Chapter 14: Sedimentation Velocity

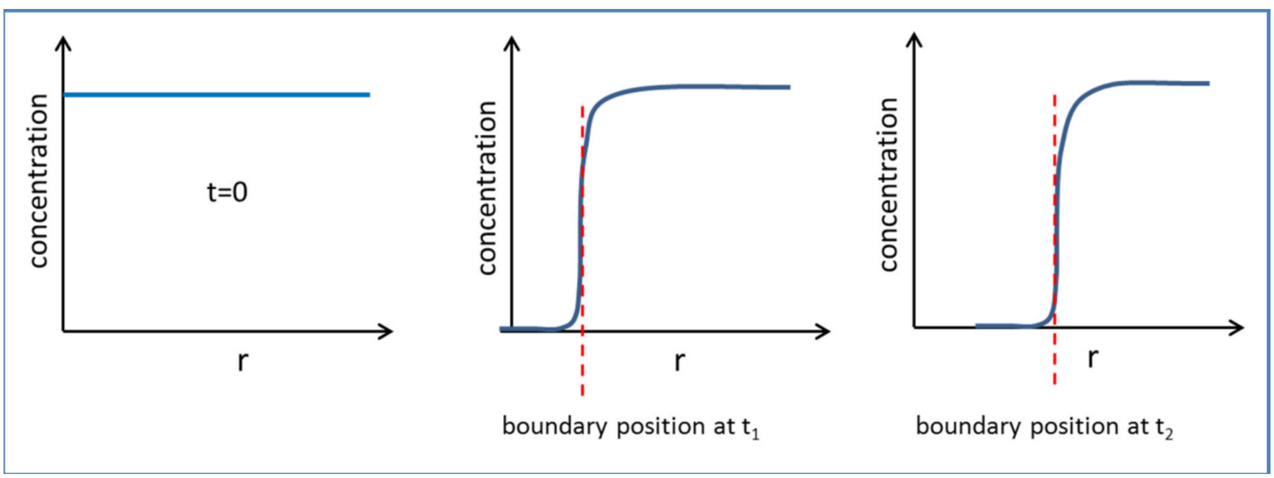
The external force (on a per molecule basis) due to centrifugation is $\mathbf{m}\phi\omega^2\mathbf{r}$, where $\boldsymbol{\phi}$ is the **density increment** , $\boldsymbol{\omega}$ is the **angular velocity**, m is the **mass**, and r is the **radius**. The **opposing frictional force** is **-fv**. When setting the sum of these forces to zero (terminal velocity), the equation yields:

$$\boldsymbol{V = \frac{m\phi\omega^2 r}{f}} \tag{1}$$

or, on a per mole basis:

$$\boldsymbol{V = \frac{M\phi\omega^2 r}{N_A f}} \tag{2}$$

How do you visualize velocity **v** in a velocity sedimentation experiment? If the sample begins with the macromolecule uniformly distributed (concentration is equal everywhere in tube), then when centrifugation begins, the **top region** (low r) of the sample will lose its macromolecules.



The sedimentation velocity **v** is the speed of the boundary: $\boldsymbol{\frac{\Delta r}{\Delta t}}$

Meaning and Measurement of Sedimentation Coefficient, s

How does the sedimentation velocity **v** relate to molecular properties? From the equation $\boldsymbol{V = \frac{M\phi\omega^2 r}{N_A f}}$, v is affected by both molecular properties (M) and experimental parameters $\boldsymbol{(\omega)}$. We want to separate the two kinds of variables on different sides of the equation:

$$\boldsymbol{\frac{v}{\omega^2 r} = \frac{m\phi}{f}} \tag{3}$$

Now, introduce **sedimentation coefficient s** to be equal to those quantities. s is obtained experimentally using $\boldsymbol{s = \frac{v}{\omega^2 r}}$ s is obtained using molecular properties through:

$$\boldsymbol{s = \frac{M\phi}{N_A f}} \text{ or } \boldsymbol{s = \frac{m\phi}{f}} \tag{4}$$

If angular velocity $\boldsymbol{\omega}$ ↑, then the sedimentation velocity v ↑ from eq. (1). But the sedimentation coefficient s is unaffected. We know this is true becuase from **equation 4**, we can relate s to molecular properties without reference to experimental parameters.

