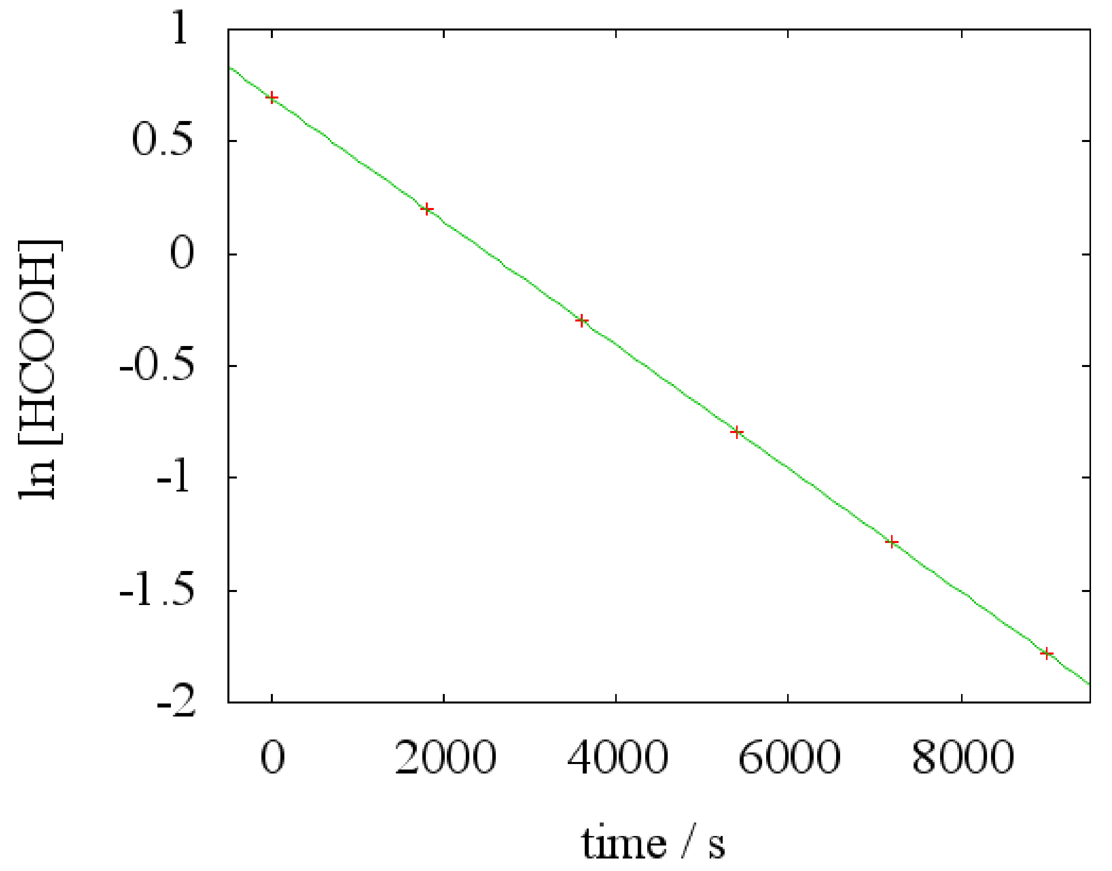


Figure 1.

- As we can see in figure 1. the straight line fits very well to points indicating a first order kinetics
- Assuming not first order reaction measured points cannot be fitted by a straight line (figure 2.)

