

# **Biochemical Spectroscopy**

by

**Richard Alan Morton**

**Volume 1**

# Biochemical Spectroscopy

by

**Richard Alan Morton, Ph.D., D.Sc., Sc.D., F.R.I.C., F.R.S.**

Emeritus Professor of Biochemistry  
University of Liverpool

**Volume 1**



**Adam Hilger**  
London

First Published February 1975

© Adam Hilger Ltd 1975

All rights reserved. No part of this publication may be reproduced, stored in a retrieval system, or transmitted, in any form or by any means, electronic, mechanical, photocopying, recording or otherwise, without the prior permission of Adam Hilger Limited.

ISBN 0 85274 286 X

Published in Great Britain by

Adam Hilger Ltd

Rank Precision Industries

29 King Street

London WC2E 8JH

Printed in Great Britain by

William Clowes & Sons, Limited,

London, Beccles and Colchester

# Preface

The first edition of my book on *The application of absorption spectra to the study of vitamins and hormones* (1939) included in its Introduction the following paragraphs:

Studies in absorption spectra interest chemists from many different points of view. Applied to diatomic molecules and the simpler polyatomic molecules, they yield valuable information on the intimate structure of the molecules, so that moments of inertia, internuclear distances, valency vibrations, valence angles and characteristic frequencies may be deduced. For the large polyatomic molecules with which this booklet is concerned information of such precision is at present out of the question.

Spectroscopic research in this field is thus much more empirical, and its value lies in its use as an *adjunct* to biochemical and organic methods of study. In spite, however, of this serious limitation, the recent developments in knowledge concerning physiologically active substances owe much to spectral absorption curves in the two directions of elucidating photochemical changes and in providing characteristic 'labels' for substances the existence or importance of which rests on biological methods of experimentation. Vitamin research must always accept the animal test as the first and last court of appeal; the main service of absorption spectra lies in the possibility (which may not, of course, always eventuate) of supplementing the physiological description of an X-sub-

stance by means of a physical criterion capable of aiding in identification and analysis.

The Preface to the second edition (1942) acknowledged my debt to E. C. C. Baly and I. M. Heilbron, and to the Medical Research Council which, through Edward Mellanby, supported my investigations on vitamins A, D and E and, incidentally, introduced me to inter-laboratory cooperation and the committee-round, from which escape is so difficult. In this Preface I wrote:

In the last twenty years the subject of Absorption Spectra has ceased to be a minor and auxiliary speciality within chemistry and has become a very versatile tool, almost indispensable in many fields of research. The subject thus affords a good vantage point. More and more workers, having other main interests, find it necessary to make use of absorption spectra, and they need a certain minimum acquaintance with the methods of experimentation and interpretation. Similarly, the spectroscopists (of whom there are several varieties) need to see their subject against a wider background. This book is a contribution to the work of liaison. It reviews many brilliant papers, distinguished by patient work, great skill and insight, but it is much more a record of small advances, the cumulative effect of which is prodigious in its implications. The great days of line spectra are over, with the developments of quantum theory in the first third of the century, sub-atomics has become neat

and tidy. Similarly, quantum mechanics is providing an adequate theory of the absorption spectra of simple molecules. A great clarification of the theory of large molecules has begun to take shape and in the next few years the study of absorption spectra will play its part in a process destined to affect the outlook of all chemists.

Much of the quotation needs no apology today, although the naive reference to sub-atomics must raise a smile. (My first war-time work concerned gas-warfare, which, to the leading chemists of the day, was a more immediate threat than atomic energy.)

At the head of the first chapter of the second edition was the following quotation:

‘Les données numériques qui caractérisent l’absorption inégale des diverses lumières conduiront peut-être prochainement à une méthode d’analyse chimique universelle.’

(BERTHELOT, *Science et philosophie*.)