Since v is defined as $\boldsymbol{\frac{dr}{dt}}$ we can determine that:

$$\boldsymbol{s = \frac{\frac{dr}{dt}}{\omega^2 r} = \frac{\frac{dln(r)}{dt}}{\omega^2}} \tag{5}$$

Measuring the position r of the boundary at a series of time points during the experiment and plotting them as **ln(boundary position) vs. t** should give a straight line with slope s$\boldsymbol{\omega^2}$, which can be used to find s.

A special unit is used to express the value of the sedimentation coefficient (natural units are seconds). The **Svedberg, S** is defined as $\mathbf{10^{-13}}$ sec.

Relating s to molecular properties

The sedimentation coefficient relates to molecular properties in two ways:

- dependence on mass
- frictional coefficient f (which depends on size → mass)

Starting with $\boldsymbol{s = \frac{m\phi}{f}}$, where f = 6$\boldsymbol{\pi\eta}$R → $\boldsymbol{s = \frac{m\phi}{6\pi\eta R}}$

But R relates to volume and mass according to $\boldsymbol{V = \frac{4}{3}\pi R^3 \rightarrow R = (\frac{3V}{4\pi})^{\frac{1}{3}}}$

Relate volume V to mass m by the density → m = V$\boldsymbol{\rho}$ and substitute V for $\frac{m}{\rho}$

$$s = \frac{m\phi}{6\pi\eta(\frac{3m}{4\pi\rho})^{\frac{1}{3}}}$$

$$s = m^{\frac{2}{3}}(\frac{\phi}{6\pi\eta})(\frac{4\pi\rho}{3})^{\frac{1}{3}} \text{ or } s = (\frac{M}{N_A})^{\frac{2}{3}}(\frac{\phi}{6\pi\eta})(\frac{4\pi\rho}{3})^{\frac{1}{3}}$$

Do larger molecules sediment move faster or slower than small molecules of equal density and similar shape? Centrifugal force on an object is proportional to mass, but opposing force is only $\frac{1}{3}$ power of the mass.

So larger molecules move faster, according to $\frac{2}{3}$ power of their mass.

This assumes that the protein or molecule is nearly spherical since we used Stoke's Equation. If the molecule of interest is **non-spherical**, then it will have the same centrifugal force but ↑ frictional force → ↓ sedimentation coefficient s. This will result in a lower estimation for molecular weight.

Combining s and D (diffusion coefficient) to get molecular weight without a spherical assumption

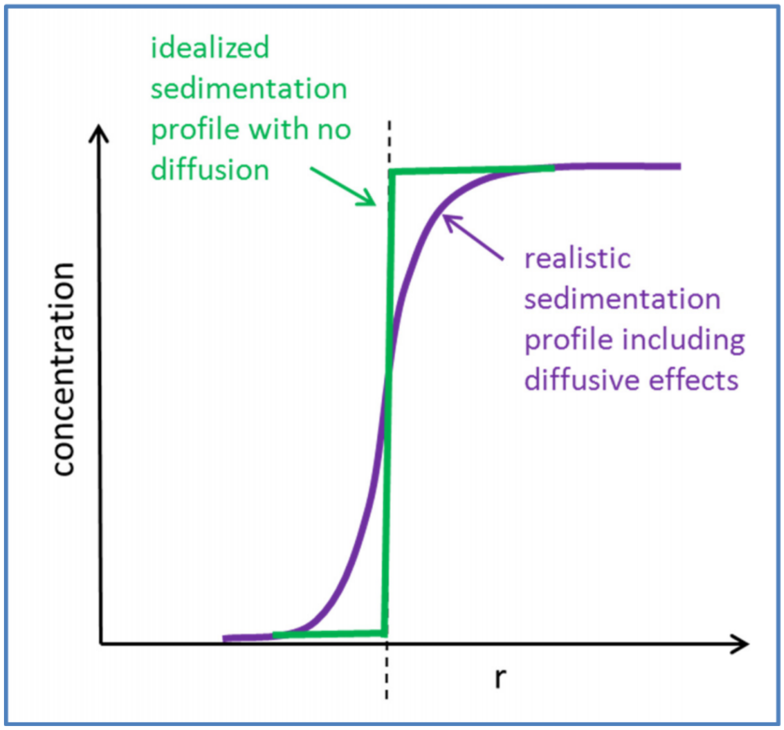
We can eliminate the assumption of spherical shape if we have measured values for s and D together. Having both values cancels frictional coefficient out. We know that $f = \frac{K_B T}{D}$ and from equation (4), $f = \frac{M\phi}{N_A s}$ If we equate the two together:

