APPENDIX 1

Kinetics and Thermodynamics

Kinetics and thermodynamics are two important fundamental branches of sciences which helps us to understand many physical process at molecular as well as macro level. As the name suggests kinetics deals with processes that changes with respect to time, for example chemical reactions, growth of organism, motion of an object, distribution of drug in body etc... On the other hand thermodynamics deals with the energy involved with such procesess to happen.

In this appendix, we will see in detail how kinetics can be approached with the help of differential equations and see some application in enzyme kinetics to calrify the concepts. Further the explicit relationship between kinetics and thermodynamics will be made clear in the latter part of this chapter.

Let us consider a person running from one point to another(A to B).In the begining he/she starts fast and as he/she reaches the finish point his/her speed will be slower compared to the initial speed. So we can say that the speed at each point within the distance A to B varies with respect to the distance travelled as well as the time. The velocity or speed measured at each point would be called the instantaneous velocity, whereas the ratio of total distance travelled to the time taken, would be the average velocity.

In order to measure the instantaneous velocity, we have to measure the small distance travelled at each point and divide it by the time taken for it. If we make that time infinitisimally small such that it approaches zero, then the velocity

can be represented as$\frac{dx}{dt}$, whereas a large time gap would result in averaged velocity for that time interval $\frac{\Delta x}{\Delta t}$.

The same argument can extrapolated to chemical reactions, where the focus would be on the rate of product formation. We will proceed with a general methodology, which is listed as follows,

- Proposing a mechanism
- Framing differential equation
- Solving the differential equations with or without assumption
- Determining the kinetic parameters by model fitting

1.1 Proposing a mechanism

The mechanism gives the details of how reactant are getting into products and what sort of reaction enables it(reversible or irreversible). For example a simple irreversible and reversible reaction of reactant A getting into B would be

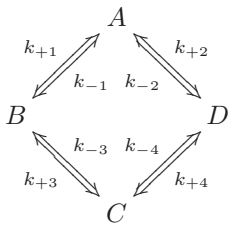
$$A \xrightarrow{k_{+1}} B \tag{1.1}$$

$$A \underset{k_{-1}}{\overset{k_{+1}}{\rightleftharpoons}} B \tag{1.2}$$

Similarly a mechanism with an intermediate [B] would be

$$A \underset{k_{-1}}{\overset{k_{+1}}{\rightleftharpoons}} B \underset{k_{-2}}{\overset{k_{+2}}{\rightleftharpoons}} C \tag{1.3}$$

A cyclic mechanism would be



(1.4)

It is important to propose a mechanism as realistic as possible to the existing physical system at hand. Simpler mechanism are always preferred in comparison to the complex mechanisms.

1.2 Framing the differential equations

Differential equations can be framed for every species involved in the mechanism. For example, considering (1.1), the reaction involves A and B, the rate of change of A and B can be written as follows,

$$\frac{dA}{dt} = -1 \times k_{+1} \times [A] \tag{1.5}$$

$$\frac{dB}{dt} = +1 \times k_{+1} \times [A] \tag{1.6}$$

Here we have three components for (1.5), -1,k_{+1},[A]which are multiplied togeather. If concentration of [A] is higher, the forward reaction will proceed resulting in more product formation (Le-chaterlier principle). so we can write this as

$$\frac{dA}{dt} \propto [A] \tag{1.7}$$

now we introduce a propotionality constant k_{+1} for equating

$$\frac{dA}{dt} = k_{+1} \times [A] \tag{1.8}$$

On considering that the forward reaction is going to decrease the concentration of [A], we multiply a prefactor of -1 to the R.H.S. which becomes,

$$\frac{dA}{dt} = -1 \times k_{+1} \times [A] \tag{1.9}$$

To clarify this further, we will consider the mechanism (1.2), here 'A' is associated with two reactions A→B (forward) and A←B (reverse), both of which affect the concentration of 'A', thereby the rate of concentration of 'A'. So the differential equation for [A] can be framed as follows,

$$\frac{dA}{dt} = \underbrace{(-1 \times k_{+1} \times [A])}_{1} + \underbrace{(+1 \times k_{-1} \times [B])}_{2} \tag{1.10}$$