In 1941 nobody could have foreseen how today ultraviolet and infrared spectroscopy, nuclear magnetic resonance and mass spectroscopy together with optical rotatory dispersion, circular dichroism and other techniques have lightened the labours of chemists and biochemists. Berthelot’s hopes have come true.

I have often been asked by the Publishers and by many scientific friends to write a third edition. My failure to do so was due first to pre-occupation with the work of a long succession of research students to whom I owe much. In the second place it was due to the burden of service on committees academic, official and semi-official, and in particular to the task of reading and understanding the relevant literature. Such work is doubtless necessary and I have felt it to be part of the price the academic scientist has to pay for the privileges he enjoys.

A good deal of water has flowed under the bridges since 1942 and it seems that an introduction to many aspects of spectroscopy has become necessary in undergraduate courses for chemists, biochemists and, indeed, many biologists. Thanks to advances in electronics and in the design of instruments generally, users are now more concerned with what an instrument can do rather than how it does it. The making of measurements has become easier, quicker and more accurate than was formerly the case. Moreover, there is no lack of introductory textbooks dealing with the theory and practice of the main spectroscopic methods.

I have therefore in the present volumes used a new

title and have aimed at displaying various spectroscopic approaches in action, sometimes concerted and at other times working selectively, in the solution of biochemical problems. The modern biochemist needs a very wide background knowledge in both biology and chemistry, but he cannot be a master of every trade. The designers of scientific instruments have relieved him of many tasks and specialists in physical methods have assembled a body of classified information which he can put to good use.

Those of us whose interest in biochemistry began some fifty years ago have seen immense advances and have enjoyed participating in them and in learning about them. I have tried in this book to interest the reader in both the biochemistry and the spectroscopy, but I have also borne in mind the worker in a particular field who needs rather full references to the literature.

In the past twenty-five years new Schools of Biochemistry have multiplied all over the world and the number of students has increased greatly. The prevailing pattern of biochemical research is a phase of surging advance leading to rapid and large-scale consolidation, followed by diminishing returns and re-direction of effort. In preparing this book I found plenty of ‘applied’ problems requiring attention and holding out promise. A mature science has responsibility to undertake research that is necessary, timely and practicable. It is encouraging to know that very many biochemists are doing that kind of work and gaining satisfaction from it.

I am indebted to the Leverhulme Trust for the award of an Emeritus Fellowship which permitted me to continue writing after retirement. Mrs M. Hilditch acted as part-time secretary under this arrangement and her assistance has been invaluable. The late Professor R. J. Pumphrey kindly offered me a room in the Zoology Department and Professor A. J. Cain extended the hospitality. I have throughout had the use of the University Library and have often visited the Library of the Chemical Society at Burlington House. I wish also to thank the various societies and authors whose diagrams I have used and to apologize for any inadvertent failure to contact authors for permission. The sources of most diagrams are given in the figure captions, with a complete reference at the end of the chapter, but the following should also be included: Fig. 2.4, Golterman & Clymo (1967); Figs. 4.1–4.7, Morton *et al.* (1934); Fig. 4.10, Morton & Stubbs (1939); Figs. 4.11–4.23, Morton & Stubbs

(1940); Figs. 6.6-6.8, 6.11-6.12, Mayneord & Roe (1935); Fig. 8.9, Wetlaufer *et al.* (1959); Fig. 8.23, Bendit (1967); Figs. 8.30-8.31, Shifrin (1965); Figs. 9.6-9.11, Mason (1959); Fig. 9.14, Hearn *et al.* (1951); Figs. 13.24-13.25, Siegel *et al.* (1959); Fig. 15.18, Crawford & Jensen (1971); Fig. 16.10, Dowling (1960);

Fig. 17.1, White *et al.* (1963); Figs. 17.3-17.4, Kishi *et al.* (1966).

Finally I must thank my wife for her tolerance and patience as well as her encouragement over the years.