$$f = \frac{K_B T}{D} = \frac{M\phi}{N_A s}$$

$$M = (\frac{RT}{\phi})(\frac{s}{D})$$

Molecular weight relates only to the ratio of s to D, shape is no longer considered (frictional coefficient).

The diffusion coefficient D can be obtained from the sedimentation experiment itself. Under ideal conditions without diffusion, the concentration profile at the boundary would be perfectly steep. At a position just before the boundary layer, there will be no macromolecules. Right at the boundary layer, there will be a concentration of macromolecules that is the same all throughout the tube after that layer.



Chapter 15: Chemical Reaction Kinetics

Reaction Velocity, v

If we consider a reaction to be reactants $\rightarrow$ products, then reaction velocity v is the frequency (#/time) wth which the event is occurring per unit volume. The units of v are $\frac{\#}{volume \times time}$ or M/sec. There is only one velocity associated with the reaction, even though multiple reactants and products may be involved.

The reaction velocity is indicated equally by the rate of change of **any** of the species involved:

$$\alpha A + \beta B + \cdots \rightarrow \gamma C + \delta D + \ldots \tag{6}$$

$$\frac{-d[A]}{dt} = \alpha v$$
$$\frac{-d[B]}{dt} = \beta v$$
$$\frac{-d[C]}{dt} = \gamma v$$
$$\frac{-d[D]}{dt} = \delta v$$

Rearranging to isolate velocity v gives

$$v = -(\frac{1}{\alpha})(\frac{d[A]}{dt}) = (\frac{1}{\beta})(\frac{d[B]}{dt}) = (\frac{1}{\gamma})(\frac{d[C]}{dt}) = (\frac{1}{\delta})(\frac{d[D]}{dt}) = \ldots$$

Evidently, if we measure the rate of change of concentration of some species, then we measured reacton velocity v.

Rate laws: how v depends on concentrations

Velocity of a reaction depends on how concentrated the reactants are. Besides concnetration, different reactions (different chemical species) will have different v according to likelihood of underlying chemical events $\rightarrow$ k

Combined dependence of v on rate constant k and concentrations $\rightarrow$ **rate law**.

Consider the equation: $\mathbf{A} \xrightarrow{\mathbf{k}} \mathbf{B}$

The rate law is $\mathbf{v} = \mathbf{k}[\mathbf{A}]$, this reaction is first order in A.

Now consider $\mathbf{2A} \xrightarrow{\mathbf{k}} \mathbf{B} \Rightarrow \mathbf{v} = \mathbf{k}[\mathbf{A}]^{\mathbf{2}}$, and the reaction is second order in A.

If the equation is now $\mathbf{A} + \mathbf{B} \xrightarrow{\mathbf{k}} \mathbf{C}$, the rate law is now $\mathbf{v} = \mathbf{k}[\mathbf{A}][\mathbf{B}]$.

Relationship of rate constants to equilibrium constants

What about if we had reversible processes? Velocities of forward and reverse reactions depend on the concentrations of reactants and products, respectively. When the concentrations are reached where forward and reverse reactons are equal, no **net** conversion is occurring. This is **chemical equilibrium**. Consider the reaction:

$$\boldsymbol{2A \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} B} \tag{7}$$

The **forward** reaction velocity is $\mathbf{v} = \mathbf{k_1}[\mathbf{A}]^{\mathbf{2}}$

The **reverse** reaction velocity is $\mathbf{v} = \mathbf{k_{-1}}[\mathbf{B}]^{\mathbf{2}}$

At **equilibrium**, $\mathrm{k}_{\mathbf{1}}[\mathrm{A}]^{\mathbf{2}} = \mathrm{k}_{\mathbf{-1}}[\mathrm{B}]^{\mathbf{2}}$ and $\boldsymbol{\frac{k_1}{k_{-1}} = \frac{[B]}{[A]^2}}$

The ratio of the rate constants is equal to the equilibrium constant K.