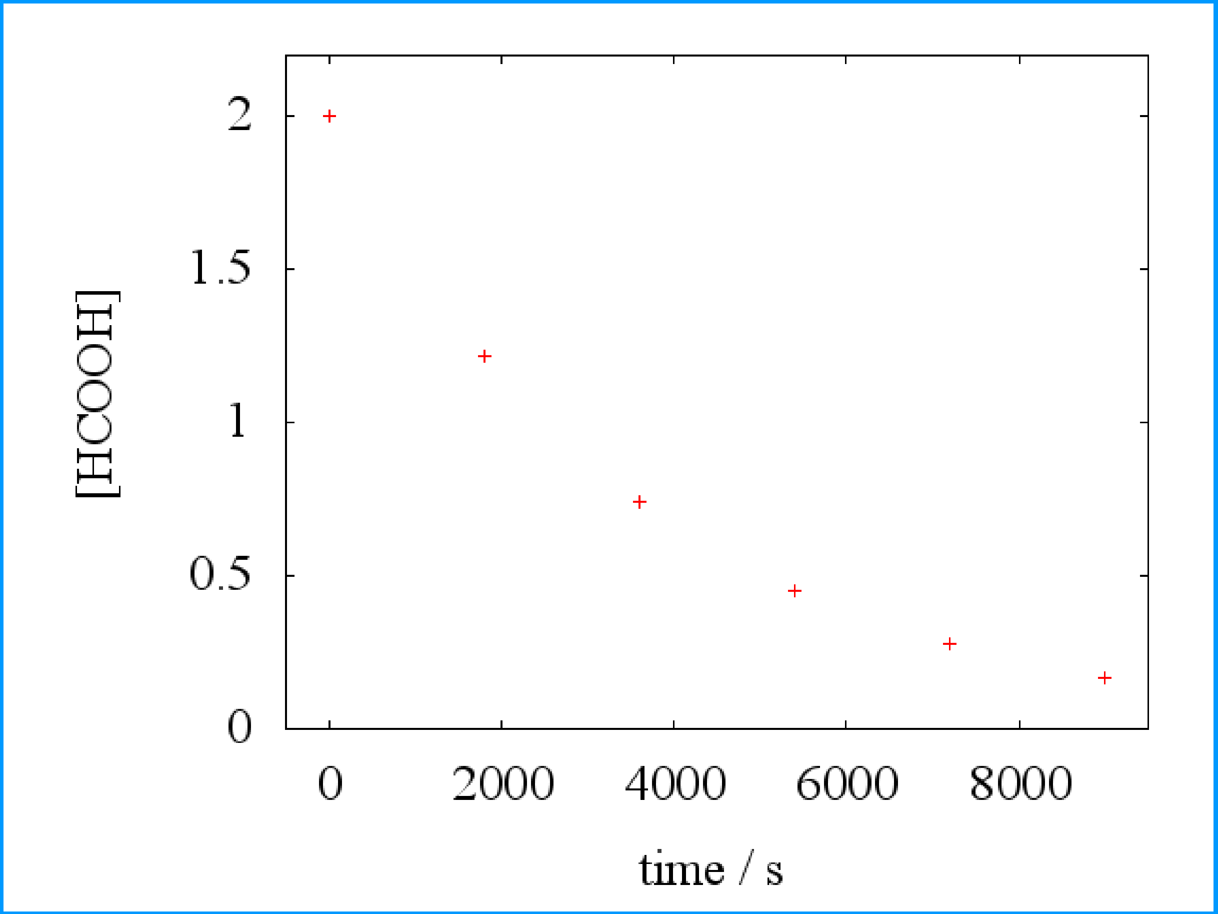


Figure 2.2 a.

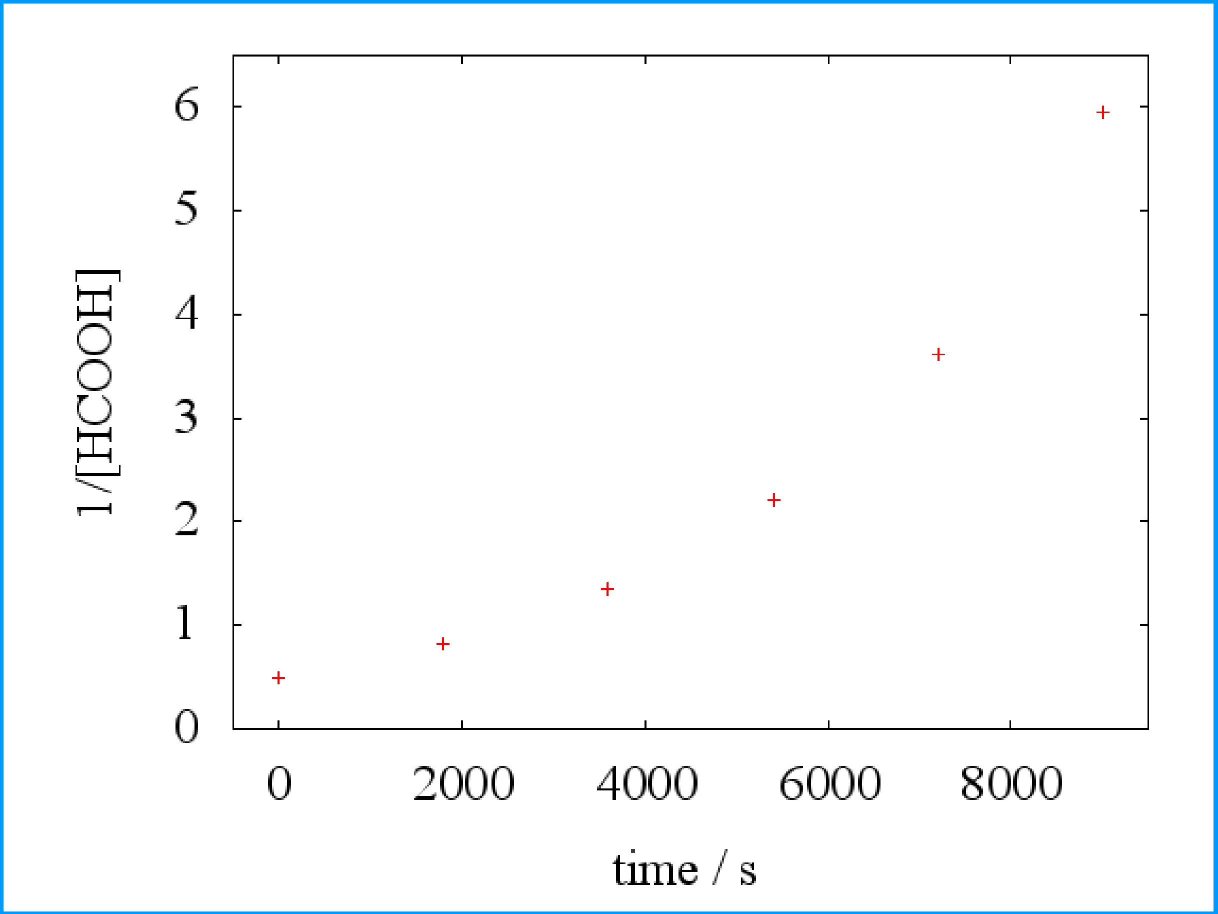


Figure 2.2 b.

