

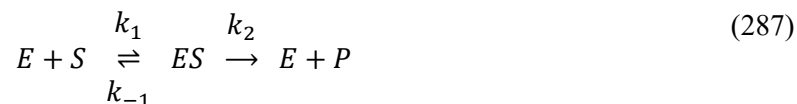
❖ Competitive and Non-Competitive Inhibition

An enzyme inhibitor is a compound that binds to an enzyme and decreases its overall activity, and the phenomenon is typically known as “enzyme inhibition”. Two of the most common enzyme inhibition processes will be discussed in this section.

➤ Competitive Inhibition

In the case of competitive enzyme inhibition, the binding of an inhibitor prevents the binding of the substrate and the enzyme. This type of behavior is actually achieved by blocking the binding site of the target molecule (the active site) by some means. The competitive enzyme inhibition can be classified into two types as discussed below.

1. Fully competitive inhibition: The fully competitive inhibition occurs when an enzyme (E) binds with the substrate (S) and inhibitor (I) separately, and it is only the enzyme-substrate complex (ES) that will convert into the product. This whole process can be described mathematically as



After applying the steady-state approximation on ES , we have

$$\frac{d[ES]}{dt} = k_1[E][S] - k_{-1}[ES] - k_2[ES] = 0 \quad (289)$$

$$k_1[E][S] = k_{-1}[ES] + k_2[ES] \quad (290)$$

$$[ES] = \frac{k_1[E][S]}{k_{-1} + k_2} \quad (291)$$

When $[S_0] \gg [E_0]$, we can assume $[S_0] \approx [S]$, and also

$$[E_0] = [E] + [ES] + [EI] \quad (292)$$

$$[E] = [E_0] - [ES] - [EI] \quad (293)$$

Now recalling the equilibrium constant for inhibition equilibria i.e.

$$K_3 = \frac{k_3}{k_{-3}} = \frac{[EI]}{[E][I]} \quad (294)$$

$$K_1 = \frac{1}{K_3} = \frac{[E][I]}{[EI]} \quad (295)$$

Hence, we can say

$$[EI] = \frac{[E][I]}{K_1} \quad (296)$$

After using the value of $[EI]$ from equation (296) into equation (293), we have

$$[E] = [E_0] - [ES] - \frac{[E][I]}{K_1} \quad (297)$$

$$[E] = \frac{K_1[E_0] - K_1[ES] - [E][I]}{K_1} \quad (298)$$

$$K_1[E] = K_1[E_0] - K_1[ES] - [E][I] \quad (299)$$

$$K_1[E] + [E][I] = K_1[E_0] - K_1[ES] \quad (300)$$

$$[E] = \frac{K_1[E_0] - K_1[ES]}{K_1 + [I]} \quad (301)$$

Using the above-derived result in equation (291), we have

$$[ES] = \frac{k_1[S]}{k_{-1} + k_2} \frac{K_1[E_0] - K_1[ES]}{K_1 + [I]} = \frac{[S]}{K_m} \frac{K_1[E_0] - K_1[ES]}{K_1 + [I]} \quad (302)$$

$$[ES] = \frac{K_1[E_0][S] - K_1[ES][S]}{K_m K_1 + K_m [I]} \quad (303)$$

Now rearranging further for $[ES]$, we get

$$K_m K_1 [ES] + K_m [I] [ES] = K_1 [E_0] [S] - K_1 [ES] [S] \quad (304)$$

$$K_m K_1 [ES] + K_m [I] [ES] + K_1 [ES] [S] = K_1 [E_0] [S] \quad (305)$$

$$[ES] = \frac{K_1 [E_0] [S]}{K_m K_1 + K_m [I] + K_1 [S]} \quad (306)$$

Since the rate of formation of product is

$$r_0 = k_2 [ES] \quad (307)$$

Using the value of $[ES]$ from equation (306) in equation (307), we get

$$r_0 = \frac{k_2 K_1 [E_0] [S]}{K_m K_1 + K_m [I] + K_1 [S]} \quad (308)$$

Since $k_2 [E_0]$ is r_{max} , the equation (308) takes the form

$$r_0 = \frac{r_{max} K_I [S]}{K_m K_I + K_m [I] + K_I [S]} \quad (309)$$

Taking the reciprocal to get Lineweaver-Burk plot

$$\frac{1}{r_0} = \frac{K_m K_I + K_m [I] + K_I [S]}{r_{max} K_I [S]} \quad (310)$$

$$\frac{1}{r_0} = \frac{K_m K_I}{r_{max} K_I [S]} + \frac{K_m [I]}{r_{max} K_I [S]} + \frac{K_I [S]}{r_{max} K_I [S]} \quad (311)$$

$$\frac{1}{r_0} = \left(\frac{K_m}{r_{max}} + \frac{K_m [I]}{r_{max} K_I} \right) \frac{1}{[S]} + \frac{1}{r_{max}} \quad (312)$$