*The University of Liverpool*  
October 1974

# Contents

## Preface

xiv

## VOLUME 1

### 1. Introduction

#### 1.1 Introductory

1

#### 1.2 Notation

3

#### 1.3 Literature

6

##### 1.3.1. Collections of Data

6

##### 1.3.2. Some Earlier References to Literature

7

##### 1.3.3. Earliest General Treatises

7

##### 1.3.4. Techniques and General References

7

#### References

10

### 2. Water and Sunlight in a Biological Context

#### 2.1 Water

11

#### 2.2 Sunlight and Ultraviolet Light

19

##### 2.2.1. Lethality

20

#### References

23

### 3. Electronic Absorption Spectra

#### 3.1 Ultraviolet Absorption Spectra

26

#### 3.2 Simple Chromophores

27

#### 3.3 Single Chromophores

28

#### 3.4 $\alpha$ - $\beta$ -Unsaturated Ketones

29

#### 3.5 Tetracyclines

35

#### 3.6 Dyes

42

#### 3.7 Conjugated Poly-enes

45

#### 3.8 Natural Poly-yne

56

#### 3.9 Ultraviolet Spectrophotometry of Fatty Acids

58

##### 3.9.1. Other Spectroscopic Aspects of Fatty Acid Research

62

#### References

70

### 4. Aldehydes, Ketones and Tautomerism

#### 4.1 Carbonyl Compounds

74

4.2	Tautomerism	77
4.2.1.	Infrared Absorption Spectra for Keto-enol Systems	86
4.3	S-acetyl-N-acetyl-cysteamine	98
4.4	Tropone and Tropolones	100
4.5	Absorption Spectra of 2,4-Dinitrophenylhydrazones	104
4.6	Infrared Spectra	108
	References	108
5.	<b>Polyprenols and Glyceryl Ethers</b>	
5.1	Polyprenols	110
5.2	Glyceryl Ethers: Batyl, Selachyl and Chimyl Alcohols	112
	References	115
6.	<b>Aromatic Compounds</b>	
6.1	Polycyclic Hydrocarbons	116
6.1.1.	Nomenclature	116
6.1.2.	Phenanthrene and its Derivatives	121
6.1.3.	Azulene and its Derivatives	124
6.1.4.	Carcinogenicity	126
6.1.5.	Polynuclear Hydrocarbons in Air-borne Particulates	128
6.2	Polyphenyls	131
6.3	Steric Hindrance	134
6.4	Aspects of Detoxication Processes	138
6.4.1.	Metabolism of Foreign Compounds	138
6.4.2.	Ultraviolet Absorption as an Aid to the Study of Detoxication Processes	138
6.4.3.	<i>m</i> -Dinitrobenzene Metabolism	147
	References	147
7.	<b>Carotenoids and Related Substances</b>	
7.1	Introduction	150
7.1.1.	<i>Cis-trans</i> -isomerism in Carotenoids	152
7.1.2.	Fucoxanthin	155
7.1.3.	Neoxanthin	157
7.1.4.	Alloxanthin	158
7.1.5.	Loroxanthin	159
7.1.6.	Oscillaxanthin	160
7.1.7.	Rubixanthin	161
7.1.8.	Myxoxanthophyll	162
7.1.9.	Carotenoproteins	169
7.2	Sporopollenin	172
	References	175
8.	<b>Aminoacids, Proteins and Enzymes. Fluorescence</b>	
8.1	Aminoacids and Proteins	178
8.1.1.	Absorption Spectra of Sulphur-containing Aminoacids	179
8.1.2.	Ultraviolet Irradiation of Proteins	181
8.1.3.	Tyrosine and Tryptophan	183
8.1.4.	Thyroxine	188
8.1.5.	Insoluble Proteins	189