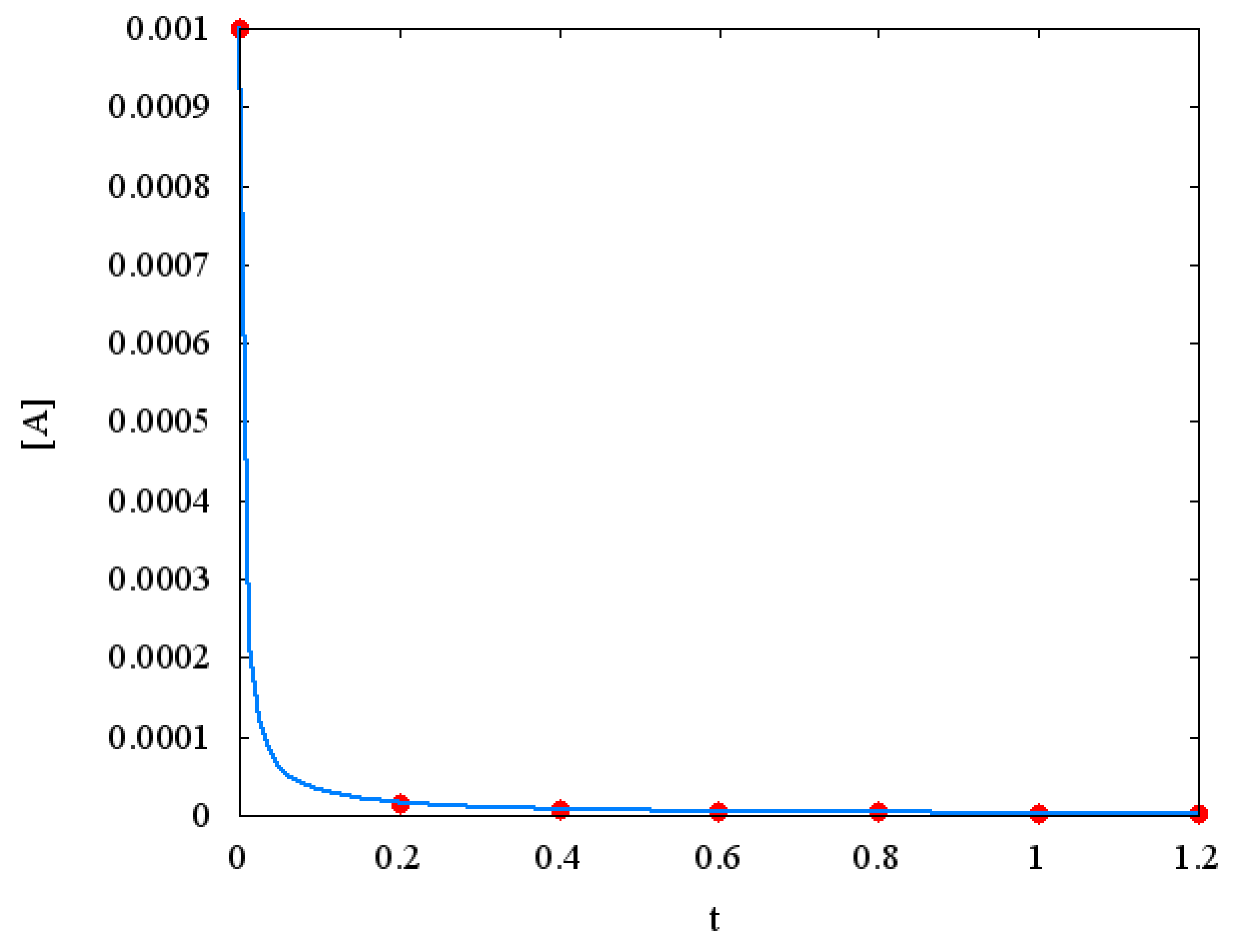
Problem 2

- We investigate the combination of two N (nitrogen) atoms to N_2 molecule
- The following values were measured for the reaction

t (ms)	0	200	400	600	800	1000	1200
[N] (M)	1	$1.60 \cdot 10^{-5}$	$8.31 \cdot 10^{-6}$	$5.48 \cdot 10^{-6}$	$4.10 \cdot 10^{-6}$	$3.36 \cdot 10^{-6}$	$2.77 \cdot 10^{-6}$

Table 2.