Integrating Rate Laws

For simple reactions, we can integrate the differential equations that come from rate law to describe how concentrations of reactants and products change over time

<u>1st Order Decay</u>

$\mathbf{A} \xrightarrow{\mathbf{k}} \mathbf{B}$

To get a differential equation in terms of [A], combine two points:

- First need $\boldsymbol{v = \frac{-d[A]}{dt}}$
- Also need $\boldsymbol{v = k[A]}$

If we equate them together, we get the following:

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

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

$$\ln[A]|_{[A]_0}^{[A]} = -\ kt|_0^t$$

This gives us the familiar first order decay equations

$$\ln(\frac{[A]}{[A]_0}) = -kt \tag{8}$$

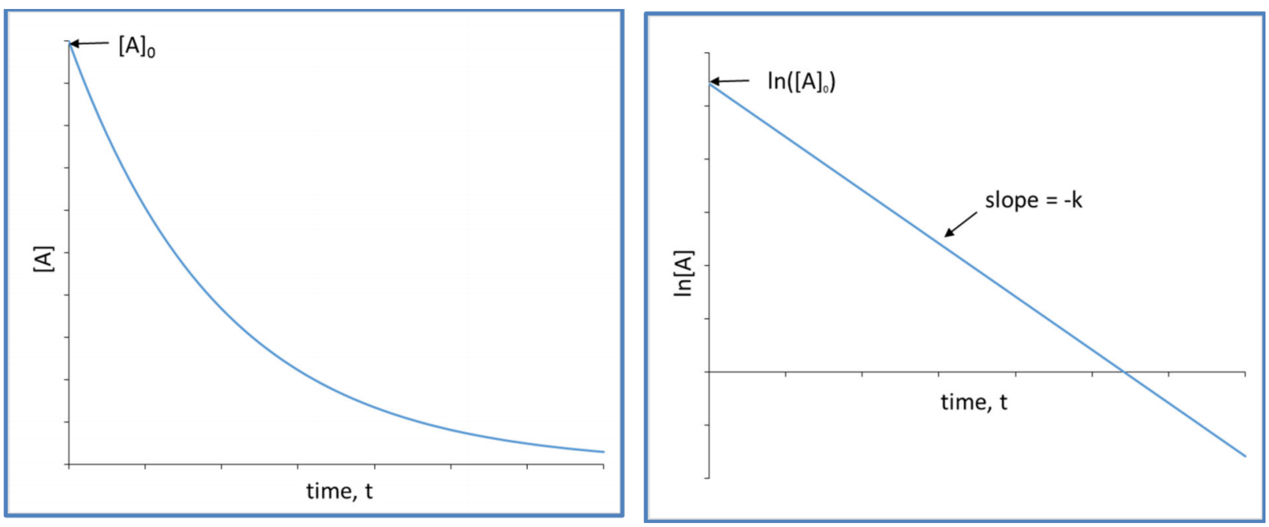


Figure 1: Behavior of [A] over time is exponential, while ln[A] is linear, with slope → k

Describing decay times for 1st order decay

Time scale of first order decay is described in terms of half-life, $t_{1/2}$, which is the time required for a reaction to reach 50% completion. Parameter τ is used to describe decay times. It gives the time required for a reaction to reach $\frac{1}{e}$ completion compared to initial condition: $\frac{[A]}{[A]_0} = \frac{1}{e}$

We can connect $\boldsymbol{t_{1/2}}$ and $\boldsymbol{\tau}$ with the following comparison:

$$\ln(\frac{1}{2}) = -kt_{\frac{1}{2}} \text{ to } \ln(\frac{1}{e}) = -1 = -k\tau \tag{9}$$

This will give:

$$t_{\frac{1}{2}} = \ln(2)\tau \tag{10}$$

For the simple first order decay reaction of [A]

