

Principles and Problems in Physical Chemistry for Biochemists

SECOND EDITION

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SECOND EDITION

CLARENDON PRESS · OXFORD
1979

Oxford University Press, Walton Street, Oxford OX2 6DP

OXFORD LONDON GLASGOW

NEW YORK TORONTO MELBOURNE WELLINGTON

IBADAN NAIROBI DAR ES SALAAM LUSAKA CAPE TOWN

KUALA LUMPUR SINGAPORE JAKARTA HONG KONG TOKYO

DELHI BOMBAY CALCUTTA MADRAS KARACHI

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FIRST EDITION 1974

SECOND EDITION 1979

British Library Cataloguing in Publication Data

Price, Nicholas C

Principles and problems in physical chemistry for biochemists. — 2nd ed.

1. Chemistry, Physical and theoretical

I. Title II. Dwek, Raymond A

541'.3'024574 0D453.2

79-40341

ISBN 0-19-855511-3

ISBN 0-19-855512-1 Pbk

PRINTED IN GREAT BRITAIN BY

J. W. ARROWSMITH LTD., BRISTOL, ENGLAND

**Principles and problems
in physical chemistry
for biochemists**

**FOR JONATHAN AND REBEKAH, DEBORAH
AND JOSHUA**

additions between editions

Preface to the Second Edition

IN preparing the second edition of this book we have drawn on the experience of those using the first edition, and we are grateful for all the helpful suggestions and comments which have been made. A number of new problems have been included and we have made alterations to every chapter. The major changes have been to the chapter on binding studies (where an account of co-operative behaviour has been included), to the chapter on electrochemical cells (where a section on oxidative phosphorylation has been added), to the chapter on chemical kinetics (where greater emphasis is placed on the transition-state theory), and to the chapter on enzyme kinetics (which has been expanded to include a discussion of two-substrate reactions). A new chapter on macromolecules includes some of the previous material but a new section on the ultracentrifuge has been added.

In this edition we have changed to SI units as far as possible. While we realize that some of these units may not be familiar to some teachers, we feel that we should follow the advice of bodies such as the Biochemical Society who recommend that SI units should be used. A note on these units is included.

August, 1978
Stirling and Oxford

NICHOLAS C. PRICE
RAYMOND A. DWEK

Preface to the First Edition

THERE is a widespread belief, to which we subscribe, that the teaching of physical biochemistry is best accomplished by the students solving problems. However all too frequently the examples chosen, while being ideal for chemistry students, have little relevance for the student of biochemistry who often has difficulty in seeing the application to his subject. We have tried to set problems which we think ought to be within the capabilities of a first or second year student, and which illustrate some of the more important present-day ideas and methods. The emphasis is on principles rather than any sophisticated mathematical manipulation (unfortunately all too common in many physical chemistry textbooks). In this light we have chosen our text specifically to provide the background for the problems at the end of each chapter, and the worked examples form an integral and important part of the text—serving to illustrate several new points. The emphasis in the treatment in the book is on equilibria and rates for we believe that it is an understanding of these phenomena that provides a secure basis for more advanced topics (such as the physical chemistry of macromolecules) to be developed.

In the first part of the book we have attempted to emphasize the universal applicability of thermodynamic equations to systems in equilibrium, not only in dealing with reactions but also with properties of solutions, acids and bases and oxidation–reduction processes. The second part of the book deals with the rates of reactions, and here it is shown how many of the basic principles of chemical kinetics can be carried over into the kinetics of enzyme-catalysed reactions. For completeness we have included two short chapters on spectrophotometry and the uses of isotopes because these are of considerable importance in biochemistry. The section dealing with solution to problems contains not only the numerical answers but sufficient comment and working to enable individual students to see if, or where, they may have made errors or misunderstood certain principles. This should enable the students to work through the problems, to a large extent, on their own. Where appropriate we have tried to suggest the importance of the results. There are several appendices containing material which can be omitted at a first reading without creating difficulties in understanding the text.