The first part of R.H.S is the same as explained above, but the second part says that the reaction B←A is going to increase the concentration of 'A', so a prefactor of +1 is multiplied to the corresponding rate constant k_{-1} and the reactant 'B'. Similar expression can be written for more compliacted species like 'C' in (1.4),

$$\frac{dC}{dt} = \underbrace{(+1 \times k_{+3} \times [B])}_{1} + \underbrace{(-1 \times k_{-3} \times [C])}_{2} + \underbrace{(+1 \times k_{+4} \times [D])}_{3} + \underbrace{(-1 \times k_{-4} \times [C])}_{4} \tag{1.11}$$

If there are more than one reactants then the reactants are multiplied togeather,

as shown in the following model.

$$A + nB \underset{k_{-1}}{\overset{k_{+1}}{\rightleftharpoons}} C \tag{1.12}$$

$$\frac{dC}{dt} = \underbrace{(+1 \times k_{+1} \times [A] \times ([B] \times [B] \times ... \; n \; times))}_{1} + \underbrace{(-1 \times k_{-1} \times [C])}_{2} \tag{1.13}$$

$$\frac{dC}{dt} = \underbrace{(+1 \times k_{+1} \times [A] \times [B]^n)}_{1} + \underbrace{(-1 \times k_{-1} \times [C])}_{2} \tag{1.14}$$

1.3 Solving the differential equations with or without steady state assumption

If there are 'n' species present in a mechanism, we need minimum 'n' coupled differential equations to solve them. What we finally get out of solving these equations is the expression for each species in terms of time 't'.When we assume steady state for all the species or only for certain species,solving them becomes easier compared to non-steady state condition.

1.3.1 Solving under steady-state assumption

Steady-state assumption for a particular species means that its concentration does not vary with repect to time. Under such case its differential equation can be equated to zero. The validity of such an assumption depends on the state of the system. For example, when a system is in equillibrium, the reactants, intermediates and the products are said to be in steady state. We will consider the mechanism (1.3), as an example to derive the apropriate expressions.

$$\frac{dA}{dt} = A' \qquad = -k_{+1}[A] + k_{-1}[B] \tag{1.15}$$

$$\frac{dB}{dt} = B' \qquad = +k_{+1}[A] - k_{-1}[B] - k_{+2}[B] + k_{-2}[C] \tag{1.16}$$

$$\frac{dC}{dt} = C' \qquad = +k_{+2}[B] - k_{-2}[C] \tag{1.17}$$

in matrix form the above equations can be written as

$$\begin{bmatrix} -k_{+1} & +k_{-1} & 0 \\ +k_{+1} & -(k_{-1}+k_{+2}) & k_{-2} \\ 0 & +k_{+2} & -k_{-2} \end{bmatrix} \begin{bmatrix} A \\ B \\ C \end{bmatrix} = \begin{bmatrix} A' \\ B' \\ C' \end{bmatrix} \tag{1.18}$$

Since we are assuming equillibrium A' = B' = C' = 0,then we have

$$\begin{bmatrix} -k_{+1} & +k_{-1} & 0 \\ +k_{+1} & -(k_{-1}+k_{+2}) & k_{-2} \\ 0 & +k_{+2} & -k_{-2} \end{bmatrix} \begin{bmatrix} A \\ B \\ C \end{bmatrix} = \begin{bmatrix} 0 \\ 0 \\ 0 \end{bmatrix} \tag{1.19}$$

expressing all the species interms of any one of the species (in our case 'A'), using (1.15) and (1.17) we have

$$B = \frac{k_{+1}}{k_{-1}}[A] \tag{1.20}$$

$$C = \frac{k_{+2}}{k_{-2}}[B] \tag{1.21}$$

substituting (1.20) into (1.21)

$$C = \frac{k_{+2}}{k_{-2}} . \frac{k_{+1}}{k_{-1}} . [A] \tag{1.22}$$

And finally using the fact (principle of conservation of mass) that the total amount of reactant is