C curve of N-N combination

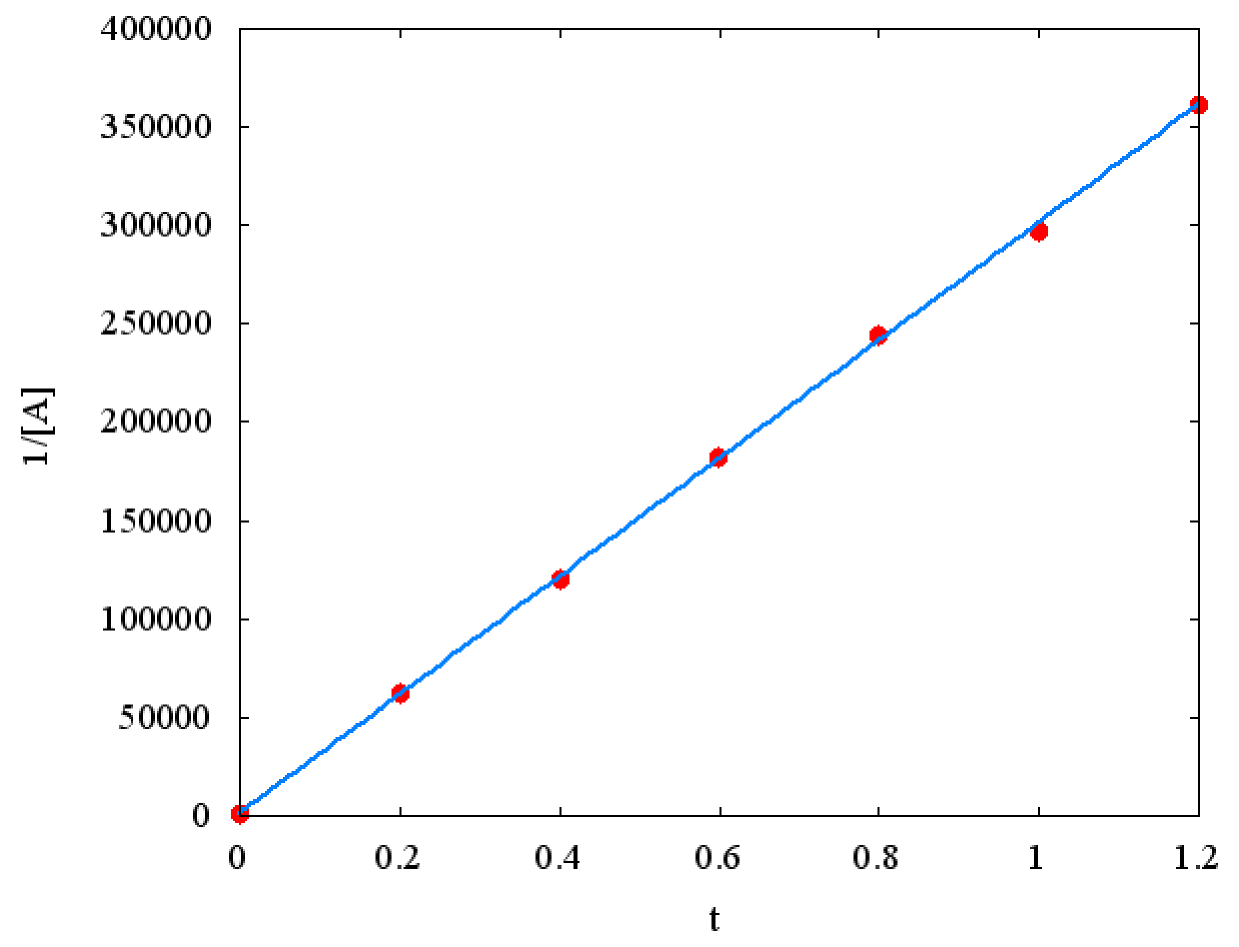


- Lets plot 1/[A] as a function of *t* to obtain a linear relationship if the reaction above is second order with respect to N

$$\frac{1}{[A]} = \frac{1}{[A]_0} + kt$$

- As we can see on the plot below the linear function fits very well for the points measured for the fusion reaction

Linear plot for N-N combination



Experimental methods for measuring reaction rate

- Classical methods
- Fast-flow methods
- Stopped-flow methods
- Flash photolysis

Classical methods

- Relatively slow reactions can be monitored by classical methods
- Reactants are mixed in a reaction space
- Some property which changes during the reaction is measured
- Usually some electrical property like conductance or voltage is measured

Fast flow methods

- In a normal mixer, reactants can be mixed only on the time scale of the reaction rate, so the obtained values are not accurate enough
- In gas phase, fast flow methods are used
- In these methods, reactants are injected into a flow tube and cover some distance to the detector
- Concentration of reactant or some product is plotted against the distance covered.
- Time scale at millisecond

Stopped flow methods

- It can be useful for reactions in liquid phase
- The speed of mixing is fast enough not to distort the results
- Injected reactants meet in a mix chamber where the reaction takes place
- Flow is stopped by a third piston and a switch (figure 2.3)

