

PHYSICAL CHEMISTRY

**Principles and Applications
in Biological Sciences**

Ignacio Tinoco, Jr.

University of California, Berkeley

Kenneth Sauer

University of California, Berkeley

James C. Wang

Harvard University

CHEMISTRY

Principles and Applications in Biological Sciences

PRENTICE-HALL, INC., ENGLEWOOD CLIFFS, NEW JERSEY 07632

Library of Congress Cataloging in Publication Data

Tinoco, Ignacio.

PHYSICAL CHEMISTRY.

Includes bibliographies and index.

1. Biological chemistry. 2. Chemistry, Physical and theoretical. I. Sauer, Kenneth, (date) joint author. II. Wang, James C., joint author. III. Title.

QH345.T56 541'.02'4574 77-25417

ISBN 0-13-665901-2

PHYSICAL CHEMISTRY

Principles and Applications in Biological Sciences

Ignacio Tinoco, Jr., Kenneth Sauer, and James C. Wang

© 1978 by Prentice-Hall, Inc., Englewood Cliffs, N.J. 07632

All rights reserved. No part of this book
may be reproduced in any form or
by any means without permission in writing
from the publisher.

Printed in the United States of America

10 9 8 7 6 5 4 3 2 1

PRENTICE-HALL INTERNATIONAL, INC., *London*
PRENTICE-HALL OF AUSTRALIA PTY. LIMITED, *Sydney*
PRENTICE-HALL OF CANADA, LTD., *Toronto*
PRENTICE-HALL OF INDIA PRIVATE LIMITED, *New Delhi*
PRENTICE-HALL OF JAPAN, INC., *Tokyo*
PRENTICE-HALL OF SOUTHEAST ASIA PTE. LTD., *Singapore*
WHITEHALL BOOKS LIMITED, *Wellington, New Zealand*

PHYSICAL CHEMISTRY

David T. Moore, Jr.

University of California, Berkeley

James E. Mayer

University of California, Berkeley

James E. Mayer

University of California

PHYSICAL

Ignacio Tinoco, Jr.

University of California, Berkeley

Kenneth Sauer

University of California, Berkeley

James C. Wang

Harvard University

Preface

We have been teaching a course in physical chemistry for the biological science majors at Berkeley for the past fifteen years. Most of the students are undergraduates from biochemistry, physiology, bacteriology, botany, and zoology departments. They include most of the premedical students. In addition a few graduate students in the biological sciences are always represented. We have found it difficult to find a suitable text for these students. Standard physical chemistry texts have too much material irrelevant to the biochemist, and they mainly ignore macromolecules and biological systems. Life science physical chemistry texts often omit too much fundamental physical chemistry, and although they discuss biological problems, they do so only at an elementary level. Modern physical methods used by molecular biologists and biochemists, such as density gradient sedimentation, fluorescence energy transfer, and electron microscopy, are usually not included.

We have written a book to provide a fundamental understanding of the physical chemistry important to biochemists and biologists. Each physical chemical principle is related and applied to biological problems. We thus try to teach basic physical chemistry, while motivating the student by stressing biochemical applications. We also discuss most of the physical methods and ideas that modern biological scientists use.

The book contains an introductory chapter describing the types of problems in biology or medicine to which physical chemistry can be applied. The next four

chapters cover thermodynamics. The emphasis is on solution thermodynamics. One complete chapter deals with the equilibrium constant, activities, and free energy. A chapter on physical equilibria includes equilibrium dialysis, active transport, surfaces, and the use of colligative properties in measuring molecular weights.

Transport properties are treated in Chapter 6. A short discussion on kinetic theory of gases is followed by a thorough description of diffusion, viscosity, sedimentation, and electrophoresis. The application of each of these properties to the determination of size and shape of macromolecules is stressed. Molecular weights, axial ratios, hydration, and flexibility of nucleic acids and proteins are described.

There are two chapters on kinetics. The kinetics and mechanisms of reactions in solution are emphasized. The chapter on enzyme kinetics includes a discussion of temperature-jump kinetics.

The first eight chapters require only a minimum of mathematical knowledge beyond algebra. Although calculus is used in these chapters, only differentiation and integration of powers of x are required. Partial derivatives are introduced, but the text and problems mainly can be understood without them. The next four chapters assume more mathematical confidence on the part of the students. The mathematical steps are explained in detail (as before), but the student will feel more confident with a course in calculus for background.