$$A_T = A + B + C \tag{1.23}$$

$$\therefore A_T = A + \frac{k_{+1}}{k_{-1}}[A] + \frac{k_{+2}}{k_{-2}}.\frac{k_{+1}}{k_{-1}}.[A] \tag{1.24}$$

in L.H.S taking out 'A' as common and rearranging, we have

$$A = \frac{A_T}{1 + K_1 + K_2K_1} \quad where K_1 = \frac{k_{+1}}{k_{-1}} \quad and\ K_2 = \frac{k_{+2}}{k_{-2}} \tag{1.25}$$

similarly substituting the value of A into (1.20) and (1.22) we have

$$B = \frac{K_1A_T}{1 + K_1 + K_2K_1} \tag{1.26}$$

$$C = \frac{K_1K_2A_T}{1 + K_1 + K_2K_1} \tag{1.27}$$

The results shows that concentration of A,B,C will not vary with respect to time as there are no time variable 't' in the final expressions. Solving (1.18) under partial steady state,(i.e. only certain species's concentration does'nt vary with respect to time) is actually a subset of solving the same under non-steady state condition.

1.3.2 Solving under non steady-state condition

Before we solve (1.18) we need to get familiar with two important mathematical routines.

1. Solving differential equation by variable seperable method

2. Diagonalisation of a matrix

1.3.3 Variable seperable method

Consider the mechanism (1.1), where the differential form of A is given as

$$\frac{dA}{dt} = -k_{+1}[A] \tag{1.28}$$

The above equation has only two variables A,t though the mechanism has three variable A,B,t. Such equations can be easily solved by seperating one to the R.H.S and the other to the L.H.S.thus,

$$\frac{dA}{A} = -k_{+1}dt \tag{1.29}$$

Taking integration on both sides

$$\int \frac{dA}{A} = \int -k_{+1}\ dt \tag{1.30}$$

$$ln[A] = -k_{+1}\ t + constant \tag{1.31}$$

to determine the value of constant, using boundary condition, that at time t = 0, A = A_0

$$ln[A_0] = -k_{+1} \times 0 + constant \tag{1.32}$$

$$\therefore constant = ln[A_0]$$

$$A = A_0 e^{-k_{+1}t} \tag{1.33}$$

Using the above expression the profile of 'A' can be obtained by plugging different values of 't' and setting the constants k_{+1},A_0 at arbitrary values. This method will not be applicable if the given differential equation has more than two variables and are combined linearly(added or substracted).We will adopt the above methodology, while solving a set of coupled differential equations as

in (1.18).

1.3.4 Diagonalisation of matrix

Given a square matrix as shown below, it is possible to represent it as a product of three matrices with middle matrix being diagonalised.Diagonolisation is a process by which all the elements of a matrix except the right diagonal becomes zero.The elements of the diagonal matrix are called the Eigenvalues and the matrix multiplied to the left of the diagonal matrix is called the Eigenvector matrix. The matrix at the right of diagonal matrix is the inverse of the Eigenvector matrix.

$$\begin{bmatrix} a1 & b1 & c1 \\ a2 & b2 & c2 \\ a3 & b3 & c3 \end{bmatrix} = \begin{bmatrix} A1 & B1 & C1 \\ A2 & B2 & C2 \\ A3 & B3 & C3 \end{bmatrix} \begin{bmatrix} \lambda_1 & 0 & 0 \\ 0 & \lambda_2 & 0 \\ 0 & 0 & \lambda_3 \end{bmatrix} \begin{bmatrix} A1 & B1 & C1 \\ A2 & B2 & C2 \\ A3 & B3 & C3 \end{bmatrix}^{-1} \tag{1.34}$$

