Biological Spectroscopy

Iain D. Campbell
and
Raymond A. Dwek

Biological Spectroscopy

Iain D. Campbell
and
Raymond A. Dwek

University of Oxford

The Benjamin/Cummings Publishing Company, Inc.
Advanced Book Program

Menlo Park, California • Reading, Massachusetts
London • Amsterdam • Don Mills, Ontario • Sydney

Sponsoring Editor Paul Elias
Production Jan deProsse, Pat Burner
Copy Edit Barbara Liguori
Cover Design Robin Gold
Book Design Richard Kharibian

Library of Congress Cataloging in Publication Data

Campbell, Iain D.
Biological spectroscopy.

(Biophysical techniques series)
Bibliography: p.
Includes index.
1. Spectrum analysis. 2. Biology—Technique.
I. Dwek, Raymond A. II. Title. III. Series.
QH324.9.S6C35 1984 574'.028 84-6232
ISBN 0-8053-1847-X
ISBN 0-8053-1849-6

ABCDEFGHIJ-HA-8987654

The Benjamin/Cummings Publishing Company, Inc.

2727 Sand Hill Road
Menlo Park, California 94025

Preface

The molecules that make up the living cell continue to excite great interest. The methods available for investigating these molecules have improved dramatically in the last decade. Some of the most powerful new techniques involve the application of electromagnetic radiation. These techniques include nuclear magnetic resonance and visible spectra, which detect transitions between energy levels, and others such as microscopy, scattering, and diffraction. In this book the term *spectroscopy* is used to cover all these methods. The close relationships among these topics are rarely emphasized in textbooks; the reading level is often either too superficial or too detailed for most readers. This book tries to bring out the recurring concepts in this field and to steer a course between the specialist text and the introductory.

Our overall aim is to lead undergraduates, graduate students, and other readers to an appreciation of the current literature. Many people find the concepts involved in spectroscopy rather difficult, so our approach here is to use worked examples and problems to emphasize important points and to illustrate biological applications rather than complex mathematical derivations. Most chapters in this text deal with one particular technique and can thus be read independently of other chapters, but we have also included a central chapter on concepts and definitions and various appendices designed to give more information on difficult points.

We owe thanks to many people who have given advice and read chapters of the manuscript. Among these we should mention D. Ashford, P. Atkins, J. Boyd, S. Easterbrook-Smith, Z. Luz, M. Moody, R. Parekh, J. Paton, S. Perkins, R. Perutz, T. W. Rademacher, W. G. Richards, J. Seelig, R. J. Simpson, B. Sutton, and A. Watts. We also thank D. Kozlow, S. Hoeft, and F. Caddick for their skillful work on the diagrams.

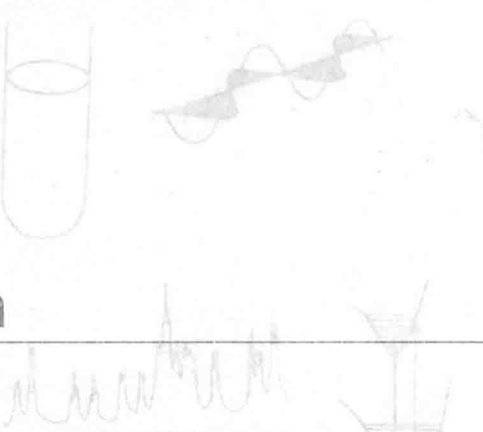
IAIN D. CAMPBELL
RAYMOND A. DWEK

Contents

5 Fluorescence 91

6 Nuclear Magnetic Resonance 127

CHAPTER 1



Introduction

Most objects we see in everyday life are visible only because they reemit part of the light that falls on them from some source, such as the sun. Interpretation of this reflected or transmitted light can yield a wealth of information not only about the color and shape of an object but also about the atomic and molecular mechanisms involved when light interacts with the object.

Light is, in fact, a form of **electromagnetic radiation** and this book is concerned with the study of the interaction of electromagnetic waves with matter and how this can be used to extract information about biological molecules and cells.

Electromagnetic radiation covers an enormous range of wavelengths (energies, frequencies). The two extremes in this range are usually taken to be radio waves, with wavelengths around 10^{-1} m, and gamma rays, with wavelengths around 10^{-11} m. Visible light covers a very small range, $4\text{-}7 \times 10^{-7}$ m.

When light interacts with an object, we can normally see only reflected or transmitted radiation. Three phenomena that occur when electromagnetic radiation interacts with matter can be defined more precisely as **scattering**, (e.g., the sky is blue because fluctuating particles in the atmosphere scatter blue light more than red light); **absorption**, (e.g., red light absorbed by a piece of glass causes the transmitted light to appear blue); and **emission**, (e.g., a fluorescent dye may emit green light after absorbing blue light). Another result of the interaction of electromagnetic interaction with matter is **photochemistry**. This is obviously extremely important in biology (such as in vision and photosynthesis) but this aspect is not dealt with here.

We thus define **spectroscopy** as the study of the interaction of electromagnetic radiation with matter, excluding chemical effects. (For the purposes of this book, neutrons and electrons are considered to give rise to electromagnetic radiation, although this is not strictly correct; see Chapter 2.)

All the techniques described in this book involve (1) irradiation of a sample with some form of electromagnetic radiation; (2) measurement of the scattering, absorption, or emission in terms of some measured parameters (e.g., scattering intensity at some angle θ, extinction coefficient at a particular wavelength, or