Rearranging further and using initial substrate concentration, we get

$$\frac{1}{r_0} = \frac{K_m}{r_{max}} \left(1 + \frac{[I]}{K_I} \right) \frac{1}{[S_0]} + \frac{1}{r_{max}} \quad (313)$$

After comparing with equation without enzyme inhibition i.e.

$$\frac{1}{r_0} = \frac{K_m}{r_{max}} \frac{1}{[S_0]} + \frac{1}{r_{max}} \quad (314)$$

it is clear that the intercepts in both the equations are the same whereas the slope has been increased from K_m/r_{max} to $(K_m/r_{max})(1 + [I]/K_I)$. This implies that enzyme inhibition changed the K_m to $K_m(1 + [I]/K_I)$.

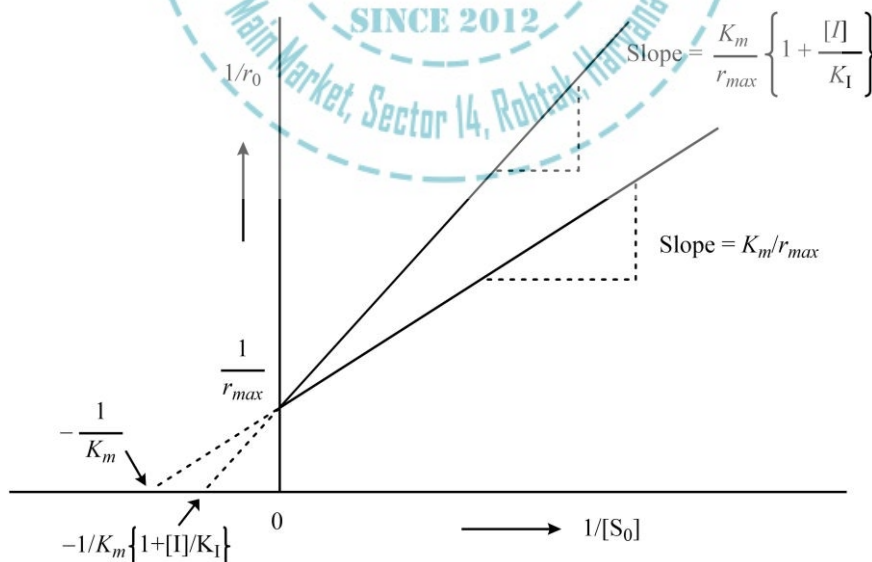


Figure 5. Lineweaver-Burk plot for enzyme-catalyzed reaction with fully competitive enzyme inhibition.

2. Partially competitive inhibition: The partially competitive inhibition occurs when the enzyme (E) binds with the substrate (S) and inhibitor (I) simultaneously, and complex ES and EI also combine with I and S to give EIS . Also, besides the enzyme-substrate complex (ES), the complex EIS will also convert into the product with the same rate of reaction. This whole process can be described mathematically as



According to classical Michaelis-Menten equation $K_m = (k_2 + k_{-1})/k_1$; however, if $k_2 \ll k_{-1}$, we have $K_m = k_{-1}/k_1$ or $K_m = 1/K'_1$. Now, set the following results

$$K_m = \frac{1}{K'_1} = \frac{[E][S]}{[ES]} \quad (320)$$

$$K_2 = \frac{1}{K'_2} = \frac{[E][I]}{[EI]} \quad (321)$$

$$K_3 = \frac{1}{K'_3} = \frac{[EI][S]}{[EIS]} \quad (322)$$

$$K_4 = \frac{1}{K'_4} = \frac{[ES][I]}{[EIS]} \quad (323)$$

Following enzyme conservation, we have

$$[E_0] = [E] + [ES] + [EI] + [EIS] \quad (324)$$

Using values of $[ES]$, $[EI]$ and $[EIS]$ from equation (320-322) into equation (324), we get

