QUANTITATIVE PROBLEMS IN BIOCHEMISTRY

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FOREWORD

In the course of the last twenty years or so Biochemistry has evolved from the descriptive and qualitative stage into a more mature phase in which it ranks as probably the most exact of all the sciences included in the field of Biology. This process of evolution has necessitated a change in the outlook and training of the student who wishes to make Biochemistry his speciality. Whereas in the past it was sufficient for him to take as his main ancillary subjects physiology and organic chemistry, he is now compelled to have more than a nodding acquaintance with physical chemistry as well and to be able to understand how the principles of physical chemistry can be applied to biological systems.

This situation in itself creates a pedagogic problem, for the number of teachers of physical chemistry who have the necessary biological background to appreciate the biochemist's problems is still all too small. Consequently any attempt made to help the student of biochemistry to a better understanding of physico-chemical problems deserves the most vigorous encouragement.

Students of chemistry have available to them a number of books dealing with chemical calculations, but no equivalent volume dealing with biochemical problems has until now been on the market, although several texts on physical biochemistry have been published during the last few years. This volume by Dr. Dawes should therefore be of considerable value in filling a gap in modern biochemical education.

Dr. Dawes has had wide experience in teaching senior students of biochemistry working for the Honours Degree in Glasgow, and at an earlier date in Leeds, and his compilation of a sufficient range of numerical problems has been a task which has spread over several years. Each group of problems is gathered into a chapter containing enough explanatory text to give the fundamental information required for their solution without at the same time converting the volume into a full fledged textbook of physical biochemistry.

Although the book has been designed to meet the specific needs of senior students of biochemistry, it may also have an

appeal to workers in allied fields who are anxious to keep their knowledge of biochemistry up to date. However this may be, it is certain of a very warm welcome from members of the Honours Biochemistry Class in the University of Glasgow, and, it is hoped, by students taking the equivalent courses in other universities.

J. N. DAVIDSON.

THE UNIVERSITY
GLASGOW.
1956.

PREFACE

'When you can measure what you are speaking about and express it in numbers, you know something about it, and when you cannot measure it, when you cannot express it in numbers, your knowledge is of a meagre and unsatisfactory kind.'—*Lord Kelvin.*

In the past the biochemist has been at a disadvantage compared with his colleagues in the more chemical and physical sciences because much of his work was, by its very nature, of a qualitative rather than a quantitative kind. Consequently the biochemist was regarded with something akin to suspicion by his colleagues in these fields, a suspicion perpetuated by the remark of that great physical chemist G. N. Lewis, when he accused living things of being 'cheats in the game of entropy'; clearly the investigator of such phenomena was himself something of an enigma!

During the past two decades the advances made in physical biochemistry and biophysics have completely revolutionized the quantitative approach to living matter and we are now in the position of having a wide field of data upon which to draw for numerical problems. Furthermore, living things are now known to 'play the game' with entropy.

This book is the outcome of a policy pursued by the author, initially at Leeds and later at Glasgow, of including numerical problems in the examinations for the Honours Degree in Biochemistry. The author feels that the greatest benefit to be derived from numerical problems is the attitude of mind engendered. All too frequently one encounters a tendency for students of biochemistry, after receiving their initial training in chemistry and physics, to become rather illogical and imprecise in thought if their course, apart from certain sections of the practical work, provides little quantitative expression. Numerical problems, properly applied, can help to combat this danger. They can also afford a clearer understanding of experimental techniques that are not usually encountered in the undergraduate laboratory course. For instance, not every biochemical laboratory possesses an analytical ultracentrifuge, and a better appreciation of the measurements that must be made before a molecular weight can be calculated is obtained if actual sample calculations are carried out.

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The original plan was to assemble a collection of suitable problems alone, but it was then felt that the book might prove to be of more value to the student if some theoretical background and worked examples were included, especially since the literature on the various topics treated is somewhat diffuse and there is no single textbook which provides the required coverage. As the intention was for emphasis to be placed on problems rather than on the development of a textbook of physical biochemistry, the greatest difficulty besetting the author was the attempt to achieve a suitable balance between inclusion and exclusion of material; the pitfalls of such a course are patently obvious. Choice of material has been quite deliberate in order to keep the book within reasonable compass, and some basic knowledge is assumed. However, to compensate for any deficiency resulting from this treatment, references and suggestions for further reading are included at the end of each chapter.