$t_{\frac{1}{2}} = \frac{\ln(2)}{k}$ and $\tau = \frac{1}{k}$

<u>Integrated rate law for a 2nd order irreversible reaction</u>

$\mathbf{2A \xrightarrow{k} B}$

Repeat the same process as above to get:

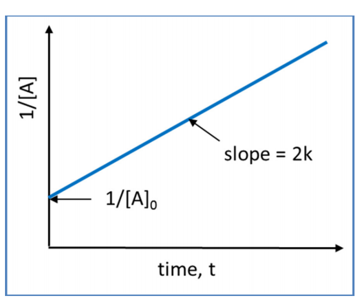
$$\frac{-d[A]}{dt} = 2k[A]^2$$

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

$$-\frac{1}{[A]}\Big|_{[A]_0}^{[A]} = -\ 2kt\Big|_0^t$$

$$\frac{1}{[A]} - \frac{1}{[A]_0} = 2kt$$

A plot of $\frac{1}{[A]}$ vs. time gives a straight line, where slope = k



Establishing a rate law from measured reaction velocities

A different way of experimentally examining a rate law is by evaluating the dependence of reaction velocity on concentrations.

If a reaction is **first order** in [A], then v will depend linearly on [A]. If [A] doubles, then v will also double.

If a reaction is **second order** in [A], then doubling [A] will quadruple the reaction velocity.

In general:

$$\boldsymbol{v = [A]^{\alpha}} \tag{11}$$

Then for rate measurements made at two different concentrations:

$$\boldsymbol{\ln(\frac{v_2}{v_1}) = \alpha \ln(\frac{[A]_2}{[A]_1})} \tag{12}$$

Behavior of more complex reaction schemes

Now, we want to consider events that are more complex than one step reactions. Consider the following example:

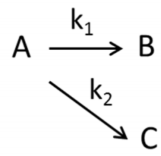
This gives the following equations:

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

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

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

For another example:



The change in concentration as a function of time is given as:

$$\frac{d[A]}{dt} = -k_1[A] - k_2[A] = -(k_1 + k_2)[A]$$

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

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

<u>Steady state assumptions for obtaining simple rate laws for complex reactions</u>

For complex reactions, dependence of the rate of the overall reaction (rate law) can depend on concentrations of species that do not contribue to the reaction itself.

When sequential reactions are invovled, simplified rate laws can be obtained by assuming steady state conditions.

Steady state is when the intermediate species (that do not contribute to overall reaction stoichiometry) concentrations have reached constant value, at least momentarily.

$$\frac{d[Intermediate]}{dt} = 0$$

Consider the following equation:

$$A \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} B \xrightarrow{k_2} C \tag{13}$$

In this equation, the intermediate is B, and the overall reaction stoichiometry is $A \longrightarrow C$. Based on equation 13, we can then state that:

$$\frac{d[B]}{dt} = k_1[A] - k_{-1}[B] - k_2[B]$$

$$= k_1[A] - [B](k_{-1} + k_2) = 0$$

Now rearrange to isolate [B]:

$$[\boldsymbol{B}] = \frac{\boldsymbol{k_1[A]}}{\boldsymbol{k_2 + k_1}} \tag{14}$$

Now go back to the original reaction scheme. The overall reaction velocity is $\boldsymbol{v = \frac{d[C]}{dt}}$, but we know that $\boldsymbol{\frac{d[C]}{dt} = k_2[B]}$. Substituting [B] from equation 14 shows that:

$$\boldsymbol{v = \frac{k_1 k_2[A]}{k_2 + k_{-1}}} \tag{15}$$

From the equation 15 above, this 2-step reaction behaves as first order in [A] at steady state.

Enzyme Kinetics under a steady-state assumption

The following model is used to treat kinetics of a simple unimolecular enzyme reaction:

$$\boldsymbol{E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_{cat}} E + P} \tag{16}$$

E is the free enzyme, S is the free/unbound substrate, ES is the enzyme-substrate complex, and P is the product. $\boldsymbol{\frac{k_1}{k_{-1}}}$ describes how the tightly the enzyme binds the substrate. $\boldsymbol{K_{cat}}$ describes the catalytic rate constant for formation of P.