Chapter 9 introduces quantum mechanics to provide a vocabulary for spectroscopy and chemical structure. A particle in a box is treated and applied to the free electron model for conjugated molecules. Qualitative discussions of molecular orbitals, and molecular structure and reactivity are given. The spectroscopy chapter deals mainly with electronic spectra of organic molecules. Ultraviolet and visible absorption, fluorescence, circular dichroism, and optical rotatory dispersion are discussed. Application of each of these methods to the study of proteins and nucleic acids is included. A short section on nuclear magnetic resonance is presented.

The application of statistical methods to macromolecules is emphasized in Chapter 11. Cooperative binding of small molecules to a macromolecule is described. The random walk is applied to diffusion and polymer dimensions. Helix-coil transitions in polypeptides and polynucleotides are discussed. The statistical thermodynamic definitions of energy, work, and entropy are described.

The last chapter gives the basic fundamentals of x-ray diffraction. Applications to structures of macromolecules are discussed. Qualitative descriptions of neutron scattering, electron microscopy, and their application to biological samples are given.

The Appendices contain tables of thermodynamic data, conversion factors, abbreviations, and structures of biological molecules mentioned in the text. The reader is encouraged to keep looking through the Appendix while using the book.

At Berkeley we have found it possible to cover most of the book in two quarters. This keeps the students and the instructor working hard. Two semesters or three quarters might be a more reasonable time to spend on the material covered in the book. The first five chapters form a complete treatment of the thermodynamics most useful for the biological scientists. The remaining chapters

can essentially stand by themselves. The instructor can teach them in any order or omit any of them. For example, the applications in the spectroscopy chapter do not require knowledge of quantum mechanics.

We have tried to make this book useful to students and instructors with a wide range of backgrounds. There are many worked examples in the text; there are many problems at the end of each chapter. Each chapter has a summary and also a review of the mathematics needed for the chapter. The first half of the book uses less mathematics (and describes it more fully) than the last half. There are references to the current literature for most chapters. We hope that the readers of this book will tell us about aspects of the book which need improving.

We are happy to acknowledge the many people who contributed to this project. In the past several years, the students of Chemistry 109A,B at Berkeley and the teaching assistants made many helpful suggestions. Our faculty colleagues who also taught the course were often properly critical. Dr. Leonard Peller, University of California, San Francisco, read essentially the entire book and made detailed criticisms. Dr. Helen Berman carefully reviewed Chapter 12. Reviewers chosen by Prentice-Hall gave useful suggestions. Drs. Paul Hartig, Che-Hung Lee, and Esther Yang kindly contributed original data. Suzanne Pfeffer and Robert Sauer solved all the problems from several chapters and helped to clarify their presentation. We wish to thank all of these people.

We are grateful to Marshall Tuttle who typed and retyped the various editions of the manuscript. His cheery disposition encouraged us to continue.

Berkeley, California

Cambridge, Massachusetts

IGNACIO TINOCO, JR.

KENNETH SAUER

JAMES C. WANG

Contents

PREFACE **xiii**

1 INTRODUCTION **1**

Energy efficiency • *Human age* • *Detection of polarization* •
Hemoglobin
 References 6
 Suggested Readings 7

2 CONSERVATION OF ENERGY **8**

The Mechanism of Energy Conservation 9
Systems and surroundings • *First law of thermodynamics* •
Energy exchanges • *Work* • *Heat* • *Radiation*
 Variables of State 23
Equations of state • *Energy and enthalpy changes* •
Heat and work changes •
Temperature and pressure changes for a liquid or solid •
Temperature and pressure changes for a gas •
Properties of $E_2 - E_1$ and $H_2 - H_1$ independent of equation of state
 Phase Changes 38

Chemical Reactions	41
<i>Heat effects of chemical reactions • Temperature dependence of ΔH • ΔE for a reaction • Standard enthalpies of formation • Bond energies</i>	
Summary	49
General Equations	50
Mathematics Needed for Chapter 2	53
References	53
Problems	54

3 SPONTANEOUS REACTIONS, ENTROPY, AND FREE ENERGY 59

Historical Development of the Second Law: The Carnot Cycle	60
Entropy Is a State Function	63
The Second Law of Thermodynamics: Entropy Is Not Conserved	64
<i>Measurement of entropy • One-way heat shield • Fluctuations</i>	
Chemical Reactions	71
Third Law of Thermodynamics	72
<i>Temperature dependence of entropy • Temperature dependence of the entropy changes for a chemical reaction • Entropy change for a phase transition • Pressure dependence of entropy • Spontaneous chemical reactions</i>	
Gibbs Free Energy	77
<i>Spontaneous reactions at constant T and P • Calculation of Gibbs free energy • Pressure dependence of Gibbs free energy • Phase changes</i>	
Helmholtz Free Energy	84
Some Thermodynamic Data of Noncovalent Reactions	85
Use of Partial Derivatives	90
Summary	93
Equations for Chapter 3	94
Reference	96
Suggested Readings	96
Problems	96