It is not intended that the chapters should necessarily be worked through in a given order; each chapter is reasonably self-contained and cross-references are provided where necessary. Many of the problems have either been taken or constructed from data in published papers and, in these instances, the references are cited. This enables the student to consult the original publication should difficulty arise. Some attempt has been made to provide problems of roughly graded difficulty in order that the book might be of use not only to honours students but also to those taking biochemistry to principal or subsidiary level, corresponding to the Double and Single Science courses of the University of Glasgow.

It is a pleasure to record my gratitude to several friends who have assisted during the preparation of this book. Professor J. N. Davidson has offered constant encouragement and advice throughout, and kindly consented to write a Foreword. I am greatly indebted to my former colleagues Dr. S. Dagley and Mr. T. J. Bowen, both of whom have given generously of their time to read the entire manuscript, and who not only criticized freely but also contributed many valuable suggestions which have been incorporated in the text. Helpful suggestions in connexion with certain portions of the text were also made by Dr. H. N. Munro, Dr. W. C. Hutchison, Mr. W. H. Holms and Dr. J. C. Speakman.

Through the kindness of Professor Eric G. Ball, Dr Fred Richards and Dr Frank Gurd of Harvard University Medical School I have been able to include some problems devised for use in the Harvard Medical Sciences 201 ab course. Many of the diagrams are the work of Mr. R. Callander, for whose help I am extremely grateful. Thanks are due to authors and publishers for permission to reproduce certain diagrams, and to Mr. T. J. Bowen for Figures 7.1 and 7.2. Answers to some of the problems were kindly checked by Mr. A. Fleck and Dr. Shelagh M. Foster, but I alone must accept responsibility for errors which may still remain. I should also like to thank the authors and publishers of the papers quoted in this book for permission to use their data. Mr. W. H. Holms, Mr. D. Murray and my wife all rendered valuable assistance with proof reading, and their help is greatly appreciated. Mr. C. Macmillan and Mr. J. Parker, of Messrs E. & S. Livingstone Limited, have given kindly guidance and have helped in many ways to facilitate the production of the book. Finally, but by no means least, I am indebted to my wife, who, starting as a novice, finished as a skilful typist in the preparation of the manuscript for the printer.

E. A. D.

Glasgow, 1956.

CONTENTS

APPENDICES

LOGARITHMIC NOTATION

THE following convention with regard to logarithms is employed throughout this book.

$$\log = \log_{10}$$
$$\ln = \log_e$$

They are related by the expression :

$$\ln x = 2{\cdot}303 \log x$$

TEMPERATURES

Unless stated to the contrary, all temperatures recorded are in degrees Centigrade.

CHAPTER I

DETERMINATION OF MOLECULAR WEIGHTS

THE molecular weight of substances of small molecular size may be determined by measurement of the colligative properties of their solutions. These include the elevation of boiling point, depression of freezing point and of vapour pressure of the pure solvent, and osmotic pressure. Many compounds of biological origin, such as proteins and polysaccharides, are macromolecules and have very large molecular weights. They can be studied only in very dilute solution; this point is made clear by an example. Molecular weight determinations of small molecules are usually carried out in 0·01-0·1 M solution. A solution 0·01 M with respect to a protein having a molecular weight of 75,000 would have to contain 750 grams of the protein per litre, and this is a physical impossibility. The most highly soluble proteins (certain albumins) will not dissolve to this extent in water, and even if this were possible to achieve, the resulting solution would be quite unsuitable for molecular weight determination. This is because the colligative properties of solutions depend on the number of molecular units present per unit volume and not on their size. Consequently a very small amount of a compound of low molecular weight may exert an effect equal to or even greater than that of the protein itself. Of the colligative properties, only osmotic pressure is used for such determinations, because in this case, by suitable choice of conditions, the effect of small molecules or ions associated with the protein may be eliminated. It is clear, therefore, that in general, to determine the molecular weights of macromolecules, other methods must be employed. These may be divided into two main groups:

1. Those based on the chemical composition, by analysis of certain elements or amino acids, or by combining weights.

2. Physico-chemical methods which include sedimentation, diffusion, viscosity, flow birefringence and light scattering. Osmotic pressure also comes under this heading. These methods find the widest application at the present time.