$$[E_0] = [E] + \frac{[E][S]}{K_m} + \frac{[E][I]}{K_2} + \frac{[E][I][S]}{K_2K_3} \quad (325)$$

$$[E] = \frac{K_m[E_0]}{K_m(1 + [I]/K_2) + [S](1 + K_m[I]/K_2K_3)} \quad (326)$$

The overall reaction rate of product formations should be

$$r_0 = k_2[ES] + k_2[EIS] \quad (327)$$

After using values of $[ES]$ and $[EIS]$ from equation (320, 322) into equation (327), we get

$$r_0 = k_2 \frac{[E][S]}{K_m} + k_2 \frac{[E][I][S]}{K_2 K_3} \quad (328)$$

Substituting the value of $[E]$ from equation (326) in (328), and rearranging at initial substrate concentration

$$r_0 = \frac{k_2[E_0][S_0]}{\{K_m(1 + [I]/K_2)/(1 + K_m[I]/K_2K_3)\} + [S_0]} \quad (329)$$

Since $k_2[E_0]$ is r_{max} , equation (329) takes the following form after the reciprocal to get Lineweaver-Burk plot

$$\frac{1}{r_0} = \frac{\{K_m(1 + [I]/K_2)/(1 + K_m[I]/K_2K_3)\}}{r_{max}} \frac{1}{[S_0]} + \frac{1}{r_{max}} \quad (330)$$

After comparing with equation without enzyme inhibition i.e.

$$\frac{1}{r_0} = \frac{K_m}{r_{max}} \frac{1}{[S_0]} + \frac{1}{r_{max}} \quad (331)$$

it is clear that intercepts in both the equations are the same whereas the slope has been varied from K_m/r_{max} to $\{K_m(1 + [I]/K_2)/(1 + K_m[I]/K_2K_3)\}/r_{max}$. This implies that enzyme inhibition changed the K_m to $\{K_m(1 + [I]/K_2)/(1 + K_m[I]/K_2K_3)\}$.

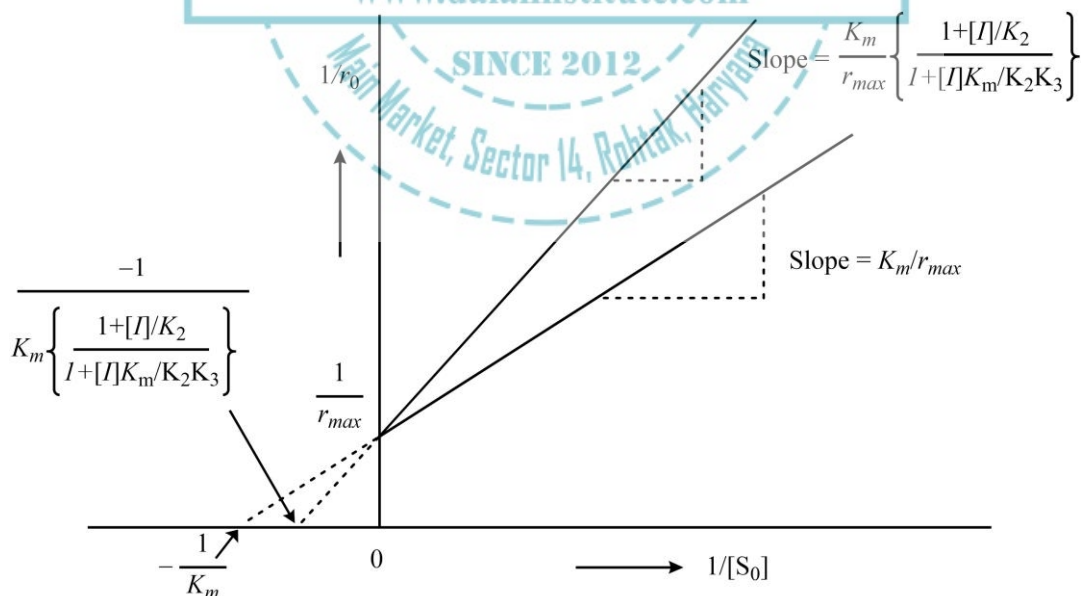


Figure 6. The Lineweaver-Burk plot for partially competitive enzyme inhibition.