8.1.6.	Ionization of Tyrosine	190
8.1.7.	Peptide Absorption in the Region 185–230 nm	192
8.1.8.	Protein Meter	193
8.1.9.	Nitration of Tyrosyl Groups	193
8.1.10.	Difference Spectra	195
8.1.11.	Measurement of Accessibility of Protein Chromophores by Solvent Perturbation of their Ultraviolet Absorption Spectra	198
8.1.12.	Ultraviolet Spectrophotometry of Serum Proteins	200
8.1.13.	G. Actin	203
8.1.14.	Optical Rotatory Dispersion	204
8.1.15.	Infrared Absorption of Proteins	207
8.1.16.	Tobacco Mosaic Virus	211
8.1.17.	Carbonic Anhydrase of Erythrocytes	213
8.1.18.	Acetylcholine Esterase	215
8.1.19.	Glyceraldehyde-3-phosphate Dehydrogenase	216
8.2	Fluorescence	220
8.2.1.	Fluorescence and Chemical Constitution	223
8.2.2.	Aromatic Aminoacids	233
8.2.3.	Laser Spectroscopy	237
8.2.4.	Vitamin B <sub>6</sub>	237
8.2.5.	Fluorescence of Steroids	238
8.2.6.	Phosphorescence and Further Aspects of Fluorescence in Relation to Proteins	239
8.2.7.	Scintillators	244
References		245

## 9. Heterocyclic Compounds Including Nucleotides and Nucleic Acids

9.1	Heterocyclic Compounds	252
9.1.1.	Pyridine and its Derivatives	255
9.2	Nucleic Acids, Nucleotides, Nucleosides and Pyrimidine and Purine Bases	273
9.2.1.	Some Thio Compounds	276
9.2.2.	Pyrimidines	282
9.2.3.	Purines	291
9.2.4.	Cytokinins	297
9.2.5.	Ethidium Bromide	300
References		303

## 10. Porphyrins, Bile Pigments and Cytochromes

10.1	Porphyrins	308
10.1.1.	Peroxidases and Catalases	315
10.1.2.	Infrared Spectra of Porphyrins	319
10.1.3.	Fluorescence Spectra	319
10.1.4.	Chlorophylli	320
10.2	Bile Pigments	324
10.2.1.	Determination of $\delta$ -Aminolaevulinic Acid and Porphobilinogen in Urine	327
10.3	Cytochromes	330
10.3.1.	Cytochrome Oxidase	339
References		343

<b>11. Copper Proteins</b>	
11.1 Metalloproteins	347
11.1.1. Absorption Spectra of Copper Compounds	348
11.2 Haemocyanins	352
11.2.1. A Note on Copper Valency: Indeterminate or Indeterminable?	355
Bibliography and References	356
<b>12. Steroids and Related Substances</b>	
12.1 Ultraviolet Absorption	358
12.2 Infrared Absorption	367
12.3 Steroidal Sapogenins	368
12.4 Cardenolides	369
12.5 Mass Spectroscopy in Steroid Analysis	370
12.6 Oestrogens	372
12.7 Adrenal Cortical Steroids	373
12.8 Determination of Urinary Steroids	374
12.8.1. Pregnanediol in Urine	376
12.9 Limonoids	377
12.10 Ultraviolet Absorption of Bile Acid Degradation Products; Colour Reactions of Bile Acids	378
12.11 The Liebermann-Burchard Reaction	379
References	380