Finally, it should be noted that biological systems are in general much more complicated than those dealt with by chemists. It is therefore often necessary to make drastic simplifications to perform any calculations from fundamental principles, at least at the level of this book.

November, 1973
Oxford

NICHOLAS C. PRICE
RAYMOND A. DWEK

Acknowledgements

IN writing this text we have drawn freely on the help and advice of our colleagues in the Department. In particular we should like to thank Drs. Keith Dalziel, David Brooks, John Griffiths and Simon van Heyningen. Professor H. Gutfreund made some useful criticisms of the manuscript.

We are also indebted to Mrs. Shirley Greenslade who carefully and patiently typed the original manuscript.

Note for second edition

WE should like to thank those friends and colleagues who have given help and advice in the preparation of this edition and in particular Drs. Simon Easterbrook-Smith, Lewis Stevens, Stuart Ferguson, and Peter Zavodszky.

A note on units

IN this edition we have tried, wherever possible, to use SI units. These are based on the metre-kilogramme-second system of measurement. Multiples of the basic units by powers of 10 are as follows:

<i>Prefix</i>			<i>Prefix</i>		
		<i>Abbreviation</i>			<i>Abbreviation</i>
10 (10 ¹)	deca	da	10 ⁻¹	deci	d
10 ²	hecto	h	10 ⁻²	centi	c
10 ³	kilo	k	10 ⁻³	milli	m
10 ⁶	mega	M	10 ⁻⁶	micro	μ
10 ⁹	giga	G	10 ⁻⁹	nano	n
10 ¹²	tera	T	10 ⁻¹²	pico	p

We do not use compound prefixes, thus 10⁻⁹ metre (m) = 1 nm, not 1 mμm.

SI units for the various physical quantities mentioned in this edition are listed below:

<i>Quantity</i>	<i>SI unit</i>
Time	<i>second</i> (s).
Length	<i>metre</i> (m). (Supplementary units retained for convenience are dm, cm.)
Mass	<i>kilogramme</i> (kg). Note multiples are based on 1 gramme (g), i.e. mg, μg rather than μkg, nkg (see above compound prefix rule).
Volume (given in units of length cubed)	<i>cubic metre</i> (m ³). For convenience the litre (1 l = 1 dm ³) and millilitre (1 ml = 1 cm ³) are retained.
Amount of substance	<i>mole</i> (mol). This quantity contains 1 Avagadro number of basic units (e.g. electrons, atoms, or molecules).
Concentration	<i>mol dm⁻³</i> used instead of molar (M) <i>mol kg⁻¹</i> used instead of molal (m)
Temperature	<i>degree kelvin</i> (K) note 0° centigrade = 273·15 K
Force	<i>newton</i> (N) (1 m kg s ⁻²)
Pressure	<i>pascal</i> (Pa) (1 Nm ⁻²). <i>In this text however we have retained the atmosphere unit (atm) since the definition of standard states is then less cumbersome, being under a pressure of 1 atm rather than 101·325 kPa</i> (1 atm = 760 mm Hg = 101·325 k Pa).
Energy	<i>joule</i> (J) (1 m ² kg s ⁻²). One calorie = 4·18 J. The gas constant, <i>R</i> = 8·31 J K ⁻¹ mol ⁻¹ .

Quantity	SI unit
Electric charge	<i>coulomb</i> (C) (1 ampere second). Faraday constant = $96\,500\text{ C mol}^{-1}$ 1 electron volt = 96.5 kJ mol^{-1}
Frequency	<i>hertz</i> (Hz) (1 s^{-1})
Viscosity	the units are $\text{kg m}^{-1}\text{ s}^{-1}$ (Note 1 centipoise = $10^{-3}\text{ kg m}^{-1}\text{ s}^{-1}$.)

