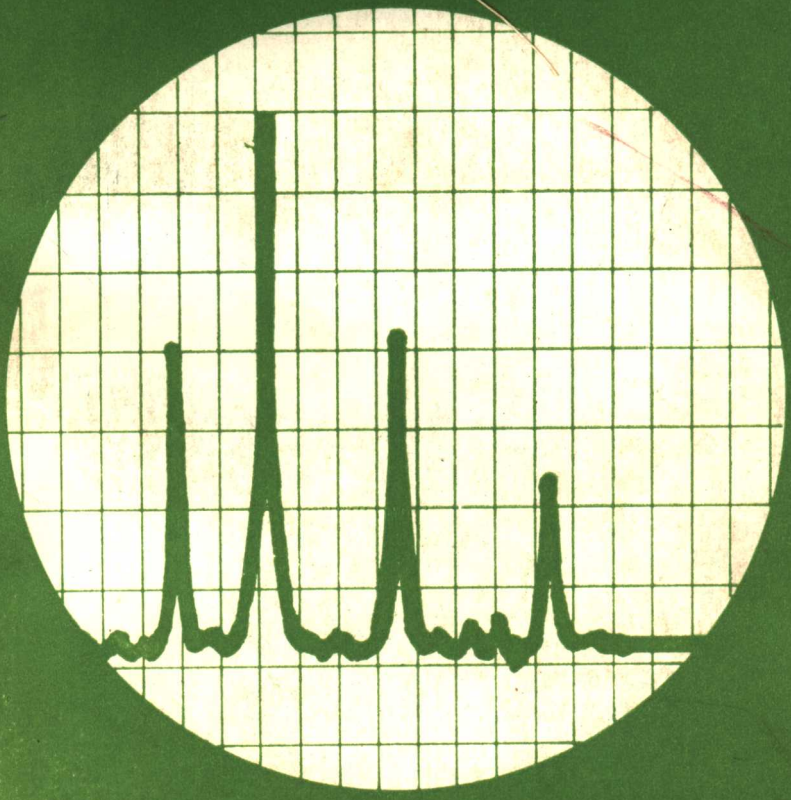
A Biologist's Guide to Principles and Techniques of Practical Biochemistry

Second Edition

Edited by Bryan L. Williams and Keith Wilson

CONTEMPORARY BIOLOGY



A Biologist's Guide to

Principles and Techniques of Practical Biochemistry

Edited by

Bryan L. Williams, M.Sc., Ph.D.

Dean of the Faculty of Science,
South Glamorgan Institute of Higher Education, Cardiff

and

Keith Wilson, B.Sc., Ph.D.

Reader in Pharmacological Biochemistry,
The Hatfield Polytechnic, Hatfield

Edward Arnold

First published 1975
by Edward Arnold (Publishers) Ltd
41 Bedford Square, London WC1B 3DQ

Reprinted 1976, 1979
Second edition 1981

British Library Cataloguing in Publication Data

A Biologist's guide to principles and techniques of practical biochemistry. - 2nd ed. - (Contemporary biology)
1. Biological chemistry - Laboratory manuals
I. Williams, Bryan L. II. Wilson, Keith
III. Series
574.19'2028 QD415.5

ISBN O-7131-2829-1

Printed in Great Britain by
Butler & Tanner Ltd
Frome and London

Preface to the Second Edition

The impact which the development of new analytical techniques and procedures has on our detailed understanding of cellular and subcellular processes has in no way diminished since the first edition of this book was published. At that time we saw the need for a book covering the basic principles of practical biochemistry but which related the techniques used to specific biological analyses. Our objective was that students should recognize that a fundamental appreciation of basic biological and physico-chemical phenomena is necessary in applying the most appropriate analytical methods for the investigation of biological phenomena. The response to the first edition that we have received from students and teachers clearly shows that the material presented has gone at least some way towards meeting this objective. Our philosophy for this new edition has therefore remained unchanged from that of the first. The approach we have adopted has been as follows:

(i) to concentrate on those analytical techniques which students might reasonably expect to use in practical classes
(ii) to cover, in less detail, some additional techniques such as electron spin resonance and nuclear magnetic resonance spectroscopy, analytical ultracentrifugation and mass spectrometry, which are unlikely to be used frequently in the undergraduate laboratory but which students may encounter in their lecture courses.
(iii) to introduce each technique by giving sufficient theoretical background to explain its principle
(iv) to describe the instruments which are associated with each technique to the extent that their working principle, limitations, potential sources of error, accuracy, advantages and disadvantages are fully appreciated
(v) to outline the fundamental experimental procedure and to describe the characteristics of the materials associated with each technique
(vi) to discuss very briefly some of the applications for which each technique is suitable.

We have attempted to incorporate as many as possible of the recommendations that we have received from colleagues in higher education both in the United Kingdom and abroad. Thus this new edition includes sections on cell counting and sizing, enzyme assays and protein fractionation. The chapter on chromatography has been extended to cover peak area analysis and recent developments in high performance liquid chromatography in view of its importance, particularly for the analysis of polar compounds. We have recognized the importance of analytical procedures based upon immunological principles by devoting a whole chapter to these techniques. It has been suggested that the book should also include reference to such techniques as optical rotatory dispersion, circular dichroism, X-ray crystallography and electron microscopy. It is obvious that any decision as to whether any of these more esoteric techniques should be included is an arbitrary one, but after careful consideration we have decided that none of them fall within the scope of our basic approach. Moreover we have deleted from the revised edition reference to Raman spectroscopy, electron nuclear double resonance spectroscopy, and certain out-dated forms of electrophoretic techniques.

The proposed readership for the book remains unchanged from that envisaged for the first edition, i.e. all students and teachers of biology from 'A' level and Technician Education Council Certificate and Diploma and Higher Certificate and Diploma courses in Applied Biology and Medical Laboratory Science to undergraduates in all disciplines within the biological sciences.

Our decision to express all quantities in SI units has, on the whole, been well received although in some quarters there is a marked reluctance to abandon μl, ml and l in favour of their SI equivalents. We have, however, decided to make two concessions. From the comments we have received, few people appreciate the Pascal units of pressure so we have expressed pressure in both Pa and psi units. We have also expressed molar concentration as M rather than mol dm^{-3}.

We are again indebted to our colleagues who have contributed to the book, particularly Dr K. Goulding for his help in the compilation of the index, and to Mr Paul Price and his staff of Edward Arnold (Publishers) Limited who have encouraged us by their helpful advice with this second edition. We continue to welcome constructive comments and criticisms from all those who use the book in conjunction with their undergraduate courses.

South Glamorgan Institute of Higher Education, Cardiff — Bryan Williams
The Hatfield Polytechnic — Keith Wilson
1981

Contributors

David H. Burrin, B.Sc., Ph.D.	Principal Lecturer in Biochemistry Coventry (Lanchester) Polytechnic Coventry
Michael G. Davis, M.Sc., Ph.D.	Senior Lecturer in Biochemistry The Hatfield Polytechnic, Hatfield
Kenneth H. Goulding, M.Sc., Ph.D.	Principal Lecturer in Biochemistry The Hatfield Polytechnic, Hatfield
Alwyn Griffiths, B.Sc., Ph.D.	Senior Lecturer in Biochemistry Oxford Polytechnic, Oxford
M. Rosalind Jenkins, M.Sc., Ph.D.	Senior Lecturer in Biochemistry The Hatfield Polytechnic, Hatfield
Ivor Simpkins, B.Sc., Ph.D.	Senior Lecturer in Biochemistry The Hatfield Polytechnic, Hatfield
Bryan L. Williams, M.Sc., Ph.D.	Dean of the Faculty of Science South Glamorgan Institute of Higher Education, Cardiff
Keith Wilson, B.Sc., Ph.D.	Reader in Pharmacological Biochemistry The Hatfield Polytechnic, Hatfield