➤ **Non-Competitive Inhibition**

Non-competitive inhibition may simply be defined as the enzyme inhibition where the inhibitor decreases the activity of the enzyme catalysis and binds equally well to the enzyme whether or not it has already bound the substrate. The non-competitive enzyme inhibition can be classified into two types as discussed below.

1. Fully non-competitive inhibition: The fully non-competitive inhibition occurs when an enzyme (E) binds with inhibitor (I), and complex ES and EI also combine with I and S to give EIS . However, it is only the enzyme-substrate complex (ES) that converts into the product. This whole process can be described mathematically as



According to classical Michaelis-Menten equation $K_m = (k_2 + k_{-1})/k_1$, however, if $k_2 \ll k_{-1}$, we have $K_m = k_{-1}/k_1$ or $K_m = 1/K'_1$. Now, set the following results

$$K_m = \frac{1}{K'_1} = \frac{[E][S]}{[ES]} = \frac{[E][S]}{[EIS]} \quad (336)$$

$$K_I = \frac{1}{K'_2} = \frac{[E][I]}{[EI]} = \frac{[ES][I]}{[EIS]} \quad (337)$$

Following enzyme conservation, we have

$$[E_0] = [E] + [ES] + [EI] + [EIS] \quad (338)$$

$$[E] = [E_0] - [ES] - [EI] - [EIS] \quad (339)$$

Using values of $[ES]$, $[EI]$ and $[EIS]$ from equation (336-337) into equation (338), we get

$$[E_0] = [E] + \frac{[E][S]}{K_m} + \frac{[E][I]}{K_I} + \frac{[E][I][S]}{K_m K_I} \quad (340)$$

$$[E] = \frac{[E_0]}{(1 + [S]/K_m)(1 + [I]/K_I)} \quad (341)$$

The overall reaction rate of product formations should be

$$r_0 = k_2[ES] \quad (342)$$

After using values of $[ES]$ from equation (336) into equation (342), we get

$$r_0 = k_2 \frac{[E][S]}{K_m} \quad (343)$$

Substituting the value of $[E]$ from equation (341) in (343), and rearranging at initial substrate concentration

$$r_0 = \frac{k_2[E_0][S_0]}{(K_m + [S_0])(1 + [I]/K_I)} \quad (344)$$

Since $k_2[E_0]$ is r_{max} , the equation (344) takes the form

$$r_0 = \frac{r_{max}[S_0]}{(K_m + [S_0])(1 + [I]/K_I)} \quad (345)$$

Taking the reciprocal to get Lineweaver-Burk plot

$$\frac{1}{r_0} = \frac{K_m}{r_{max}} \left(1 + \frac{[I]}{K_I}\right) \frac{1}{[S_0]} + \frac{1}{r_{max}} \left(1 + \frac{[I]}{K_I}\right) \quad (346)$$

After comparing with equation without enzyme inhibition i.e.

$$\frac{1}{r_0} = \frac{K_m}{r_{max}} \frac{1}{[S_0]} + \frac{1}{r_{max}} \quad (347)$$

it can be clearly seen that intercept is increased from $1/r_{max}$ to $(1/r_{max})(1 + [I]/K_I)$ whereas the K_m has remained the same.

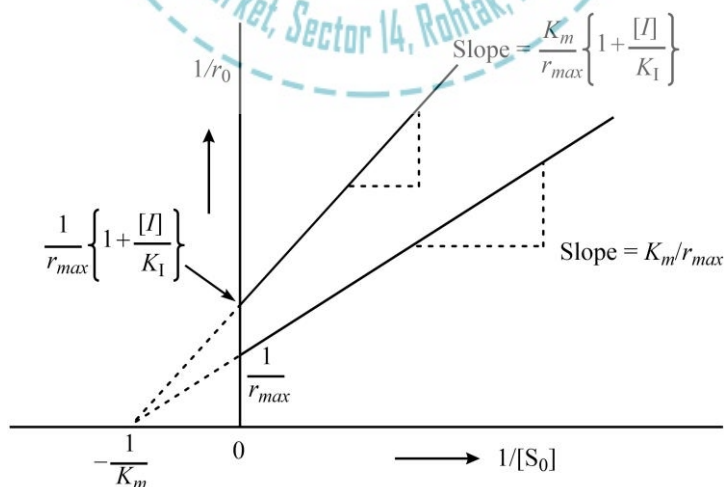
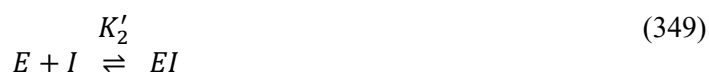


Figure 7. The Lineweaver-Burk plot for fully non-competitive enzyme inhibition.