The implication of these units for the topics discussed in this book are as follows:

- (i) Temperatures are quoted in degrees kelvin (or absolute). Thus 25°C is 298.15 K (in practice, this is given as 298 K).
- (ii) Enthalpy, internal energy, and free energy changes are given in J mol^{-1} .
- (iii) Entropies are quoted in $\text{JK}^{-1}\text{ mol}^{-1}$.
- (iv) Molar concentrations are given in terms of mol dm^{-3} rather than M.
- (v) Enzyme activities are quoted in terms of katal (the amount of enzyme catalysing the transformation of 1 mol substrate per second). Specific activities are quoted in terms of katal kg^{-1} .
- (vi) The curie (Ci) is redundant as a unit of radioactivity; $1\text{ Ci} = 3.7 \times 10^{10}\text{ s}^{-1}$. (We have retained this unit, however, in this text.)

For fuller discussions SI units the following may be consulted:

Quantities, units, and symbols (2nd edn). The Royal Society, London (1975). *Physicochemical quantities and units* (2nd edn). M. L. McGlashan, Royal Institute of Chemistry, London (1971).

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1 The first law of thermodynamics

What is thermodynamics?

THERMODYNAMICS is concerned with the bulk behaviour of substances. Certain empirical laws are used to derive equations which can then be used to calculate the final results of processes. It does not deal with the rates of such processes; this aspect is encompassed in the subject of kinetics. To the biochemist, the importance of thermodynamics lies in its ability to predict the position of equilibrium in a system. For example, we might wish to study the equilibrium position of, and energy changes involved in, the reaction:

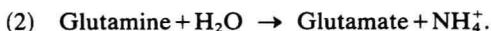


As we shall see later, determination of the energy changes would involve measuring the concentrations of the various substances taking part in the reaction when the system has come to equilibrium. In this system, however, the equilibrium concentration of ATP is too small to be accurately measured.

However, we can make measurements of the equilibrium concentrations of reactants in the reactions:



and

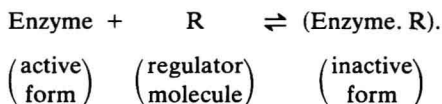


Using thermodynamics we could then predict the position of equilibrium and calculate the energy changes involved in the reaction of interest, i.e.



(which is the sum of reactions (1) and (2) above).

The position of equilibrium in a biochemical system is often of crucial importance. Many processes are regulated by the binding of one molecule to another. For instance, the catalytic activity of many enzymes is affected by the binding of small molecules:



2 The first law of thermodynamics

An example of such a system might be, for instance, pyruvate dehydrogenase which can be inhibited by the binding of acetyl CoA (which is in fact a product of the reaction).

Knowledge of the thermodynamic quantities involved in this reaction allows us to predict the position of equilibrium under any specified set of conditions (concentration of reactants, temperature, etc.) and hence to calculate the amount of enzyme in the active form under these conditions.

Some basic definitions

A *system* consists of matter which is capable of undergoing a change. A complete definition includes the matter contained and the pressure, volume, and temperature.

The *surroundings* are anything in contact with the system which can influence its state.

A *process* is any change which is taking place in the system.

Systems can interact with their surroundings via a flow of heat or work, or matter. Systems such as the cell which interact via a flow of matter are called *open* systems but initially we will consider *closed* systems where only heat and work changes occur. If two systems, or a system and its surroundings, are at the same temperature they are said to be in thermal equilibrium.

Statement of the First Law of Thermodynamics

The First Law is the law of conservation of energy and defines a quantity known as *internal energy*. If a system is isolated (that is it does not interact with its surroundings) the First Law states that its energy will remain constant irrespective of any processes taking place. Suppose however the system interacts with its surroundings. The First Law states that 'a change in internal energy ΔU occurs such that

$$\Delta U = \Delta q - \Delta w .$$

Δq is the heat *absorbed* by the system during the interaction and Δw is the work done *by* the system *on* its surroundings.' Any surplus heat energy is gained as internal energy so no energy is destroyed or created. We should note that the change in internal energy is independent of the path taken to go from the initial to the final state:†

$$\Delta U = U_f - U_i.$$

† Properties of a system which depend only on the initial and final states of the system are termed *state functions*. Examples that we shall meet include internal energy, enthalpy, entropy, and Gibbs free energy.