Abbreviations and SI units

The following abbreviations have been used throughout this book without definition:

ADP	Adenosine 5′-diphosphate
ATP	Adenosine 5′-triphosphate
DDT	2,2-bis-(*p*-chlorophenyl)-1,1,1-trichloroethane
DNA	Deoxyribonucleic acid
EDTA	Ethylenediaminetetra-acetate
mol. wt.	Molecular weight
NAD^+	Nicotinamide adenine dinucleotide (oxidized)
NADH	Nicotinamide adenine dinucleotide (reduced)
$NADP^+$	Nicotinamide adenine dinucleotide phosphate (oxidized)
NADPH	Nicotinamide adenine dinucleotide phosphate (reduced)
RNA	Ribonucleic acid
s.t.p.	Standard temperature and pressure
e.m.f.	Electromotive force
e	Electron

SI UNITS (SYSTÈME INTERNATIONAL D'UNITÉS)

Physical Quantity	*Name of SI Unit*	*Symbol*
Basic Units		
length	metre	m
mass	kilogramme	kg
time	second	s
electric current	ampere	A
thermodynamic temperature	kelvin	K
amount of substance	mole	mol

Physical Quantity	*Name of SI Unit*	*Symbol*
Derived Units		
energy	joule	J
force	newton	N
pressure	pascal	Pa
power	watt	W
electric charge	coulomb	C
electric potential difference	volt	V
electric resistance	ohm	Ω
frequency	hertz	Hz
magnetic flux density	tesla	T

POWERS OF SI UNITS - PREFIXES

Multiple	*Prefix*	*Symbol*
10^{6}	mega	M
10^{3}	kilo	k
10	deca	da
10^{-1}	deci	d
10^{-2}	centi	c
10^{-3}	milli	m
10^{-6}	micro	μ
10^{-9}	nano	n
10^{-12}	pico	p
10^{-15}	femto	f

VOLUME

The SI unit of volume is the cubic metre, m^3. The litre has been redefined as being exactly equal to the cubic decimetre. Although the term litre still remains in common usage, it is recommended that both the litre and fractions of it (e.g. millilitre) are abandoned in exact scientific work.

$$1 \text{ litre (l)} = 1\,dm^3 = 10^{-3}\,m^3$$
$$1 \text{ millilitre (ml)} = 1\,cm^3 = 10^{-6}\,m^3$$
$$1 \text{ microlitre } (\mu l) = 1\,mm^3 = 10^{-9}\,m^3$$

CONVERSION TABLE FOR COMMON UNITS TO SI EQUIVALENTS

Unit	*SI equivalent*
ångström (Å)	$100\,\text{pm} = 10^{-10}\,\text{m}$
atmosphere (standard) (760 mmHg at s.t.p.)	101 325 Pa
calorie	4.186 J
centigrade (°C)	(t°C + 273)K
curie, Ci	$3.7 \times 10^{10}\,\text{s}^{-1}$
cycles/second	1 Hz
erg	10^{-7} J
gauss (G)	10^{-4} T
micron, μ	1 μm
millimetre mercury (mmHg)	133.322 Pa
molar, M (1 mol l^{-1})	1 mol dm^{-3}
pound-force/sq in (lb f in^{-2}) (p.s.i.)	6894.76 Pa

VALUES OF SOME PHYSICAL CONSTANTS IN SI UNITS

Gas constant (R)	$8.314\,\text{J K}^{-1}\,\text{mol}^{-1}$
Planck's constant (h)	$6.63 \times 10^{-34}\,\text{J s}$
Molar volume of ideal gas at s.t.p.	$22.41\,\text{dm}^3\,\text{mol}^{-1}$

Contents

SECTION 1

General Considerations

Chapter 1 General Principles of Biochemical Investigations

This section considers fundamental concepts and approaches which must be considered when designing biochemical experiments.