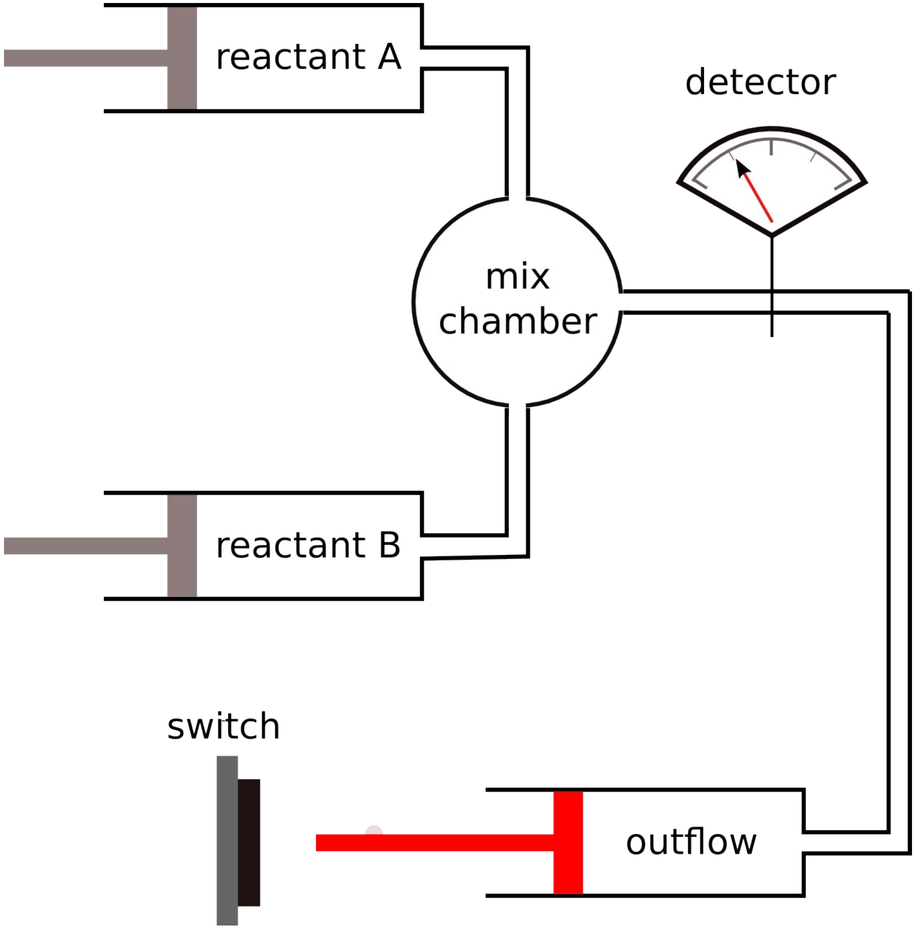


Figure 2.3

Flash photolysis

- Mixing finishes before beginning of reaction
- Reaction is started by a flash of light
- In the case of a homogeneous ray, the distribution of reactants will be also homogeneous
- Time scale depends on the width of impulse but it can reach the nanosecond interval or smaller

Simplifying methods for obtaining the rate law

- Complex reactions can be so complicated that the rate law cannot be obtained purely by mathematical rearrangements
- In such cases, we need to simplify the expression describing kinetics of the reaction
- Several methods are known to do this:
    - Steady-state approximation
    - Pseudo-first-order approximation
    - Rate limiting step approximation

Steady state approximation

- Let us consider a serial reaction

$$A \xrightarrow{k_1} B \xrightarrow{k_2} C$$

- Steady-state approximation assumes that the concentration of the intermediate can be considered constant
- The corresponding rate laws are

$$\frac{d[A]}{dt} = k_1[A] \qquad \frac{d[B]}{dt} = k_1[A] - k_2[B] \qquad \frac{d[C]}{dt} = k_2[B]$$

- In steady state approximation we assume

$$\frac{d[B]}{dt}=k_1[A]-k_2[B]=0$$

thus

$$[B]=\frac{k_1}{k_2}[A]$$

and thus

$$\frac{d[C]}{dt}=\frac{k_2\cdot k_1}{k_2}[A]=k_1[A]$$

- Integrating the rate law gives us the concentration of component C as a function of time
- Let us substitute [A] from the expression above to get

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

and integrating the expression we obtain

$$[C]=[A]_0\int_0^t e^{-k_1 t}\,dt=[A]_0\left(1-e^{-k_1 t}\right)$$

Example of steady state approximation

- Let us consider a protein with the states: unfolded (U), first intermediate (I_1), second intermediate (I_2) and native (N)
- For the folding of the protein the following scheme was proposed

$$U \xrightarrow{k_1} I_1 \qquad I_1 \xrightarrow{k_2} I_2$$

$$I_1 \xrightarrow{k_3} N \qquad I_2 \xrightarrow{k_4} N$$

- The corresponding rate laws are

$$\frac{dU}{dt}=-k_1[U] \qquad \frac{d[I_1]}{dt}=k_1[U]-(k_2+k_3)[I_1]$$

$$\frac{d[I_2]}{dt}=k_2[I_1]-k_4[I_2] \qquad \frac{d[N]}{dt}=k_3[I_1]+k_4[I_2]$$

- Using the steady state approximation we assume

$$\frac{d[I_1]}{dt}=k_1[U]-(k_2+k_3)[I_1]=0 \qquad \frac{d[I_2]}{dt}=k_2[I_1]-k_4[I_2]=0$$

- The concentrations of the intermediates thus

$$[I_1]=\frac{k_1}{k_2+k_3}[U]$$

$$[I_2]=\frac{k_2}{k_4}[I_1]=\frac{k_1 k_2}{(k_2+k_3)k_4}[U]$$

- Substituting the concentrations of the intermediates we obtain a first order rate law for the end-product

$$\frac{d[N]}{dt}=\frac{k_1 k_3+k_1 k_2}{k_2+k_3}[U]$$

- It can be seen that the unfolded state takes part in only a first order decay so its concentration is