The above matrices can be compactly written for better representation as

$$[\mathbf{a}] = [\mathbf{U}][\lambda][\mathbf{U}]^{-\mathbf{1}} \tag{1.35}$$

1.3.5 Solutions for coupled differential equations

Rewriting (1.18) in a compact matrix notation (as mentioned above),

$$[\mathbf{M}'] = [\mathbf{C}][\mathbf{M}] \tag{1.36}$$

where M',C,M are matrices of (1.18) written from right to left. This form resembeles the differential form of(1.28).Infact the same approach can be adopted

and the solution for such matrix forms can be given as,

$$\mathbf{M} = \mathbf{e}^{[\mathbf{C}]\mathbf{t}}\mathbf{M_0} \tag{1.37}$$

where M,M_0 and C represents matrices, please take note that the order of multiplication matters in matrix form, but not important for scalar forms like(1.33). The only difficulty in evaluating the above expression is the calculation of exponential of the matrix C. Though the exponentials can be expanded by taylor series and evaluated upto second or third order terms.The better way is to diagonalize the C matrix and proceed as follows,

$$[\mathbf{C}] = [\mathbf{U}][\lambda][\mathbf{U}]^{-\mathbf{1}} \tag{1.38}$$

$$\mathbf{e}^{[\mathbf{C}]\mathbf{t}} = [\mathbf{U}]\mathbf{e}^{[\lambda]\mathbf{t}}[\mathbf{U}]^{-\mathbf{1}} \text{(Obtained using standard formula)} \tag{1.39}$$

$$\therefore [\mathbf{M}] = [\mathbf{U}]\mathbf{e}^{\lambda\mathbf{t}}[\mathbf{U}]^{-\mathbf{1}}[\mathbf{M_0}] \tag{1.40}$$

Representing the elements of each matrix explicitly we have,

$$\begin{bmatrix} A \\ B \\ C \end{bmatrix} = \begin{bmatrix} A1 & B1 & C1 \\ A2 & B2 & C2 \\ A3 & B3 & C3 \end{bmatrix} \begin{bmatrix} e^{\lambda_1 t} & 0 & 0 \\ 0 & e^{\lambda_2 t} & 0 \\ 0 & 0 & e^{\lambda_3 t} \end{bmatrix} \begin{bmatrix} A1' & B1' & C1' \\ A2' & B2' & C2' \\ A3' & B3' & C3' \end{bmatrix} \begin{bmatrix} A_0 \\ B_0 \\ C_0 \end{bmatrix} \tag{1.41}$$

$$\begin{bmatrix} A \\ B \\ C \end{bmatrix} = \begin{bmatrix} A1 * e^{\lambda_1 t} & B1 * e^{\lambda_2 t} & C1 * e^{\lambda_3 t} \\ A2 * e^{\lambda_1 t} & B2 * e^{\lambda_2 t} & C2 * e^{\lambda_3 t} \\ A3 * e^{\lambda_1 t} & B3 * e^{\lambda_2 t} & C3 * e^{\lambda_3 t} \end{bmatrix} \begin{bmatrix} A1' & B1' & C1' \\ A2' & B2' & C2' \\ A3' & B3' & C3' \end{bmatrix} \begin{bmatrix} A_0 \\ B_0 \\ C_0 \end{bmatrix} \tag{1.42}$$

$$\begin{bmatrix} A \\ \\ B \\ \\ C \end{bmatrix} = \begin{bmatrix} A1'A1e^{\lambda_1 t} + A2'B1e^{\lambda_2 t} + A3'C1e^{\lambda_3 t} & B1'A1e^{\lambda_1 t} + B2'B1e^{\lambda_2 t} + B3'C1e^{\lambda_3 t} \\ & C1'A1e^{\lambda_1 t} + C2'B1e^{\lambda_2 t} + C3'C1e^{\lambda_3 t} \\ A1'A2e^{\lambda_1 t} + A2'B2e^{\lambda_2 t} + A3'C2e^{\lambda_3 t} & B1'A2e^{\lambda_1 t} + B2'B2e^{\lambda_2 t} + B3'C2e^{\lambda_3 t} \\ & C1'A2e^{\lambda_1 t} + C2'B2e^{\lambda_2 t} + C3'C2e^{\lambda_3 t} \\ A1'A3e^{\lambda_1 t} + A2'B3e^{\lambda_2 t} + A3'C3e^{\lambda_3 t} & B1'A3e^{\lambda_1 t} + B2'B3e^{\lambda_2 t} + B3'C3e^{\lambda_3 t} \\ & C1'A3e^{\lambda_1 t} + C2'B3e^{\lambda_2 t} + C3'C3e^{\lambda_3 t} \end{bmatrix} \begin{bmatrix} A_0 \\ \\ B_0 \\ \\ C_0 \end{bmatrix} \tag{1.43}$$