2. Partially non-competitive inhibition: The partially non-competitive inhibition occurs when an enzyme (E) binds with inhibitor (I), and complex ES and EI also combine with I and S to give EIS . However, unlike fully non-competitive inhibition, besides the enzyme-substrate complex (ES), the complex will also convert into the product. This whole process can be described mathematically as



and



and



and



and



According to classical Michaelis-Menten equation $K_m = (k_2 + k_{-1})/k_1$; however, if $k_2 \ll k_{-1}$, we have $K_m = k_{-1}/k_1$ or $K_m = 1/K'_1$. Now, set the following results

$$K_m = \frac{1}{K'_1} = \frac{[E][S]}{[ES]} \quad (353)$$

and

$$K_I = \frac{1}{K'_2} = \frac{[E][I]}{[EI]} \quad (354)$$

and

$$K_m = \frac{1}{K'_1} = \frac{[EI][S]}{[EIS]} \quad (355)$$

and

$$K_I = \frac{1}{K'_2} = \frac{[ES][I]}{[EIS]} \quad (356)$$

Following enzyme conservation, we have

$$[E_0] = [E] + [ES] + [EI] + [EIS] \quad (357)$$

Using values of $[ES]$, $[EI]$ and $[EIS]$ from equation (353-356) into equation (357), we get the following expression.

$$[E_0] = [E] + \frac{[E][S]}{K_m} + \frac{[E][I]}{K_I} + \frac{[E][I][S]}{K_m K_I} \quad (358)$$

or

$$[E] = \frac{[E_0]}{(1 + ([S]/K_m)(1 + [I]/K_I))} \quad (359)$$

The overall reaction rate of product formations should be

$$r_0 = k_2[ES] + k'[EIS] \quad (360)$$

After using the values of $[ES]$ and $[EIS]$ from equation (353-356) into equation (360), we get the following result.

$$r_0 = k_2 \frac{[E][S]}{K_m} + k' \frac{[E][I][S]}{K_m K_I} \quad (361)$$

Now, substituting the value of $[E]$ from equation (359) in (361), and rearranging at the initial substrate concentration

$$r_0 = \frac{(k_2[E_0][S_0] + k'[E_0][S_0][I]/K_I)/(1 + [I]/K_I)}{K_m + [S_0]} \quad (362)$$

Using $k_2[E_0]$ is r_{max} and then taking the reciprocal to get the Lineweaver-Burk plot, we get the following relation.

$$\frac{1}{r_0} = \frac{K_m(1 + [I]/K_I)}{(r_{max} + k'[E_0][I]/K_I)} \frac{1}{[S_0]} + \frac{(1 + [I]/K_I)}{(r_{max} + k'[E_0][I]/K_I)} \quad (363)$$

After comparing the result given above with the general equation without enzyme inhibition, i.e., we can conclude some important points.

$$\frac{1}{r_0} = \frac{K_m}{r_{max}} \frac{1}{[S_0]} + \frac{1}{r_{max}} \quad (364)$$

it can be clearly seen that intercept is increased from $1/r_{max}$ to $(1 + [I]/K_I)/(r_{max} + k'[E_0][I]/K_I)$ whereas the K_m has remained the same.