## VOLUME 2

### 13. Vitamins and Coenzymes

<b>13.1 Provitamins and Vitamins A (Retinol and 3-Dehydroretinol)</b>	382
13.1.1. Ultraviolet Absorption and $\text{SbCl}_3$ Colour Test	383
13.1.2. Correction Procedures for Spectrophotometric Analyses	384
13.1.3. Anhydroretinol	387
13.1.4. Interpretation of Vitamin $\text{A}_2$ Spectrum	388
13.1.5. Low Temperature Spectroscopy	393
<b>13.2 Kitol: Occurrence, Properties, Structure</b>	394
<b>13.3 Provitamins and Vitamins D</b>	396
13.3.1. Ketone 250	399
13.3.2. Origin of Vitamin D in Fish Liver Oils	400
13.3.3. Provitamin $\text{D}_3$ : 7-Dehydrocholesterol	401
13.3.4. Ultraviolet Absorption of Purified Ergosterol	401
13.3.5. Determination of Ergosterol in Yeast	403
13.3.6. Vitamin D in Man as a Photochemical Product	403
13.3.7. Vitamins $\text{D}_2$ and $\text{D}_3$ : Ergocalciferol and Cholecalciferol	403
13.3.8. Differentiation and Assay of Vitamins $\text{D}_2$ and $\text{D}_3$	405
13.3.9. Metabolism	407
13.3.10. 1,25-Dihydroxycalciferol	408
13.3.11. Multicomponent Systems	408
<b>13.4 Vitamin E. Tocopherols and Tocotrienols</b>	410
13.4.1. Spectrophotometric Determination of $\alpha$ -Tocopherol	413
13.4.2. Metabolism of Vitamin E	414
13.4.3. Oxidative Rancidity	415
13.4.4. $\text{NN}'$ -Diphenyl-p-phenylenediamine (DPPD)	416
<b>13.5 Vitamin B<sub>1</sub>. Thiamine</b>	417
13.5.1. Spectrophotometric Determination of Thiamine	420
13.5.2. Colorimetric Method of Melnick and Field (1939)	421
13.5.3. Thiamine Pyrophosphate	421
<b>13.6 Ultraviolet Absorption Spectra of Pyridine Nucleotides</b>	423
13.6.1. Nicotineadeninedinucleotide (NAD) and Analogues	425
<b>13.7 Riboflavin and Flavoproteins</b>	427
13.7.1. Historical	427
13.7.2. Flavin of Succinate Dehydrogenase: SD-flavin	435
<b>13.8 Vitamin B<sub>6</sub></b>	436
13.8.1. Metabolites and Fluorimetric Methods	447
<b>13.9 Vitamin C</b>	448
13.9.1. Ascorbate-3-sulphate	451
13.9.2. Reductic Acid	451

13.10 Biotin	451
13.11 Folic Acid Group	453
13.12 Vitamin B <sub>12</sub>	465
13.12.1. Co-enzymes Containing Vitamin B <sub>12</sub>	466
References	469
<b>14. Quinones and Related Substances</b>	
14.1 Introduction	478
14.2 Naphthazarin and its Derivatives	484
14.3 Naturally Occurring Quinones	486
14.4 Plastoquinones	488
14.5 Ubiquinones	490
14.5.1. NADH-Ubiquinone Reductase	493
14.6 Vitamin K	494
14.7 Other Naphthoquinones and their Spectra	495
14.8 Anthraquinones	499
14.9 Further Studies on Quinones	508
References	513
<b>15. Photosynthesis</b>	
15.1 Introduction	515
15.2 Ferredoxins and Chlorophylls	526
15.3 Cytochromes and Cytochromoids	532
15.4 Flash Photolysis	534
15.5 Phytochrome	536
References	539
<b>16. Vision Research</b>	
16.1 Introductory	543
16.2 Retinenes	545
16.3 Rhodopsins and Porphyropsins	546
16.4 Other Pigments, Mixtures and Partial Bleaching	549
16.5 Roles of Retinols and Retinaldehydes	551
16.6 A Complex Situation	552
16.7 Adaptation Through Pigments	553
16.8 Photochemical Aspects	556
16.9 Implications of Photochemical Changes	559
16.10 Flash Photolysis	560
16.11 Theoretical Considerations	561
16.12 The Binding of Retinal	563
16.13 Insect Pigments	570
16.14 Human Visual Responses	573
16.15 Pigments in Halophilic Bacteria	576
References	578
<b>17. Bioluminescence and Chemiluminescence</b>	
17.1 Luciferase and Other Systems	582
17.2 Chemiluminescence	591
References	592

