An Introduction to Electronic Absorption Spectroscopy in Organic Chemistry

A. E. GILLAM *and* E. S. STERN

AN INTRODUCTION TO ELECTRONIC ABSORPTION SPECTROSCOPY IN ORGANIC CHEMISTRY

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FOREWORD

THE majority of papers on organic chemistry published today contains visible or ultra-violet light absorption data, whereas twenty years ago such data were rare and their significance was but little understood. Today there is an ever-growing appreciation of the value of such spectrographic determinations in relation to the purification, identification and structural elucidation of organic compounds and the application of these methods has been greatly facilitated in recent years by the availability of excellent equipment.

It is most appropriate that the first book on this subject should have come from the pen of the late Dr. A. E. Gillam. He was a pioneer in the use of ultra-violet absorption spectra for the solution of analytical and structural problems in organic chemistry and his personal contributions assisted materially in paving the way for the truly remarkable use made of these methods today. In 1940 he published the first paper in a series in which it was " planned to collect experimental data on the absorption spectra of compounds containing specific light-absorbing groups of atoms with the object of discovering the effect of various well-defined molecular environments on the resulting absorption spectra ". This paper can justly be regarded as the precursor of the many similar and more extensive studies that have since been made and which have proved to be of great assistance in many fields.

The need for a new edition some three years after its first appearance may be taken as indicating that this eminently readable book has proved to be helpful alike to both undergraduate students and research workers. Examination of the present-day literature reveals that the techniques which this book describes are being increasingly employed throughout the whole range of organic chemical activity; in analysis, in kinetic investigations and in the solution of structural problems whether by synthesis or degradation. The already large stock of semi-empirical knowledge of the relationship between electronic absorption spectra and molecular structure is being continuously augmented and it is essential that the latest information should be carefully sifted and made available in a readily accessible form. This new edition, containing much additional material, will be indispensable to all who are trying to keep abreast with modern developments in organic chemistry.

E. R. H. JONES,
Oxford.

PREFACE TO THE FIRST EDITION

ALTHOUGH absorption spectroscopy has been applied to organic chemistry from 1875 onwards by several workers, notably Hartley and his collaborators, and, later, Henri and Baly among others, the early methods were largely qualitative or, at best, semi-quantitative. The "quantitative" methods introduced by Henri and others were rather cumbersome, and it is probably true that it was not until Twyman's sector photometer was made available commercially in 1913 that a firm foundation for quantitative absorption spectrophotometry was created.

The war of 1914–1918 interfered with the development of spectroscopic techniques and instruments and with their application, but the two decades between the wars saw the rapid growth of absorption spectroscopy as a tool in the hands of the organic chemist. The discovery, in 1928, that the absorption band of the elusive vitamin *A* could be used both for its identification and for its determination with high accuracy, probably did more to popularise the use of absorption spectrophotometry in this field of chemistry than any other single application of the technique.

Numerous workers in many parts of the world have investigated the absorption spectra of organic compounds, so that the data and literature on the subject have now assumed enormous proportions. The recent development of photo-electric methods, especially in the United States, and the simplification of the technique, together with the increasing usefulness of the results, promise to make absorption-spectrophotometric apparatus essential to every analytical and research laboratory.

The present book aims only at introducing the subject to the chemist of graduate or senior-student level. The book has grown out of lectures given by Dr. Gillam to student chemical societies and local sections of the Chemical Society and the Royal Institute of Chemistry, as well as post-graduate courses in chemical spectroscopy which were started at Manchester University in 1935.

The untimely death of Dr. Gillam in January 1950 left the book unfinished; it has now been completed and the subject matter brought up to date (to the end of 1951) without extensive alterations to the original lay-out. Despite the rapidly increasing importance of infra-red absorption spectroscopy, the scope of the book has been limited to electronic absorption in the visible and ultra-violet regions between 2000 and 10,000 A., since these have, in the past, proved of most direct application to problems in organic and biochemistry. Moreover, the experimental techniques in this field are now so highly standardised as to permit ready comparisons and discussion of the data.

Because the data on electronic absorption spectra are now so numerous and the literature is growing so rapidly, a complete review is far outside the scope of this introduction to the subject. Instead, the material for discussion has been selected so as to illustrate the elementary principles and modern concepts of organic absorption spectrophotometry, and some of its more important applications.

The book, therefore, falls into three distinct parts, with little overlap. The first part introduces the basic concepts and the laws of absorption spectroscopy, and deals very briefly with some experimental methods of determining absorption spectra in solution. This leads naturally to discussions of the relation of colour to chemical constitution and of the origin of spectra.