The velocity of the overall reaction is described by $\boldsymbol{v = \frac{d[P]}{t}}$. According to the rate law, $\boldsymbol{v = k_{cat}[ES]}$. [ES] is an intermediate, and so we need to replace [ES] by adopting steady state assumption (where $\boldsymbol{\frac{d[ES]}{dt} = 0}$).

$$\boldsymbol{\frac{d[ES]}{dt} = k_1[E][S] - (k_{-1} + k_{cat})[ES] = 0}$$

$$\boldsymbol{[ES] = \frac{k_1[E][S]}{k_{-1} + kcat}}$$

Now plug in [ES] from above into $\boldsymbol{v = k_{cat}[ES]}$:

$$\boldsymbol{v = \frac{k_{cat} k_1[E][S]}{k_{-1} + k_{cat}}} \tag{17}$$

This equation is a start, but it only describes the reaction velocity in terms of the free enzyme concnetration. In an experiment, we usually only have control over the total enzyme concnetratoin. We want to rewrite the equation in terms of total enzyme concentration and in terms of the ratio of the reaction velocity to the maximum possible value ($[\boldsymbol{ES}] = [\boldsymbol{E}]_{\boldsymbol{total}}$) The maximum velocity is $\boldsymbol{k_{cat}}$ $\times$ max value for $[\boldsymbol{ES}]$, which is:

$$\boldsymbol{v_{max} = k_{cat}[E]_{total} = k_{cat}([ES] + [E])} \tag{18}$$

The ratio of reaction velocities relative to its maximum is given:

$$\boldsymbol{\frac{v}{V_{max}} = \frac{k_{cat}[ES]}{k_{cat}([E] + [ES])}}$$

$$\boldsymbol{\frac{v}{V_{max}} = \frac{[ES]}{([ES] + [E])}}$$

$$\boldsymbol{\frac{v}{V_{max}} = \frac{1}{1 + \frac{[E]}{[ES]}}}$$

Taking the previous equation $\boldsymbol{[ES] = \frac{k_1[E][S]}{k_{-1}+k_{cat}}}$ and rearranging so that it becomes $\boldsymbol{\frac{[E]}{[ES]} = \frac{k_{-1}+k_{cat}}{k_1[S]}}$, substitute this equation above to give:

$$\boldsymbol{\frac{v}{V_{max}} = \frac{1}{1 + \frac{k_{-1}+k_{cat}}{k_1[S]}}}$$

$$\boldsymbol{\frac{v}{V_{max}} = \frac{[S]}{[S] + \frac{k_{-1}+k_{cat}}{k_1[S]}}}$$

$$\boldsymbol{\frac{v}{V_{max}} = \frac{[S]}{[S] + K_M} \longrightarrow K_M = \frac{k_{-1} + k_{cat}}{k_1}}$$

Or, we can also convert from fractional velocity to v using equation 18 for $\boldsymbol{V_{max}}$:

$$\boldsymbol{v = \frac{k_{cat}[E]_{total}[S]}{[S] + K_M}} \tag{19}$$

Relaxation kinetics: how systems approach equilibrium

The T-Jump Method

The temperature jump method was developed to study fast reactions and their "relaxation" back towards equilibrium. If energy is rapidly delivered to a solution containing reactant and product at equilibrium, the temperature of the system can be heated nearly instantaneously. Recalling the **van't Hoff equation**, if **Δ**H for the reaction is non-zero, then the equilibrium constant will be different at a new temperature. Then you can observe how fast the reaction returns to its new equilibrium.

The speed at which equilbrium is achieved depends on the forward and backward rate constants of the reaction.