1. Calculation of Molecular Weight from Chemical Composition.

(*a*) Elementary and Amino Acid Analysis

The molecular weight of a compound such as a protein may be calculated if its elementary composition is known. Since a compound must contain in its molecule at least one atom of every element shown by analysis to be present, the mass of the compound which contains one gram-equivalent of such an element will be the minimal molecular weight. In other words, the molecular weight cannot be smaller than this amount. The compound may contain more than one atom of the element, and the molecular weight is then an integral multiple of the minimal value based on the assumption that only one atom is present. Thus, if the percentage of the element present in the compound is known, the minimal molecular weight is given by the expression:

$$\text{Minimal molecular weight} = \frac{\text{atomic weight of element}}{\text{percentage of element in compound}} \times 100 \quad . \quad . \quad (1.1)$$

and true molecular weight

$$= n \times \text{minimal molecular weight} \quad . \quad . \quad (1.2)$$

where n is the number of atoms of the element present in the molecule.

Example 1.1.—The nitrogen content of serine is 13·33 per cent. Calculate the minimal molecular weight.

$$\text{Min. mol. wt.} = \frac{14}{13{\cdot}33} \times 100 = 105.$$

Serine contains one nitrogen atom, so that $n = 1$ and the true molecular weight is also 105.

Example 1.2.—Lysine contains 19·17 per cent. nitrogen. What is the minimal molecular weight?

$$\text{Min. mol. wt.} = \frac{14}{19{\cdot}17} \times 100 = 73.$$

Lysine contains two nitrogen atoms and therefore the true molecular weight is 146.

To obtain the true molecular weight it is necessary, therefore, to know the value of n. This is usually obtained by some other

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method such as the measurement of one of the colligative properties of a solution of the compound. As the value of n increases, its evaluation becomes more difficult. This is because the value for the minimal molecular weight becomes correspondingly smaller, until, eventually, it may be within the range of experimental error in the measurement of the colligative property being used to determine n. For accuracy it is necessary to select an element which is present in the molecule in a relatively small amount and which can be determined with accuracy.

Further information may be obtained if analytical figures are available for more than one element which is present in the compound. For instance, if in Example 1.1 the additional information had been given that serine contains 45·71 per cent. oxygen, we should have been able to obtain the minimal molecular weight of $\frac{16}{45{\cdot}71} \times 100 = 35$ from the oxygen data and 105 from the nitrogen value. If the serine molecule contains n_1 atoms of nitrogen and n_2 atoms of oxygen, then the molecular weight of serine must be represented by the equation

$$n_1 \times 105 = n_2 \times 35.$$

This is satisfied by $n_1 = 1$ and $n_2 = 3$, and hence the molecular weight is 105 or some multiple of it. The data given do not permit a decision on the latter point, and in the absence of further evidence the molecular weight might be 210 or 315.

For the determination of the molecular weight of proteins, the elements normally used are sulphur and, in the case of metalloproteins, the metal present in the prosthetic group. The minimal molecular weight of haemoglobin, for example, has been determined by analysis of its iron content. The sulphur present in a protein may exist as disulphide, sulphydryl or thio-ether, and the sulphur determination may be expressed in these terms or simply as the percentage total sulphur. Elements such as carbon and nitrogen are present in too large a percentage for minimal molecular weight determinations and they give very large values of n.

Some proteins do not contain any element in sufficiently small quantity to be used in these calculations, but in many cases the percentage content of certain amino acids has been used for the determination of the minimal molecular weight. The procedure

is exactly analogous to that used when the basis is the elementary composition. Choice is made of an amino acid present in small percentage; the minimal molecular weight must contain at least one of these amino acid molecules. Provided the amino acid analysis is accurate, this method offers advantages, because, unlike an element, it is not likely that an amino acid will be present as a contaminant of the protein. Amino acids finding greatest application in this way are tyrosine, tryptophan and cystine, for they are usually present in small amount and can be accurately determined.

There are many data now available on the composition of various proteins, but unfortunately not all of them are reliable for the calculation of minimal molecular weights because of uncertainty in the state of purity of the protein analysed. There is also the possibility that in hydrolysing the protein prior to analysis some of the amino acids may be destroyed.