<b>18. Flavonoids, Anthocyanins and Flavylum Salts</b>	
18.1 Occurrence, Distribution and Nature of Flavonoids	594
18.2 Chemistry and Spectroscopy of Flavonoids	602
18.3 Anthocyanins and Anthocyanidins	610
18.4 Flavylum Salts	613
References	615
<b>19. Indole, Quinoline, Isoquinoline Families; Melanin, Acridine</b>	
19.1 Indole and Related Simple Substances	616
19.1.1. Serotonin	630
19.1.2. Violacein	631
19.2 Indole Alkaloids	632
19.2.1. LSD	637
19.3 Quinolines and Isoquinolines	639
19.4 Some Related Alkaloids	643
19.4.1. Other Quinoline Alkaloids	647
19.5 Aspects of Melanin Chemistry	649
19.6 Acridines	655
References	660
<b>20. Some Phytochemical Topics</b>	
20.1 Absciscic Acid: Quiesone	663
20.1.1. The Racemate Dilution Method	664
20.2 Phycoerythrins and Phycocyanins	666
20.3 Phytoalexins	669
20.4 Phaseolic Acid	672
20.5 Brassins	673
20.6 Coleons	674
20.7 Substances from Ferns and Hops	679
20.8 Rottlerin and its Derivatives	683
20.9 Substances from Lichens	689
20.10 Cucurbitanes	694
20.11 Lignins	696
References	703
<b>21. Some Aspects of Insect Biochemistry</b>	
21.1 Pheromones	706
21.2 Nectar Guides	715
21.3 Pyrethrin Insecticides	716
References	719
<b>22. A Selection of Toxic Substances (Based on Spectroscopic Properties)</b>	
22.1 Saxitoxin	721
22.2 Amatoxins and Phallotoxins	722
22.3 Teratogenic Agents	725
22.4 Pesticides	726

22.5	Tetrodotoxin-Tarichatoxin	727
22.6	Fungal Toxins	729
22.7	Rust Fungi	730
22.8	Urushiol	731
22.9	Gossypol	732
22.10	Favism	734
22.11	Cycasin	735
22.12	Fungal Metabolites Containing Furofuran Groups	735
22.13	Antifungal Compounds	741
	References	742
<b>23.</b>	<b>Some Antibiotics and Medicinal Substances</b>	
23.1	Antibiotics	745
23.2	Streptimidone	746
23.3	Penicillins	746
23.4	Amicetin	752
23.5	Phenazine and Phenoxazine	753
23.6	Actinomycin	755
23.7	Quinomycins and Echinomycin	757
23.8	Pyo Compounds	759
23.9	Antimycin	760
23.10	Chloramphenicol	761
23.11	Rifamycins	762
23.12	Penicillins and Hypericin	764
23.13	Frequentin	766
23.14	Thiostrepton	767
23.15	Prodigiosin	768
23.16	Arsanilic Acid	769
23.17	Amphetamines	771
23.18	Valium and Librium	771
	References	773
<b>24.</b>	<b>Some Problems in Food Science</b>	
24.1	Natural and Synthetic Antioxidants, Food Additives	776
24.2	Properties of Some Flavouring Principles and of Spice Constituents	781
24.3	Spectroscopic Properties of Essential Oils	794
24.4	Analytical Chemistry of Essential Oils	797
	References	803
<b>25.</b>	<b>Illustrative Bio-Analytical Examples</b>	
25.1	Sulphides	806
25.2	Dehydrogenases	808
25.3	Indirect Determination of Sugars	810
25.4	Determination of Nitrogen	810
25.5	Determination of Sulphate	811
25.6	The Bratton and Marshall Method of Estimating Aromatic Amines	811
25.7	Starch-Iodine Coloration	812
25.8	Dissociation Constants	813