If this were not so, a cyclic process could create or destroy energy and perpetual motion would be possible.

The symbols Δ in the above equations refer to large measurable changes in the quantities U , q , w . We could, of course, also express the First Law in terms of very small changes in these quantities:-

$$\delta U = \delta q - \delta w.$$

Applications of the First Law of Thermodynamics

In biochemical systems several forms of work are carried out. Some examples are: the mechanical work performed by muscles, the electrical work required to charge nerve membranes, and the chemical work in synthesis of large molecules, or to produce the light emitted by glow worms. The First Law of Thermodynamics can be applied to each of these processes, provided the appropriate Δw (work) term is used. However initially we will consider only the work done against a pressure when chemical reactions proceed.

Consider a small change (δV) in volume when such a reaction proceeds. The work done (δw) against the pressure is given by

$$\delta w = P \delta V.$$

(Note that if δV is small enough, the pressure P can be considered as effectively constant.)

Now from the First Law

$$\delta U = \delta q - P \delta V.$$

For large measurable changes

$$\Delta U = \Delta q - \int P dV.$$

The pressure must be known as a function of volume before the work term (Δw) can be calculated by integration.

Reactions are usually studied at constant volume or pressure to simplify the integral above. At *constant volume* dV will be zero, so that $\Delta U = \Delta q_v$, while at *constant pressure*

$$\int_{V_i}^{V_f} P dV = P(V_f - V_i) = P \Delta V$$

and so

$$\Delta U = \Delta q_p - P \Delta V.$$

Clearly measuring Δq_v , the heat absorbed at constant volume, e.g. in a bomb calorimeter, gives ΔU for the reaction directly. From the above

4 The first law of thermodynamics

equation however, Δq_p , the heat absorbed at constant pressure, equals $\Delta U + P\Delta V$. For convenience we will define an energy function H (known as *enthalpy*) such that

$$H = U + PV$$

Then at constant pressure,

$$(H_f - H_i) = (U_f - U_i) + P(V_f - V_i)$$

or

$$\Delta H = \Delta U + P\Delta V$$

for any reaction.

Consequently ΔH (the change in enthalpy of the reaction) equals Δq_p . Since most chemical reactions are studied at constant pressure, enthalpy changes (ΔH) rather than internal energy changes (ΔU) are usually quoted. For ideal gases a simple relationship between ΔH and ΔU exists. Since $PV = nRT$, then for a reaction at constant temperature and pressure, which involves a change Δn in the number of moles of gas, we have

$$P\Delta V = \Delta n(RT)$$

$$\Delta H = \Delta U + \Delta n(RT)$$

For reactions in solution, the volume changes are normally negligible and so $\Delta H = \Delta U$.

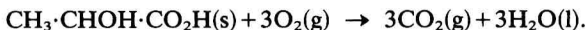
Worked examples

(1) The oxidation of solid lactic acid was studied in a bomb calorimeter at 291 K. The heat released was 1367 kJ mol^{-1} ; find ΔH for the process.

Solution

Now Δq_v is the heat *absorbed* at constant volume and so

$$\Delta U = \Delta q_v = -1367 \text{ kJ mol}^{-1}$$



Δn equals the change in the number of moles of gaseous components and here Δn is zero. So ΔH is also $-1367 \text{ kJ mol}^{-1}$ for this reaction.

(2) In a bomb calorimeter, the combustion of fumaric acid released 1330 kJ mol^{-1} ; calculate ΔH for this process ($T = 291 \text{ K}$). $R = 8.31 \text{ J K}^{-1} \text{ mol}^{-1}$.