$$[U]=[U_0]e^{-k_1 t}$$

- Substituting this into the rate law for the native state we get

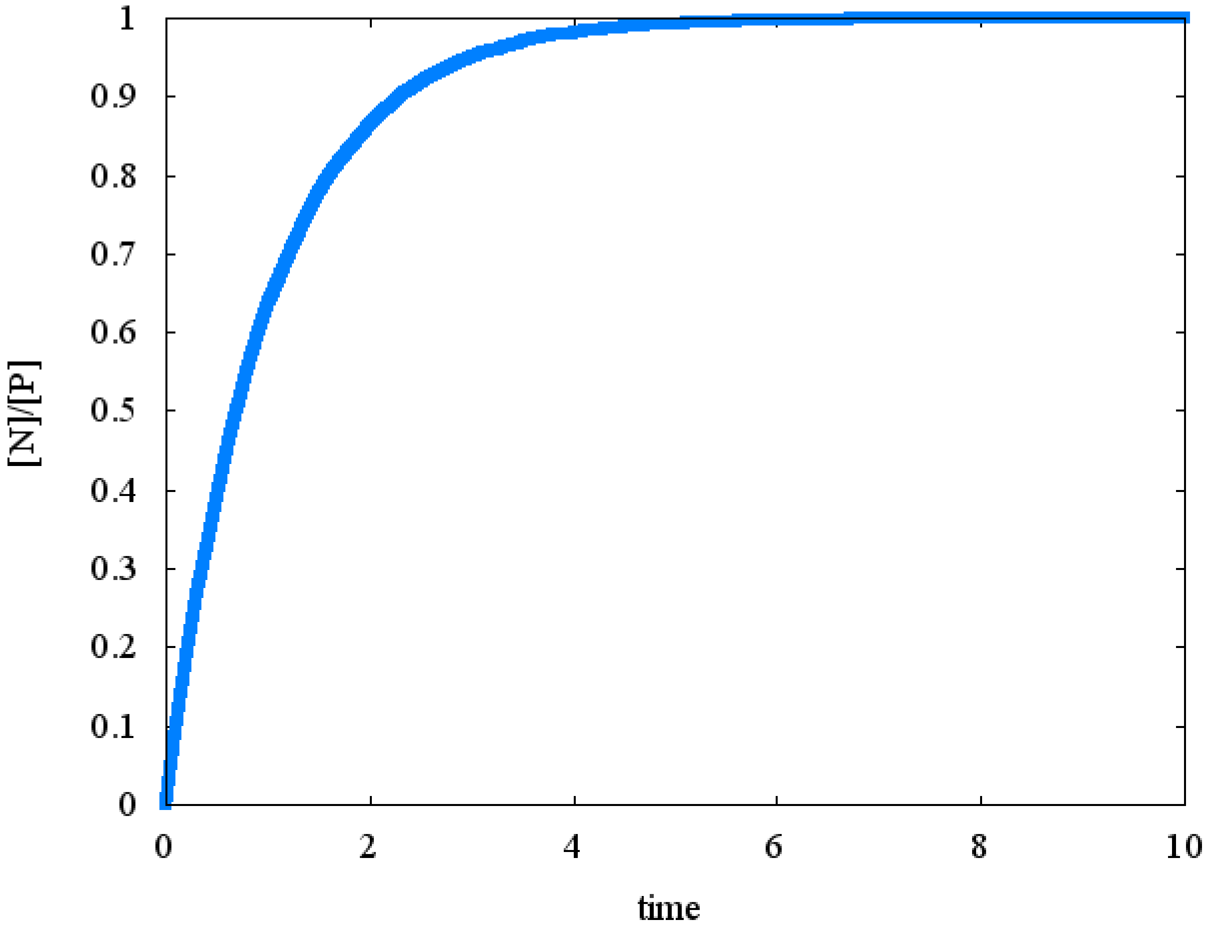
$$\frac{d[N]}{dt}=\frac{k_1 k_3+k_1 k_2}{k_2+k_3}e^{-k_1 t}[U_0]=k_1 e^{-kt}[U_0]$$

- Integrating between 0 and t we get the concentration of the native state

$$[N]=[U_0]k_1\int_0^t e^{-k_1 t}=[U_0](1-e^{-k_1 t})$$

- In kinetic measurements this folding process will resemble to a two-state folding

Kinetic measurement of the folding of a protein



Rate limiting step

- Consider a serial reaction

$$A \xrightarrow{k_1} B \xrightarrow{k_2} C$$

- If one of the rate constants is far smaller than the other one, the process characterized by that smaller rate constant is considered as the process which limits the rate of the whole reaction
- Some simplification can be carried out using of rate limiting step approximation