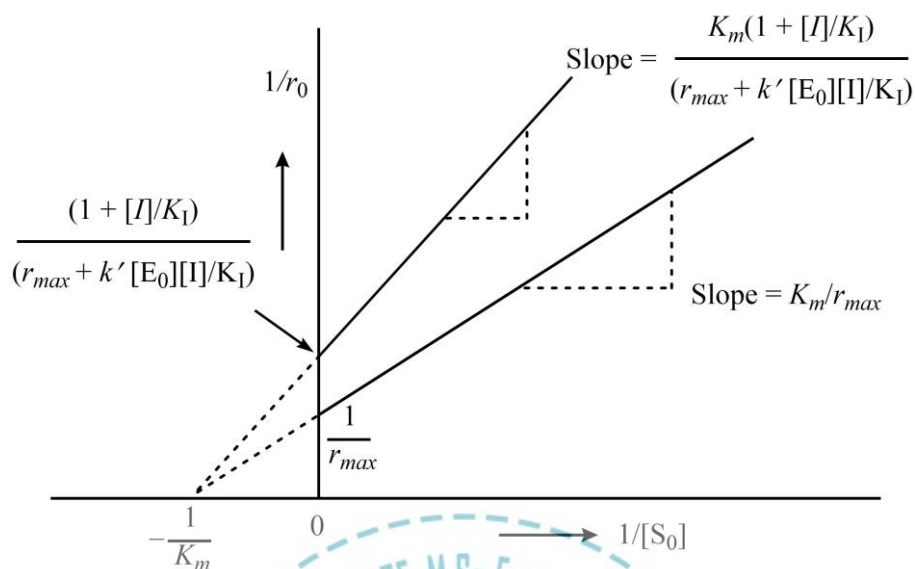


Figure 8. The Lineweaver-Burk plot for partially non-competitive enzyme inhibition.



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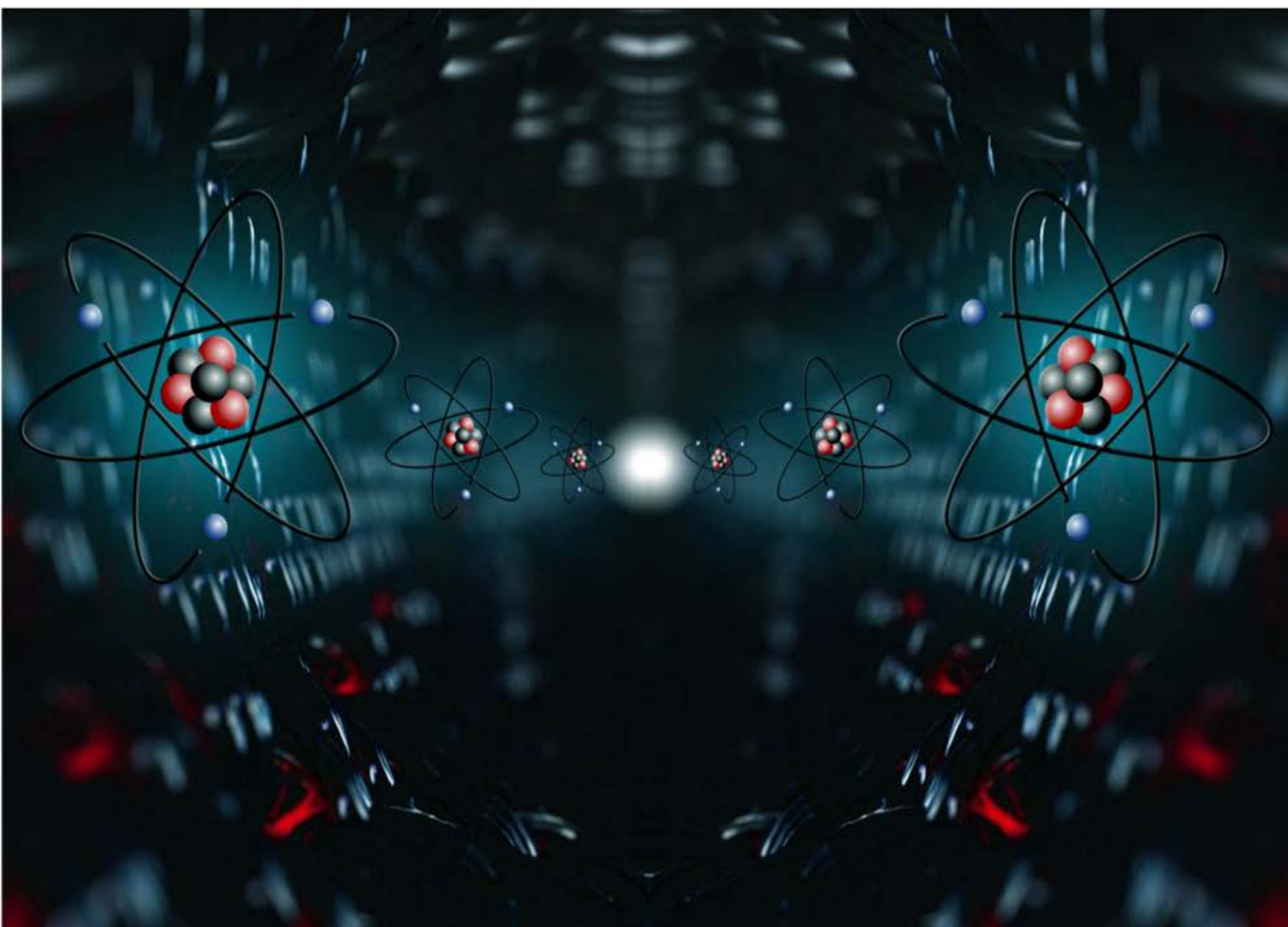
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Volume I