25.9	Sulphur Dioxide	814
25.10	Ozone	815
25.11	Determination of Ammonia	815
25.12	Spectrophotometric Determination of Nitrate (Particularly in Soils)	816
25.13	3-Methoxy-4-hydroxymandelic Acid in Urine	816
25.14	Determination of Lead in Urine	817
25.15	Determination of Copper in Urine	818
25.16	Phosphatase Activity	818
25.17	Spectrophotometric Methods in Clinical Biochemistry	819
	References	820
<b>26.</b>	<b>Miscellaneous</b>	
26.1	Spectrophotometry of Turbid Preparations	822
26.2	Reflection Spectra	826
	26.2.1. Internal Reflection Spectroscopy	827
26.3	The Near Infrared Region	828
26.4	Actinometers	830
	26.4.1. Potassium Ferrioxalate as a Chemical Actinometer	830
	26.4.2. Sunlight	831
26.5	Urinary Pregnanetriol ( $5\beta$ -pregnane $3\alpha$ , $17\alpha$ , $20\alpha$ -triol)	832
26.6	Purification of Solvents for Ultraviolet Spectroscopy	833
	References	834
	<b>Author Index</b>	837
	<b>Subject Index</b>	857
	<b>Corrigenda</b>	873

# 1 Introduction

## 1.1 Introductory

In recent years the task of establishing the structures of most new natural products has been made far easier than once seemed possible. High resolution mass spectroscopy leads to accurate molecular weights and sets definite limits to possible empirical formulae. Fragmentation patterns, nuclear magnetic resonance, infrared absorption, Raman spectra, ultraviolet absorption, optical rotatory dispersion and circular dichroism all provide evidence such that chemists today enjoy a flying start. X-ray crystallography too makes its own superb contribution.

The designers and makers of elaborate scientific instruments have greatly facilitated the gathering of reliable information. Most of the techniques are now explained, at least in principle, in undergraduate courses. Each physical method has, however, specialists thrusting into new territory and revealing more and more sophisticated aspects of structure and function. An important aspect of the new situation is that a good part of the effort once needed for recognizing and characterizing the molecules participating in complex biological processes is now deflected to elucidating modes of action. Biochemists should be able to interpret the information derived from a wide range of spectroscopic investigations. They will then be more free, and more competent, to clarify the roles of individual compounds in living organisms.

In the period immediately after the 1914-1918 War experimental work on infrared absorption spectra of organic compounds was a task for specialists. Coblenz

had laid down firm foundations but serious technical difficulties persisted and even as late as 1938 it could be said that 'the technique for accurate work is neither easily nor cheaply acquired'. The advances in instrumentation which came later gradually reduced to simple routine much of the gathering of data.

The pioneers of absorption spectroscopy in the ultraviolet and visible regions (Hartley, Dobbie, Hantzsch, Baly and others) managed to obtain only semi-quantitative curves. They had to use photographic methods for recording spectral transmission by solutions of organic substances, and although the blackening of the developed photographic plate could be measured accurately, both theoretical and practical difficulties remained. In particular when the product  $I$  was constant ( $I$  = light intensity,  $t$  = time of exposure) the blackening was not constant. In fact a relation  $It^n = \text{constant}$  held, but  $n$  (the Schwarzschild exponent) showed considerable variation between different kinds of photographic plates. The advent of suitable photometers permitted reasonable accuracy to be achieved in the measurement of absorption spectra in the visible and ultraviolet regions. Twyman's sector photometer (invented just before the 1914-1918 War) came into wide use among specialists. The light source was a high tension condensed electric spark between metallic electrodes. A long-focus sector photometer was made specially for Baly's group at Liverpool so as to allow an intense arc between iron and nickel rods to be used instead of a spark. Various improved photometers