$$\begin{bmatrix} A \\ \\ B \\ \\ C \end{bmatrix} = \begin{bmatrix} A_0[A1'A1e^{\lambda_1 t} + A2'B1e^{\lambda_2 t} + A3'C1e^{\lambda_3 t}] & +B_0[B1'A1e^{\lambda_1 t} + B2'B1e^{\lambda_2 t} + B3'C1e^{\lambda_3 t}] \\ & +C_0[C1'A1e^{\lambda_1 t} + C2'B1e^{\lambda_2 t} + C3'C1e^{\lambda_3 t}] \\ A_0[A1'A2e^{\lambda_1 t} + A2'B2e^{\lambda_2 t} + A3'C2e^{\lambda_3 t}] & +B_0[B1'A2e^{\lambda_1 t} + B2'B2e^{\lambda_2 t} + B3'C2e^{\lambda_3 t}] \\ & +C_0[C1'A2e^{\lambda_1 t} + C2'B2e^{\lambda_2 t} + C3'C2e^{\lambda_3 t}] \\ A_0[A1'A3e^{\lambda_1 t} + A2'B3e^{\lambda_2 t} + A3'C3e^{\lambda_3 t}] & +B_0[B1'A3e^{\lambda_1 t} + B2'B3e^{\lambda_2 t} + B3'C3e^{\lambda_3 t}] \\ & +C_0[C1'A3e^{\lambda_1 t} + C2'B3e^{\lambda_2 t} + C3'C3e^{\lambda_3 t}] \end{bmatrix} \tag{1.44}$$

From the above equation we have obtained explicit expression relating A, B, C with the only variable time 't', and the rest constants like A1, A2, A3, B1,... which inturn are made up of different combinations of rate constants like k_{+1}, k_{-1}, k_{+2}, k_{-2} etc...

1.4 Application in Enzyme Kinetics

In many of the enzme kinetic problem, the main objective would be to determine the rate of product formation rather than predicting the profile of product concentration. Indirectly from the velocity or rate of product formation, it is possible to unravel the mechanism of enzyme reaction. The classical Michaelis-

Menten mechanism is given as follows,

$$E + S \underset{k_{-1}}{\overset{k_{+1}}{\rightleftharpoons}} ES \xrightarrow[\text{E}]{k_{+2}} + P \tag{1.45}$$

In the presence of inhibitors similar such mechanisms can be proposed according to the nature of interaction.For example, the mechanisms for competitive, non-competitive, un-competitive inhibition with reversible binding are given as follows Competitive Model:

$$E + S \underset{k_{-1}}{\overset{k_{+1}}{\rightleftharpoons}} [ES] \xrightarrow[\text{E}]{k_{+2}} + P \tag{1.46}$$

$$E + I \underset{k_{-1}}{\overset{k_{+1}}{\rightleftharpoons}} [EI] \tag{1.47}$$

Non-Competitive Model (Inhibitor binds to E even in presence of S):

$$E + S \underset{k_{-1}}{\overset{k_{+1}}{\rightleftharpoons}} [ES] \xrightarrow[\text{E}]{k_{+2}} + P \tag{1.48}$$

$$E + S + I \underset{k_{-1}}{\overset{k_{+1}}{\rightleftharpoons}} [ESI] \tag{1.49}$$

Non-Competitive Model (Inhibitor binds to E even in presence of S):

$$\begin{array}{ccccc} E + S & \underset{k_{+1}}{\overset{k_{-1}}{\Longleftrightarrow}} & [ES] & \longrightarrow & E + P \\ & & \Big\Updownarrow{\scriptstyle k_{-2}}_{+[I]k_{+2}} & & \\ & & [ESI] & & \end{array} \tag{1.50}$$

for irreversible mechanism just the forward reaction alone is considered ommitting the reverse reaction.