MANDEEP DALAL



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Table of Contents

CHAPTER 1	11
Quantum Mechanics – I	11
❖ Postulates of Quantum Mechanics	11
❖ Derivation of Schrodinger Wave Equation.....	16
❖ Max-Born Interpretation of Wave Functions	21
❖ The Heisenberg's Uncertainty Principle.....	24
❖ Quantum Mechanical Operators and Their Commutation Relations.....	29
❖ Hermitian Operators – Elementary Ideas, Quantum Mechanical Operator for Linear Momentum, Angular Momentum and Energy as Hermitian Operator	52
❖ The Average Value of the Square of Hermitian Operators	62
❖ Commuting Operators and Uncertainty Principle (x & p ; E & t).....	63
❖ Schrodinger Wave Equation for a Particle in One Dimensional Box.....	65
❖ Evaluation of Average Position, Average Momentum and Determination of Uncertainty in Position and Momentum and Hence Heisenberg's Uncertainty Principle.....	70
❖ Pictorial Representation of the Wave Equation of a Particle in One Dimensional Box and Its Influence on the Kinetic Energy of the Particle in Each Successive Quantum Level	75
❖ Lowest Energy of the Particle	80
❖ Problems	82
❖ Bibliography	83
CHAPTER 2	84
Thermodynamics – I	84
❖ Brief Resume of First and Second Law of Thermodynamics.....	84
❖ Entropy Changes in Reversible and Irreversible Processes.....	87
❖ Variation of Entropy with Temperature, Pressure and Volume	92
❖ Entropy Concept as a Measure of Unavailable Energy and Criteria for the Spontaneity of Reaction	94
❖ Free Energy, Enthalpy Functions and Their Significance, Criteria for Spontaneity of a Process ...	98
❖ Partial Molar Quantities (Free Energy, Volume, Heat Concept).....	104
❖ Gibb's-Duhem Equation.....	108
❖ Problems	111
❖ Bibliography	112

CHAPTER 3	113
Chemical Dynamics – I	113
❖ Effect of Temperature on Reaction Rates.....	113
❖ Rate Law for Opposing Reactions of 1st Order and 2nd Order.....	119
❖ Rate Law for Consecutive & Parallel Reactions of 1st Order Reactions	127
❖ Collision Theory of Reaction Rates and Its Limitations	135
❖ Steric Factor.....	141
❖ Activated Complex Theory	143
❖ Ionic Reactions: Single and Double Sphere Models	147
❖ Influence of Solvent and Ionic Strength.....	152
❖ The Comparison of Collision and Activated Complex Theory	157
❖ Problems.....	158
❖ Bibliography	159
CHAPTER 4	160
Electrochemistry – I: Ion-Ion Interactions	160
❖ The Debye-Huckel Theory of Ion-Ion Interactions	160
❖ Potential and Excess Charge Density as a Function of Distance from the Central Ion.....	168
❖ Debye-Huckel Reciprocal Length	173
❖ Ionic Cloud and Its Contribution to the Total Potential	176
❖ Debye-Huckel Limiting Law of Activity Coefficients and Its Limitations.....	178
❖ Ion-Size Effect on Potential.....	185
❖ Ion-Size Parameter and the Theoretical Mean - Activity Coefficient in the Case of Ionic Clouds with Finite-Sized Ions.....	187
❖ Debye-Huckel-Onsager Treatment for Aqueous Solutions and Its Limitations.....	190
❖ Debye-Huckel-Onsager Theory for Non-Aqueous Solutions.....	195
❖ The Solvent Effect on the Mobility at Infinite Dilution	196
❖ Equivalent Conductivity (Λ) vs Concentration $C^{1/2}$ as a Function of the Solvent	198
❖ Effect of Ion Association Upon Conductivity (Debye-Huckel-Bjerrum Equation)	200
❖ Problems.....	209
❖ Bibliography	210
CHAPTER 5	211
Quantum Mechanics – II	211
❖ Schrodinger Wave Equation for a Particle in a Three Dimensional Box	211