were devised (Judd-Lewis, Spekker) but the Twyman instrument proved easier to use. It was not theoretically impeccable (because of the Schwarzschild factor) but there was a compensatory intermittency effect on the blackening of the plate. The measurements remained tedious and some skill and experience was needed for  $\pm 2\%$  accuracy in measuring extinction coefficients. The light sources moreover emitted line spectra, a fact which militated against the measurement of absorption displaying fine structure, particularly in vapour spectra. Light sources with continuous spectra were available for work in the visible and near-ultraviolet regions but there was still need for a stable light source having a spectrum continuous down to 200 nm. An early attempt in that direction was a condensed spark between aluminium electrodes under water. The spectrum was continuous but the spark was erratic and very noisy. An underwater spark between tungsten electrodes (Fulweiler & Barnes, 1922) was perfected at the Bureau of Standards by Brode. It involved a rotatory auxiliary spark gap in air and a spark under water actuated by a Tesla discharge at a very high voltage. This light source, in conjunction with a short-focus Twyman sector photometer, proved highly satisfactory apart from its noise and the fact that it was a source of electrical interference.

A hydrogen discharge tube associated with the names of Bay and Steiner was a further development. Different versions of hydrogen 'lamps' were all at first quite sizable and had to be connected to a source of (purified) hydrogen; they were however noiseless and provided a continuous spectrum down to 210 nm.

Considerable advances in electronics led to the use of small and highly reliable hydrogen 'lamps' which today form part of standard photoelectric spectrophotometers. They are now taken very much for granted but they were however the result of a prolonged effort to find the best light source for a new generation of spectrophotometers.

Visual spectrophotometry over the range 440–760 nm continues to have some advantages and the combination of the Hilger constant deviation spectrometer and the Nutting (or König-Martens) photometer has a fine record of work done. Nevertheless this technique is obsolescent and for many purposes recording spectrophotometers meet the needs better.

Photoelectric spectrophotometry began with very 'individual' assemblies using a mercury vapour lamp as light source and the work of von Halban (von Halban & Siedentopf, 1922) overcame numerous

difficulties. Historians of the subject will find many other significant developments (cf. Suhrmann & Kollath, 1928; Warburg *et al.*, 1929). The work of Michaelson & Liebafsky (1936) assisted Hardy in his important studies which led to recording ultraviolet spectrophotometers.

The appearance of the Cary & Beckman spectrophotometers, followed by many rather similar instruments, led to an enormous widening of the field of users of ultraviolet absorption. The manually operated photoelectric spectrophotometers were capable of an accuracy of  $\pm 0.2\%$  and this allowed a fresh approach to analytical work particularly in respect of correction for irrelevant absorptions. On the technical side Edisbury's book (1966) is of great value to new entrants to this field.

With respect to infrared spectroscopy applied to organic substances, here too remarkable advances in instrumentation have been made with parallel developments in the interpretation of spectra. Rock salt prisms remain widely used although prisms of KBr, LiF, CaF<sub>2</sub> and CsBr are also used. Prism-grating spectrophotometers and precision-grating spectrometers are now available. Selection of an instrument depends on the nature of the problem to be tackled. Some relatively cheap recording spectrophotometers are easy to use and maintain and give excellent service for semi-quantitative work, while the better performance of more expensive instruments meets special needs. The literature distributed by manufacturers is very helpful and most centres of organic or biochemical research now have workers experienced in the field. Infrared instrumentation is discussed briefly by Rao and more fully by Conn & Avery (1960), Goddu (1960) and West (1960), and many others.

Nuclear magnetic resonance was first observed in 1946 but its advantages in the study of organic structures only began to emerge in 1953 (Meyer *et al.*). In twenty years a vast amount of work has been done and the technique has undergone progressive improvements. The main reasons for this effort are that the information provided is different from, but complementary to, that obtained by ultraviolet and infrared investigations. Nuclear magnetic resonance (n.m.r.) has added a new dimension to structural studies. In many problems it can be used easily but the subject has complications demanding subtlety and expertise.

At the time that Aston carried out his early work on mass spectra, the notion that a molecular weight could for example be observed as 346.177 and calculated to