(*b*) Combining Weights

Minimal molecular weights may be determined as the weight of the compound which combines or reacts with 1 gram-molecule of a suitable chemical reagent such as a monovalent acid or base. Proteins, for example, contain a number of free carboxyl and amino groups in their molecules and these may be titrated with base and acid respectively. In this way the maximal base and acid-binding capacities are determined. If there are, say, x carboxyl groups per molecule, then one gram-molecule of protein will combine with x equivalents of base and the minimal molecular weight will be $1/x$ of the true molecular weight. x may be evaluated in a similar manner to n as described in the preceding section. The main disadvantage of this method is the large number of free acidic and basic groups which are present in most protein molecules and which thus yield very small minimal molecular weights with attendant difficulties in the evaluation of x.

Titration with acidic and basic dyes has also been used to determine the combining weights of proteins, and in the special case of respiratory proteins, combination with oxygen has been accurately measured (see Problem 1.6).

Example 1.3.—The maximal acid-combining capacity of egg albumin is $8{\cdot}7 \times 10^{-4}$ equivalents per gram protein. Calculate the minimal molecular

weight. The molecular weight of this protein, as determined by diffusion and sedimentation velocity measurements, is 43,800. Determine the approximate number of basic groups per molecule.

$8{\cdot}7 \times 10^{-4}$ equivalents of acid combine with 1 g. protein

$\therefore$ 1 equivalent of acid combines with $\dfrac{1}{8{\cdot}7 \times 10^{-4}}$

$=1149$ g. protein.

Hence the minimal molecular weight is 1149.

The number of basic groups per egg albumin molecule will therefore be

$$\frac{43800}{1149} \simeq 38.$$

(*c*) End-group Analysis

This method presupposes a known or postulated structure of the molecular units on which basis the results are interpreted. Not unnaturally, this constitutes a major limitation to its usefulness. Furthermore, where a linear chain type of structure is assumed, any branching, unless quantitatively assessed, will introduce error. The classical example of end-group analysis is the estimation of the chain length of cellulose by Haworth and his collaborators. Assuming that cellulose consists of linear chains of glucose molecules joined by 1 : 4 linkages, the cellulose is completely methylated by the gentlest possible means and then hydrolysed under conditions which permit breakage of glucose-glucose links but not hydrolysis of the methyl groups. At one end of each cellulose chain will be a 2,3,4,6 tetramethyl glucose molecule, whereas all the other members of the chain will be 2,3,6 trimethyl glucose units. Accordingly analysis of the products of hydrolysis permits an estimate of the chain length. In this way the value of 100 to 200 β-glucose units, corresponding to a molecular weight of 20,000 to 40,000, was obtained. A further difficulty encountered in this work is the possibility of degradation of the chain during the methylation procedure so that the values must be regarded as minimal limits.

2. Physico-chemical Methods.

These are based on molecular kinetic theory and fall into two main categories depending on (1) the colligative properties of solutions, i.e. dependent on the *number* of molecular units present per given volume and (2) the *weight* of the units present. The number-average methods include measurement of osmotic pressure, spread monolayers, as well as end-group assay, whilst

weight-average methods embrace sedimentation, diffusion, viscosity and light-scattering.

The number-average molecular weight, M_n, is given by the expression

$$M_n = \frac{\Sigma n_1 M_1}{\Sigma n_1} = \frac{\Sigma C_1}{\Sigma n_1} \quad . \quad . \quad . \quad (1.3)$$

and the weight-average value, M_w, by

$$M_w = \frac{\Sigma n_1 M_1^2}{\Sigma n_1 M_1} = \frac{\Sigma C_1 M_1}{\Sigma C_1} \quad . \quad . \quad . \quad (1.4)$$

where n_1 is the number of molecules of molecular weight M_1, and C_1 is their concentration, equal to $n_1 M_1$.

The number-average and weight-average molecular weights afford information as to the dispersity of high molecular weight substances in solution. If a protein consists of molecular units all of the same size, it is said to be *monodisperse* and the molecular weight determined by number-average and weight-average methods will be the same. But if it consists of molecular units of different sizes, i.e. if it is *polydisperse*, the determined molecular weights are not the same, the weight-average figure always being greater than the number-average value. The ratio of these averages is a rough indication of the *polydispersity* of the protein.

Example 1.4.—The composition of a protein corresponds to 5 moles of molecular weight 15,000 and 10 moles of molecular weight 30,000.