- The rate laws for the reaction above are

$$\frac{d[A]}{dt}=-k_1[A]$$

$$\frac{d[B]}{dt}=k_1[A]-k_2[B]$$

$$\frac{d[C]}{dt}=k_2[B]$$

- The concentration change for the substances are

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

$$[C]=[A]_0+[B]_0+[C]_0-[A]-[B]$$

Introduction to biophysics: Reaction kinetics

$$\frac{d[B]}{dt}=k_1[A]-k_2[B]$$

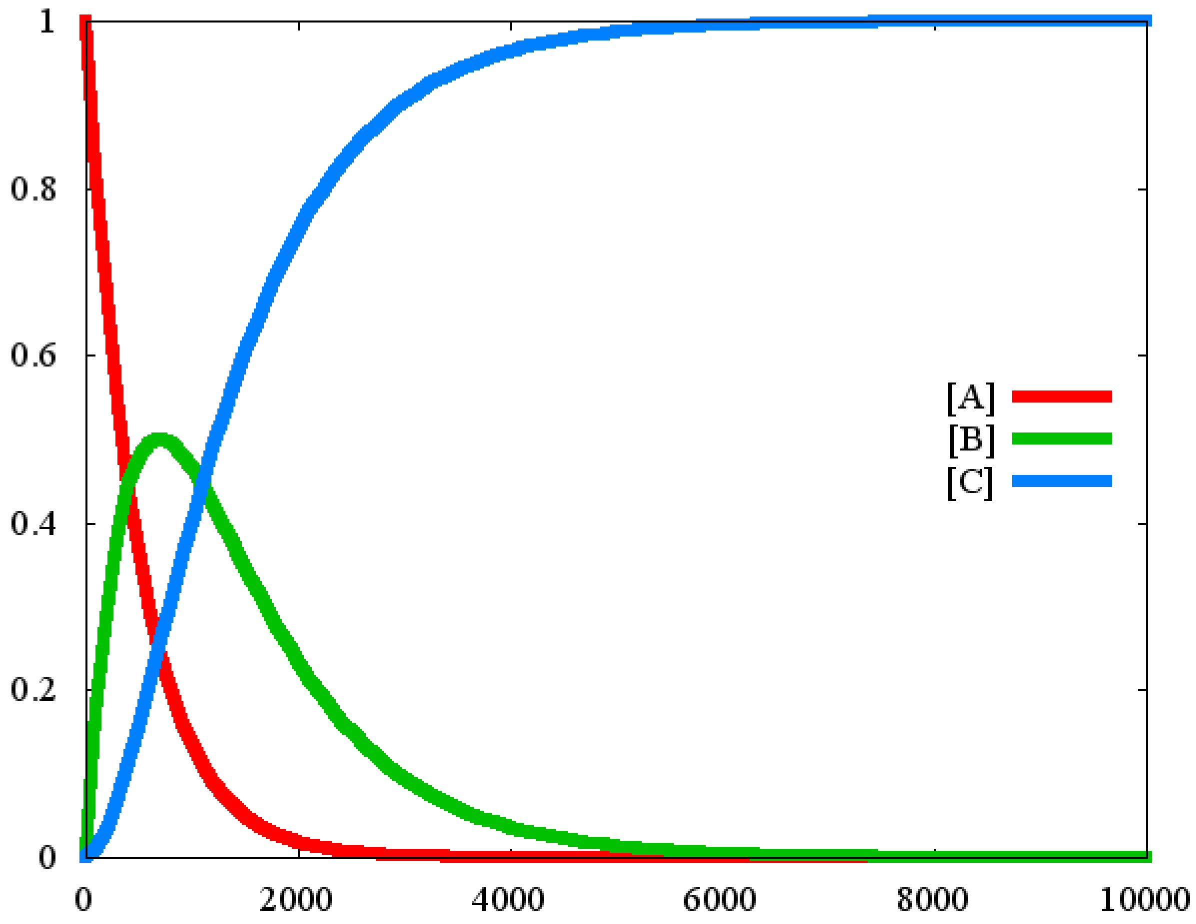
$$[A]=[A]_0 e^{-k_1 t}$$

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

$$e^{-k_2 t}\frac{d}{dt}\left([B]e^{k_2 t}\right)=k_1[A]_0 e^{-k_1 t}$$

$$\int_0^t \frac{d}{dt}\left([B]e^{k_2 t}\right)dt=k_1[A]_0\int_0^t e^{(k_2-k_1)t}\,dt$$

$$[B]=\frac{k_1[A]_0}{k_2-k_1}\left[e^{-k_1 t}-e^{-k_2 t}\right]+[B]_0 e^{-k_2 t} \qquad (k_2\neq k_1)$$



$$[A]_0 = 1 \qquad [B]_0 = 0 \qquad [C]_0 = 0$$

- These complex expressions can be simplified using of the rate limiting approximation
- If $k_1 << k_2$ then