Consider a simple system:

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

Let **x** be the distance of each species from equilibrium. $\bar{A}$ will be $[A]_{equilbrium}$ at the new temperature, same for $\bar{B}$. The concentrations of A and B are related to the equilibrium conc. by:

$$[A] = \bar{A} + x$$
$$[B] = \bar{B} - x$$

Now examine the approach to equlibrium by writing an equation for time dependence of **x**.

$$\frac{d[A]}{dt} = \frac{d[\bar{A} + x]}{dt} = \frac{dx}{dt}$$

$$\frac{d[A]}{dt} = -k_1[A] + k_{-1}[B]$$

$$\frac{d[A]}{dt} = -k_1[\bar{A} + x] + k_{-1}[\bar{B} - x]$$

$$= k_{-1}\bar{B} - k_{-1}\bar{A} - x(k_1 + k_{-1})$$

The term $k_{-1}\bar{B} - k_{-1}\bar{A} = 0$ because $\frac{\bar{B}}{\bar{A}} = K = \frac{k_1}{k_{-1}}$

So, after setting the above to 0, the equation yields:

$$\frac{dx}{dt} = -(k_1 + k_{-1})x \tag{20}$$

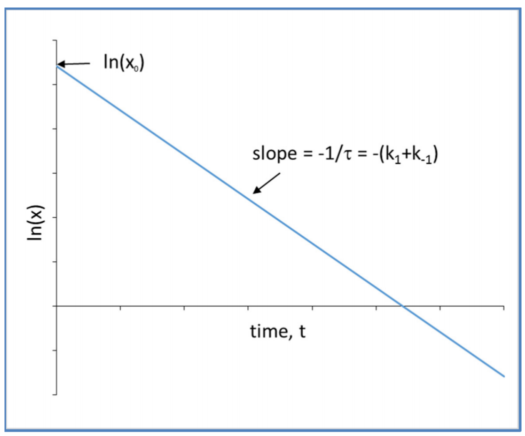
X follows first order kinetics. Skipping the familiar detials for handling a first order differential equation:

$$x = x_o^{-(k_1+k_{-1})} \text{ and } \ln(\frac{x}{x_0}) = -(k_1 + k_{-1})t \tag{21}$$

Convert these to even more general forms:

$$x = x_0 e^{\frac{-t}{\tau}} \text{ and } \ln(\frac{x}{x_0}) = \frac{-t}{\tau} \tag{22}$$

where $\tau = \frac{1}{k_1+k_{-1}}$



If you can find [A] or [B] as a function of time, then you can also find **x** as a function of time.

$\mathbf{x} = [A] - \bar{A} = \bar{B} - [B]$

You can use this to measure τ, and thus also $k_1 + k_{-1}$

$$1/\tau = k_1 + k_{-1} \text{ and } k_1 = Kk_{-1}$$

$$1/\tau = Kk_{-1} + k_{-1} = (K+1)k_{-1}$$

$$k_{-1} = \frac{1}{\tau(K+1)} \text{ and } k_1 = \frac{K}{\tau(K+1)}$$

Higher Order Reactions Approaching Equilibrium

Now consider this second order reversible reaction:

$$\boldsymbol{A + B \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} C}$$

There is only one transformation, st he distance can still be described by **x**:

$$\boldsymbol{[A] = \bar{A} + x}$$

$$\boldsymbol{[B] = \bar{B} + x}$$

$$\boldsymbol{[C] = \bar{C} - x}$$

We can use the same approach from the above to describe the change in concentrations:

$$\boldsymbol{\frac{d[A]}{dt} = \frac{d\bar{A} + x}{dt} = \frac{dx}{dt}}$$

$$\boldsymbol{\frac{d[A]}{dt} = -k_1(\bar{A} + x)(\bar{B} + x) + k_{-1}(\bar{C} - x)}$$

$$\boldsymbol{= k_{-1}\bar{C} - k_1\bar{A}\bar{B} - x(k_1(\bar{A} + \bar{B}) + k_{-1}) - k_1x^2}$$

$\boldsymbol{k_{-1}\bar{C} - k_1\bar{A}\bar{B}}$ cancels to 0, and if we are close enough to equilibrium then x is small, so we can neglect the $\boldsymbol{x^2}$ term. So,

$$\boldsymbol{\frac{dx}{dt} = -(k_1(\bar{A} + \bar{B}) + k_{-1})x} \tag{23}$$

So, the distance from equilibrium x shows first order behavior close to equilibrium, with $\boldsymbol{\tau = \frac{1}{k_1(\bar{A}+\bar{B})+k_{-1}}}$

Kinetics from Single Molecule Studies