1.4.1 Michaelis-Mentin general enzymen kinetic model

$$E + S \underset{k_{-1}}{\overset{k_{+1}}{\rightleftharpoons}} ES \xrightarrow[\text{E}]{k_{+2}} + P \tag{1.51}$$

The differential equations are framed as follows,

$$\frac{dE}{dt} = -k_{+1}[E][S] + k_{-1}[ES] \tag{1.52}$$

$$\frac{d[ES]}{dt} = +k_{+1}[E][S] - k_{-1}[ES] - k_2[ES] \tag{1.53}$$

$$\frac{dP}{dt} = +k_2[ES] \tag{1.54}$$

We are interested in $\frac{dP}{dt}$, which is the rate of product formation (v).From equation (1.54),if we know the concentration of [ES], we can calculate 'v' easily. But it is very difficult to find $[ES]$ as it is transient, and difficult to measure experimentally. The other way arond would be to express $[ES]$ in terms of either [S] or [P] concentration. for example, when starch is hydrolysed by amylase, Iodimetry titrations can be carried to quantify the remaining starch substrate at different time intervals, or the hydrolysed product, glucose can be quantified by colorimetric methods.To express $[ES]$ in terms of $[S]$, we will make use of a valid assumption that the concentration of $[ES]$, does not vary with respect to time. So, we can write (1.53) as

$$\frac{d[ES]}{dt} = +k_{+1}[E][S] - k_{-1}[ES] - k_2[ES] = 0$$

On rearranging

$$[E] = \frac{(k_{+1} + k_2)[ES]}{k_{+1}[S]} \tag{1.55}$$

Now we know that $[E]$, the free enzyme concentration is also difficult to measure, so we use the principle of conservation of mass to exress $[ES]$ in terms of $[E]_T$

(Total enzyme is the sum of free and bound enzyme concentration),

$$[E_T] = [E] + [ES] \tag{1.56}$$

$$= \frac{(k_{+1} + k_2)[ES]}{k_{+1}[S]} + [ES]$$

$$= [ES]\left(\frac{(k_{+1} + k_2)}{k_{+1}[S]} + 1\right)$$

$$\therefore [ES] = \frac{E_T}{1 + \frac{k_{-1} + k_2}{k_{+1}[S]}} \tag{1.57}$$

Substituting (1.57) into (1.54), we have

$$v = k_2 \frac{E_T}{1 + \frac{k_{-1} + k_2}{k_{+1}[S]}} \tag{1.58}$$

In the ratio $\frac{k_{-1}+k_2}{k_{+1}}$, the Numerator is actually the sum of the rate constants of two reactions that dissociates [ES] into either back to substrate or product.

$$E + S \xleftarrow{k_{-1}} ES \xrightarrow{k_2} E + P$$

Whereas k_{+1} is the rate constant that brings about association of [ES].

$$E + S \xrightarrow{k_{+1}} [ES]$$

Thus the above fraction is the ratio of sum of dissociation rate constants to the association rate constant.In general this is representedtermed as K_D, the

'Dissociation constant'.

$$v = k_2 \frac{E_T}{1 + \frac{k_D}{[S]}} \tag{1.59}$$

$$= k_2 \frac{E_T[S]}{[S] + k_D} \tag{1.60}$$

The term $k_2 E_T$ is similar to $v = k_2[ES]$, except for the difference that E_T, is in the place of bound enzyme$[ES]$. Which means 'v' is propotional to bound enzyme and if all the enzyme are present in bound form, then that is the maximum velocity that could be reached for the given E_T.

$$v' = k_2[ES] \tag{1.61}$$

$$in\ [E_T] = [E] + [ES],\ if\ [E] = 0,\ (i.e.\ no\ free\ enzyme\ present), then\ [E_T] = [ES]$$

$$= k_2[E_T] = v_{max} \quad Substituting\ back\ into\ (1.60) \tag{1.62}$$

$$v = \frac{v_{max}[S]}{K_D + [S]} \tag{1.63}$$

1.4.2 Competitive models

1.5 Thermodynamics

1.5.1 Gibb's Free energy, Activity, Equillibrim constants

1.5.2 Enthalpy and entropy

1.5.3 Partial derivatives... & Maxwell equations