$$[C] \approx [C]_0 + [A]_0 e^{-k_1 t}$$

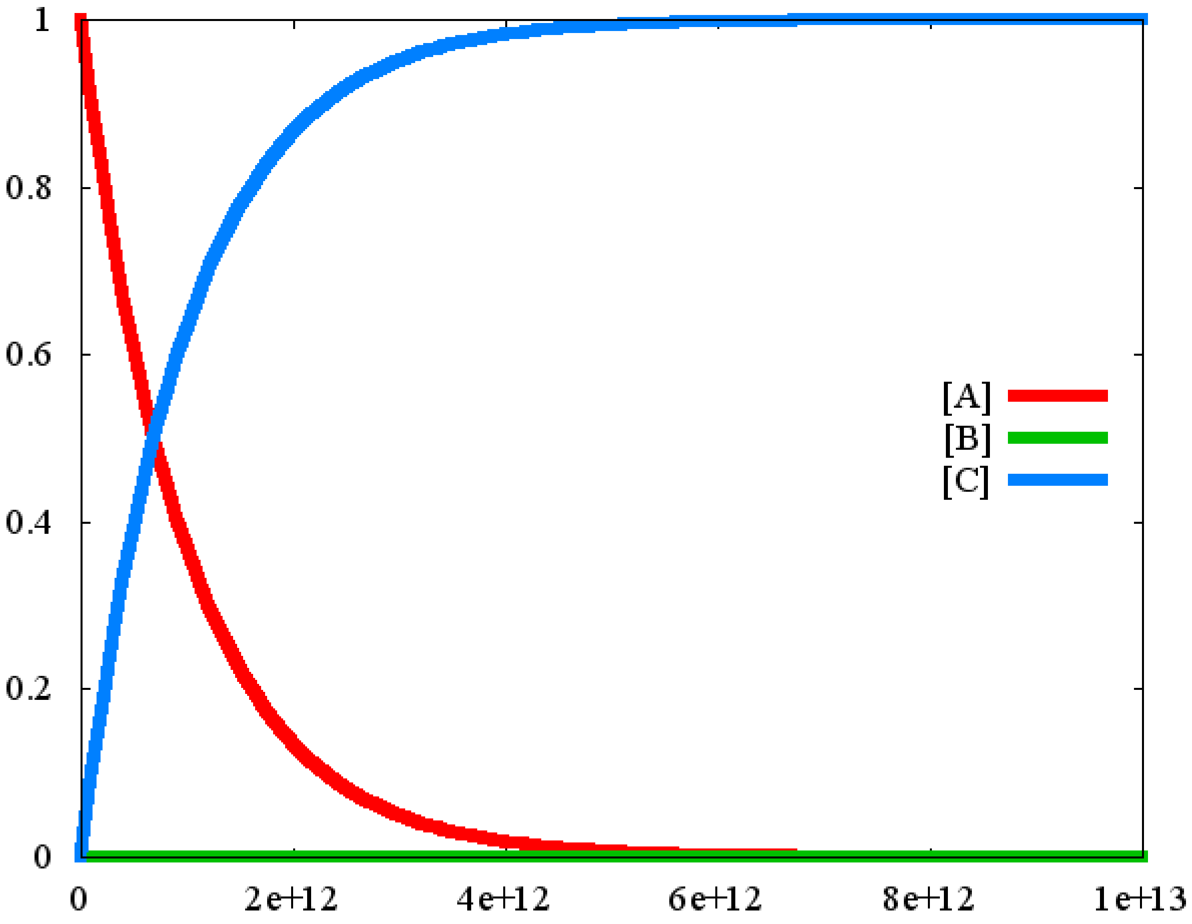
- If however $k_2 << k_1$ then

$$[C] \approx [C]_0 + [A]_0 e^{-k2t}$$

- If C is not present at the beginning of the reaction then

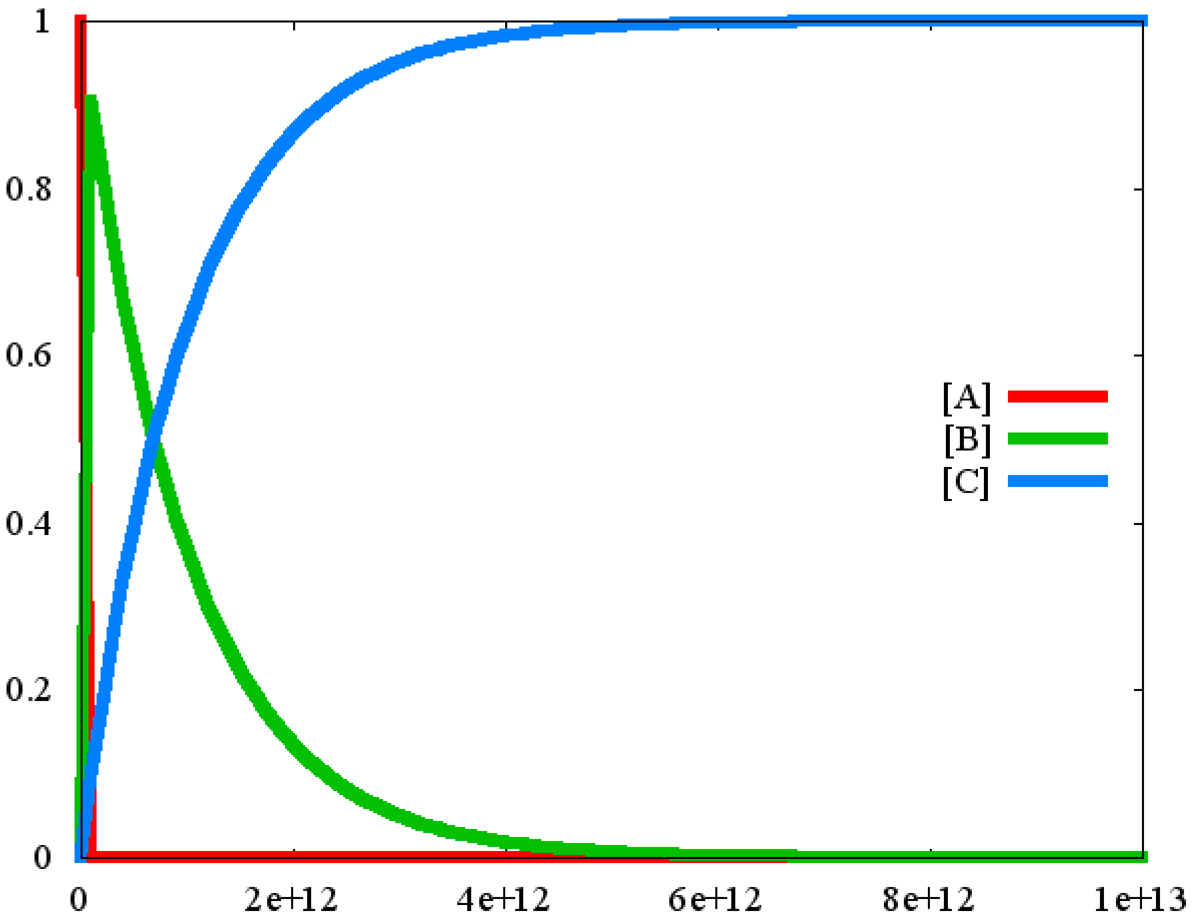
$[C] = [A]_0 e^{-k_1 t}$ and $[C] = [A]_0 e^{-k_2 t}$ respectively

Introduction to biophysics: Reaction kinetics



$[A]_0 = 1$ $[B]_0 = 0$ $[C]_0 = 0$ $k_1 = 10^{-12}$ $k_2 = 10^{-3}$

Introduction to biophysics: Reaction kinetics



$[A]_0 = 1$ $[B]_0 = 0$ $[C]_0 = 0$ $k_1 = 10^{-3}$ $k_2 = 10^{-12}$