The number-average molecular weight

$$= \frac{5 \times 15000 + 10 \times 30000}{5 + 10} = 25{,}000,$$

and the weight-average molecular weight

$$\frac{5 \times (15000)^2 + 10 \times (30000)^2}{5 \times 15000 + 10 \times 30000} = 27{,}000.$$

Molecular weight determinations on proteins are complicated by the shape and the electrical charge of the molecules. The former factor has a profound effect on molecular movement such as that measured in sedimentation and diffusion studies. Spherical molecules behave in a normal manner under these experimental conditions, but elongated, thread-like molecules of fibrous proteins deviate from the normal due to increased

frictional and also hydration effects; as a consequence their rate of diffusion is reduced.

The possibility of aggregation of molecules is increased in concentrated solution. Colligative phenomena, with the exception of osmotic pressure, are of little value for the determination of the molecular weights of macromolecules. Not only are the elevation of boiling point and depression of freezing point or vapour pressure too small to be measured, but, as already mentioned, the presence of traces of low molecular weight compounds, such as salts, would produce an effect equal to or even greater than that of the macromolecule itself. By correct choice of conditions the effect of such compounds can be eliminated in osmotic pressure measurements, the basis of which is now described.

(*a*) Osmotic Pressure

If a solution is separated from the pure solvent by a membrane permeable to molecules of solvent but impermeable to those of solute, then solvent molecules pass through the membrane into the solution until equilibrium is reached. The pressure which must be exerted on the solution to prevent the passage of solvent molecules across the membrane is a measure of the osmotic pressure of the solution. Osmotic pressure was first studied by the botanist Pfeffer in 1877, which perhaps emphasizes the importance of the phenomenon in biological systems. Van't Hoff showed that for very dilute solutions

$$\Pi V = RT \quad . \quad . \quad . \quad (1.5)$$

where Π is the osmotic pressure, V the volume containing 1 gram-molecule of solute, R the gas-constant and T the absolute temperature. Alternatively,

$$\Pi = CRT \quad . \quad . \quad . \quad . \quad (1.6)$$

where C is the concentration of solute in moles per litre. The value of R is 0·082 when Π is expressed in atmospheres and V in litres. The molar concentration C is equal to c/M, where c is the concentration in grams per litre and M the molecular weight of the solute. Thus:

$$M = \frac{cRT}{\Pi} \quad , \quad , \quad . \quad (1.7)$$

and by measuring the osmotic pressure of a solution of known concentration at a given temperature the molecular weight of the solute may be obtained. The van't Hoff equation holds only for very dilute solutions, however, and in practice it is often found that the osmotic pressures determined experimentally are considerably below those computed from equation 1.7. Now, since $\Pi/c = RT/M$, it follows that Π/c (sometimes called the

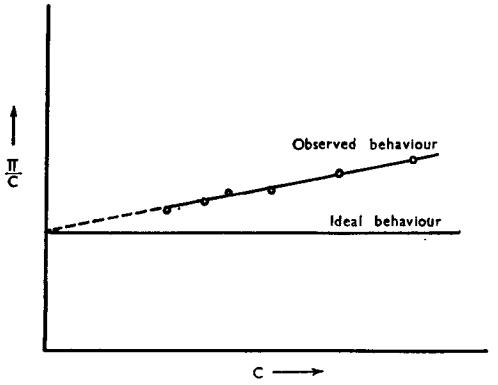

FIG. 1.1

Determination of the osmotic pressure Π at infinite dilution, by extrapolation to zero concentration, for molecular weight calculation.

reduced osmotic pressure) should be constant for all concentrations if the solution behaves ideally. Accordingly, Π/c is measured at several different concentrations and then Π/c is plotted as a function of c. In many cases this gives an almost linear curve which may be extrapolated to zero concentration, i.e. infinite dilution, and the Π/c intercept thus obtained used in the van't Hoff equation to evaluate M. This is shown in Fig. 1.1. The slope of the line is a measure of the interaction between solute and solvent and becomes greater when the solvent has a large solvating effect. It is also dependent on the shape of the molecule; the greater the asymmetry, the greater is the deviation from ideal behaviour because the elongated molecules, by solvation, immobilize solvent molecules.

With protein solutions there is the additional complication of the Donnan equilibrium (p. 43) whereby the free diffusion of inorganic ions through the membrane is restricted. Here the

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