4 THE CONCENTRATION DEPENDENCE OF FREE ENERGY 102

Ideal Gases	103
<i>Free energy changes • Equilibrium constant</i>	
Solutions	108
<i>Activity and chemical potential • Standard states • Biochemist's standard state</i>	
Standard Free Energy and the Equilibrium Constant	115
<i>Calculation of equilibrium concentrations: Ideal solutions • Temperature dependence of the equilibrium constant</i>	
Biochemical Applications of Thermodynamics	126
<i>Thermodynamics of metabolism</i>	

Galvanic Cells	133
<i>Standard electrode potentials</i>	• <i>Concentration dependence of \mathcal{E}</i>
<i>Applications of galvanic cells</i>	• <i>Activity coefficients of ions</i>
Partial Molal Quantities	142
Summary	144
References	147
Problems	147

5 PHYSICAL EQUILIBRIA 153

Phase Equilibria	153
<i>Free energies of transfer between phases</i>	•
<i>Equilibrium dialysis and Scatchard plots</i>	
Surfaces, Membranes, and Surface Tension	159
<i>Surface tension</i>	• <i>Biological membranes</i>
Active and Passive Transport	165
Colligative Properties	168
<i>Boiling point and freezing point of a pure component</i>	•
<i>Colligative properties of ideal solutions</i>	
Molecular-weight Determination	176
<i>Vapor-pressure lowering</i>	• <i>Boiling points and freezing points</i>
<i>Osmotic pressure</i>	• <i>Number-average molecular weights</i>
<i>Weight-average molecular weights</i>	
Activity of the Solvent and Colligative Properties	181
<i>Vapor pressure</i>	• <i>Boiling point</i> • <i>Freezing point</i> • <i>Osmotic pressure</i>
Phase Rule	185
Summary	186
Reference	190
Suggested Reading	190
Problems	190

6 TRANSPORT PHENOMENA 196

Kinetic-Molecular Model of Gases	197
<i>Velocities of molecules</i>	• <i>Molecular collisions</i> • <i>Mean free path</i>
Diffusion	205
<i>Diffusion in a gas</i>	• <i>Diffusion coefficient and Fick's first law</i>
<i>Fick's second law</i>	• <i>Determination of the diffusion coefficient</i>
<i>Relation between the diffusion coefficient and the mean-square displacement</i>	•
<i>Determination of the diffusion coefficient by laser light scattering</i>	•
<i>Diffusion coefficient and molecular parameters</i>	•
<i>Solvation</i>	• <i>Shape factor</i> • <i>Diffusion coefficients of random coils</i>
Sedimentation	219
<i>Determination of the sedimentation coefficient</i>	•
<i>Standard sedimentation coefficient</i>	•
<i>Dependence of s on molecular size and shape</i>	•
<i>Sedimentation-diffusion equilibrium</i>	

Viscosity	228
<i>Measurement of viscosity</i> • <i>Viscosity of solutions of rigid macromolecules</i> •	
<i>Viscosity of random coils</i>	
Combination of Hydrodynamic Measurements	234
Electrophoresis	236
<i>Isoelectric point</i> • <i>Gel electrophoresis</i> • <i>Gel electrophoresis of proteins</i> •	
<i>Gel electrophoresis of double-stranded DNA</i> •	
<i>Gel electrophoresis of single-stranded nucleic acids</i>	
Size and Shape of Macromolecules	242
Summary	243
References	248
Problems	249

7 KINETICS 253

Rate Law	255
<i>Order of a reaction</i> • <i>Experimental rate data</i> • <i>Zero-order reactions</i> •	
<i>First-order reactions</i> • <i>Second-order reactions</i> • <i>Reactions of other orders</i> •	
<i>Determining the order and rate constant of a reaction</i>	
Reaction Mechanisms and Rate Laws	276
<i>Parallel reactions</i> • <i>Series reactions (first order)</i> •	
<i>Equilibrium and kinetics</i> • <i>Complex reactions</i> •	
<i>Deducing a mechanism from kinetic data</i>	
Temperature Dependence	290
<i>Transition-state theory</i>	
Ionic Reactions and Salt Effects	298
Diffusion-controlled Reactions	300
Photochemistry and Photobiology	303
<i>Vision</i> • <i>Photosynthesis</i>	
Summary	311
Mathematics Needed for Chapter 7	317
References	318
Suggested Readings	319
Problems	319