❖ The Concept of Degeneracy Among Energy Levels for a Particle in Three Dimensional Box	215
❖ Schrodinger Wave Equation for a Linear Harmonic Oscillator & Its Solution by Polynomial Method	217
❖ Zero Point Energy of a Particle Possessing Harmonic Motion and Its Consequence	229
❖ Schrodinger Wave Equation for Three Dimensional Rigid Rotator.....	231
❖ Energy of Rigid Rotator	241
❖ Space Quantization.....	243
❖ Schrodinger Wave Equation for Hydrogen Atom: Separation of Variable in Polar Spherical Coordinates and Its Solution	247
❖ Principal, Azimuthal and Magnetic Quantum Numbers and the Magnitude of Their Values.....	268
❖ Probability Distribution Function.....	276
❖ Radial Distribution Function	278
❖ Shape of Atomic Orbitals (<i>s</i> , <i>p</i> & <i>d</i>).....	281
❖ Problems.....	287
❖ Bibliography	288
CHAPTER 6	289
Thermodynamics – II.....	289
❖ Clausius-Clapeyron Equation.....	289
❖ Law of Mass Action and Its Thermodynamic Derivation	293
❖ Third Law of Thermodynamics (Nernst Heat Theorem, Determination of Absolute Entropy, Unattainability of Absolute Zero) And Its Limitation.....	296
❖ Phase Diagram for Two Completely Miscible Components Systems	304
❖ Eutectic Systems (Calculation of Eutectic Point).....	311
❖ Systems Forming Solid Compounds A_xB_y with Congruent and Incongruent Melting Points	321
❖ Phase Diagram and Thermodynamic Treatment of Solid Solutions.....	332
❖ Problems.....	342
❖ Bibliography	343
CHAPTER 7	344
Chemical Dynamics – II	344
❖ Chain Reactions: Hydrogen-Bromine Reaction, Pyrolysis of Acetaldehyde, Decomposition of Ethane.....	344
❖ Photochemical Reactions (Hydrogen-Bromine & Hydrogen-Chlorine Reactions).....	352
❖ General Treatment of Chain Reactions (Ortho-Para Hydrogen Conversion and Hydrogen-Bromine Reactions).....	358

❖ Apparent Activation Energy of Chain Reactions	362
❖ Chain Length	364
❖ Rice-Herzfeld Mechanism of Organic Molecules Decomposition (Acetaldehyde)	366
❖ Branching Chain Reactions and Explosions (H_2-O_2 Reaction)	368
❖ Kinetics of (One Intermediate) Enzymatic Reaction: Michaelis-Menten Treatment	371
❖ Evaluation of Michaelis's Constant for Enzyme-Substrate Binding by Lineweaver-Burk Plot and Eadie-Hofstee Methods	375
❖ Competitive and Non-Competitive Inhibition	378
❖ Problems	388
❖ Bibliography	389
CHAPTER 8	390
Electrochemistry – II: Ion Transport in Solutions	390
❖ Ionic Movement Under the Influence of an Electric Field	390
❖ Mobility of Ions	393
❖ Ionic Drift Velocity and Its Relation with Current Density	394
❖ Einstein Relation Between the Absolute Mobility and Diffusion Coefficient	398
❖ The Stokes-Einstein Relation	401
❖ The Nernst-Einstein Equation	403
❖ Walden's Rule	404
❖ The Rate-Process Approach to Ionic Migration	406
❖ The Rate-Process Equation for Equivalent Conductivity	410
❖ Total Driving Force for Ionic Transport: Nernst-Planck Flux Equation	412
❖ Ionic Drift and Diffusion Potential	416
❖ The Onsager Phenomenological Equations	418
❖ The Basic Equation for the Diffusion	419
❖ Planck-Henderson Equation for the Diffusion Potential	422
❖ Problems	425
❖ Bibliography	426
INDEX	427



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