8 ENZYME KINETICS 327

Kinetics of Enzymatic Reactions	331
<i>Kinetic data analysis</i>	
Relaxation Methods	339
<i>Steady state</i> • <i>Relaxation kinetics</i>	
Mechanisms of Enzymatic Reactions	350
<i>Competitive inhibition</i> • <i>Noncompetitive inhibition</i>	
Summary	354
Mathematics Needed for Chapter 8	356
References	357
Suggested Readings	358
Problems	358

9 QUANTUM MECHANICS 366

Electrons as waves • Wave mechanics and wavefunctions

Schrödinger's Equation 374

Solving Wave Mechanical Problems 376

Outline of wave mechanical procedures

Particle in a Box 380

Simple Harmonic Oscillator 388

Hydrogen Atom 392

Electron Distribution 394

Electron distribution in a hydrogen atom •

Many-electron atoms •

Molecular orbitals and linear combinations of atomic orbitals •

Hybridization • Delocalized orbitals •

Molecular structure and molecular orbitals •

Hydrogen bonds

Summary 411

Mathematics Needed for Chapter 9 414

References 415

Suggested Readings 416

Problems 416

10 SPECTROSCOPY 421

Absorption and Emission of Radiation 425

Radiation-induced transitions • Classical oscillators •

Quantum mechanical description • Beer-Lambert law

Proteins and Nucleic Acids: Ultraviolet Absorption Spectra 436

Amino acid spectra • Polypeptide spectra •

Secondary structure •

Origin of spectroscopic changes • Nucleic acids •

Rhodopsin: A chromophoric protein

Fluorescence 445

Excited-state properties • Fluorescence quenching •

Excitation transfer • Molecular rulers • Phosphorescence

Optical Rotatory Dispersion and Circular Dichroism 454

Polarized light • Optical rotation • Circular dichroism

Circular Dichroism of Nucleic Acids and Proteins 460

Induced circular dichroism of chromophores

Nuclear Magnetic Resonance 463

Chemical shifts and spin-spin splitting

Additional Spectroscopic Methods 471

Summary 472

References 474

Suggested Readings 475

Problems 475

11

**MOLECULAR DISTRIBUTIONS
AND STATISTICAL THERMODYNAMICS 481**

- Binding of Small Molecules by a Polymer 483
- Identical-and-independent-sites model • Langmuir adsorption isotherm •*
 - Nearest-neighbor interactions, three sites •*
 - Cooperative binding, anticooperative binding, and excluded-site binding •*
 - N identical sites in a linear array with nearest-neighbor interactions*
- The Random Walk 498
- Calculation of some mean values for the random-walk problem •*
 - Diffusion • Average dimension of a linear polymer*
- Helix-coil Transitions 508
- Helix-coil transition in a polypeptide •*
 - Helix-coil transition in a double-stranded nucleic acid*
- Statistical Thermodynamics 520
- Statistical mechanical internal energy • Work • Heat •*
 - Distributions • Quantum mechanical rules governing a distribution •*
 - Most probable distribution •*
 - Partition function: The most probable distribution for an isolated system •*
 - Entropy and the most probable distribution •*
 - Statistical mechanical entropy •*
 - Examples of entropy and probability • Partition function: Applications*
- Summary 540
- Mathematics Needed for Chapter 11 543
- References 544
- Problems 544

12

X-RAY DIFFRACTION AND RELATED TOPICS 548

- X Rays 549
- Emission of x rays • Image formation • Scattering of x rays •*
 - Laue's equations for the diffraction of x rays by a crystal •*
 - Measuring the diffraction pattern •*
 - Physical meaning of the indices (h, k, l) in Laue's equations •*
 - Bragg's interpretation •*
 - Monochromatization of x rays by Bragg reflection •*
 - Intensity of diffraction • Unit-cell • Summing waves •*
 - Summing scattered rays •*
 - Phase problem • Symmetry considerations •*
 - Scattering of x rays by noncrystalline materials •*
 - Absorption of x rays and x-ray filters •*
 - Extended fine structure of edge absorption •*
 - X rays from synchrotron radiation*
- Electron Diffraction 578
- Neutron Diffraction 579
- Electron Microscopy 581
- Resolution • Contrast • Radiation damage •*
 - Transmission and scanning electron microscopes •*
 - Image enhancement and reconstruction*

Summary	590
References	593
Suggested Readings	593
Problems	593

APPENDIX 596

Table A.1. Useful Physical Constants	596
Table A.2. Definition of Prefixes	597
Table A.3. Energy Conversion Factors	597
Table A.4. Miscellaneous Conversions and Abbreviations	597
Table A.5. Inorganic Compounds	598
Table A.6. Hydrocarbons	599
Table A.7. Organic Compounds	600
Table A.8. Atomic Weights of the Elements	602
Table A.9. Biochemical Compounds	603

ANSWERS TO SELECTED PROBLEMS 609

INDEX 613

Introduction

Physical chemistry is a group of principles and methods that are helpful in solving many different types of problems. Just as the methods of algebra can be applied to many problems, so can the methods of thermodynamics. In the following chapters we shall present the principles of thermodynamics, kinetics, quantum mechanics, and statistical thermodynamics. We shall also discuss various experimentally measurable properties, such as viscosity, light absorption, and x-ray diffraction. All these experimental and theoretical methods can give us useful information about the part of the universe we are interested in. We will stress biochemical and biological applications in this book, but it is up to the reader to see how the methods presented can be applied to other specific problems of interest. For applications of physical chemistry to other areas, the reader is directed to standard physical chemistry texts. Biochemistry and molecular biology texts can provide specific information about such areas as enzyme mechanisms, metabolic paths, and structure of membranes. Finally, a good physics textbook is useful for learning or reviewing the fundamentals of forces, charges, photons, and energy. A list of such books is given at the end of the chapter.

In this introductory chapter we shall give a few examples of the types of problems that physical chemistry can solve.

Energy Efficiency

The minimum amount of food that an average, adult human being at rest needs every day to survive corresponds to about 1500 kcal of energy. This is the basal metabolic rate of a 60-kg human; it is equal to 70 W of power. Light daily activity, such as walking, studying, and eating, increases the human energy requirement to about 2000 kcal day⁻¹, or roughly 100 W. The energy we eat is food energy converted from solar energy by photosynthesis. We convert this food energy directly to heat or to some form of work outside the body. There is obviously a long chain of energy-converting steps involved in this solar-energy to human-energy process. It is to our advantage to understand these steps and to be able to improve their efficiency. The efficient conversion of energy from one form to another is the subject of thermodynamics.

The amount of solar energy reaching the earth's atmosphere is 1.4 kW m⁻² (2 cal min⁻¹ cm⁻²), but only about one half of this reaches the surface of the earth. This is the total energy input available to us for food productivity. Naturally occurring photosynthesis in plants is only about 1% efficient, so about 100 kcal of food can be grown per day per square meter of the earth's surface. This means that a minimum of 20 m² is needed to raise food for one person. However, in the United States we spend about 10 cal of energy to produce and prepare 1 cal of food (Steinhart and Steinhart, 1974). Figure 1.1 shows how this added energy has increased over the years. The energy input occurs about one fourth at the farm (fertilizer, machinery, irrigation), one third in refrigeration and cooking, and the rest in the processing industry. How can knowledge of physical chemistry and thermodynamics help? Just knowing the amounts of energy involved may make us more conscious of the real cost of eating highly processed foods. But more important is the fact that thermodynamics can tell us the maximum possible efficiency for performing a certain task. The first and second laws of thermodynamics tell us the maximum amount of heat or work that can be transferred in a particular process for a given energy input. Analysis of U.S. energy uses shows an average efficiency of only 10% of the maximum efficiency possible (*Physics Today*, 1975). For example, refrigeration efficiency is only 4% of theoretical thermodynamic efficiency, and truck transportation is 10% of theoretical efficiency. Improving refrigeration and transport efficiency would allow us to have food variety all year round yet not spend 10 times its food-energy value.

How about human efficiency? The energy cost of transportation can be defined as the amount of power used divided by the weight and velocity of the moving object. The energy cost is unitless; it is energy input per time (power) divided by the weight (a force) times distance moved per time. The minimum cost of walking occurs at a velocity of 3.8 mph for a 150-lb human. The metabolic rate for this fast walker is about 450 W and the cost of transport is 0.38. The minimum cost of transport for a bicyclist is 0.1 and is the most efficient transportation by an animal; only large fish or whales may be as efficient. Freight trains and freight steamers are the most efficient form of any kind of transportation; their minimum cost is about 0.01 (Tucker, 1975).