In the middle sections of the book, light absorption data for compounds containing relatively simple chromophoric systems are collected and analysed. The number and the classes of compounds selected had to be severely limited, but an effort has been made to include typical and important examples not only from the aliphatic, alicyclic (including steroid and triterpene), and aromatic, but also from the heterocyclic series.

The third part of the book illustrates, by reference to a few simple examples, the application of absorption-spectroscopic methods to the identification of organic compounds, to the quantitative analysis of mixtures, and to the elucidation of molecular structures and configurations.

Throughout this introductory book an attempt has been made to overlap as little as possible with textbooks on spectroscopy; the references included in the text should, however, make it easy for anyone wishing to study any particular topic more intensively to locate the original work and the more comprehensive reviews. An appendix listing some of the most important reference books, textbooks, and reviews has, moreover, been included.

The junior author gratefully acknowledges contributions made to several portions of the book by Dr. Gillam's former colleagues at Manchester University, and most valuable discussions with Professor E. R. H. Jones, F.R.S., and Dr. E. A. Braude.

E. S. S.

PREFACE TO THE SECOND EDITION

THE early exhaustion of the first edition of this volume has encouraged the preparation of a revised edition. Whilst the overall scheme of the first edition has been retained, it is hoped that the usefulness of the book to the organic chemist may be increased by the many additions and alterations made. These take account of the more recently published spectral data and of newer developments in technique; in particular, some of the latest photo-electric spectrophotometers are discussed and illustrated. It is felt that the introduction of these recording instruments has caused so great a simplification in the determination of absorption spectra that they deserve special emphasis. Further, Chapters 9 and 10 have been considerably enlarged in an attempt to illustrate how some generalisations have been and are being derived from sets of experimental results.

It is intended, however, that this book should maintain its character as an introduction to a subject which is being widely applied and growing rapidly. The book is not meant to be a hand-book for the specialist, whether analyst or dye-stuffs chemist, but will, it is hoped, be of help to the graduate entering a specialised branch of organic chemistry and perhaps also to those workers who are interested in extending the use of spectrophotometric methods within their own fields of work.

1957 E. S. S.

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Thanks are due to the manufacturers of the instruments discussed in Chapter 2 for the information given and for the blocks of Figures and photographs for reproduction. Thus Messrs. Hilger and Watts, Ltd. kindly supplied blocks for Figs 2.1, 2.3, 2.9, 2.17, and 2.18 and Unicam Instruments (Cambridge) Ltd. the block for Fig. 2.7. Photographs for reproduction and information pertaining to the respective instruments were kindly made available by: Messrs. Hilger and Watts Ltd. (St Pancras Way, London, N.W.1) (Figs. 2.2 and 2.8); Beckman Instruments Inc. (Fullerton, California) (Figs. 2.4, 2.11, and 2.12); Applied Physics Corporation (Pasadena 1, California) (Fig. 2.10); Perkin-Elmer Corporation (Norwalk, Connecticut) (Figs. 2.13 and 2.14); the American Optical Co. (Buffalo 15, New York) (Fig. 2.15); and the General Electric Company (Schenectady, New York) (Fig. 2.16).

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CHAPTER 1

INTRODUCTION

The visible spectrum. The science of spectroscopy dates from the discovery by Newton, about 1672, that the amount of refraction undergone when light is passed through a prism varies with the colour of the light. This discovery was made following the observation that when pieces of coloured material are viewed through a prism their images are displaced to a greater or less extent, depending on the different colours. Equally important was the observation that the image of the blue material is displaced more than that of the red.

In one of his later experiments, Newton, using a hole in a window-blind as a light-source, allowed a beam of sunlight to pass through a glass prism, and thereby produced a spectrum. It had been vaguely known earlier that colours could be seen in prisms, but it was thought that the prism supplied the colours. Newton, however, concluded from these and other experiments that white light is composite, and we now speak of seven *primary colours*—red, orange, yellow, green, blue, indigo, and violet—that make up white light.

In Newton's first experiment, in which he used a round hole, there would only be recognised five or six of these primary colours, owing to the overlapping of images which produces an *impure spectrum*. Improvements were effected by introducing a lens between the light-source and the prism, and finally (Wollaston, 1802) by using a narrow slit as source instead of a round hole. In conjunction with a lens and a prism, this produced a *pure spectrum*.

In those days almost the only convenient light-source was the sun, so that the spectrum most commonly studied was the solar spectrum. In 1802 Wollaston noticed a number of dark lines superimposed on this continuous spectrum and parallel to the slit. These were later studied in detail by Fraunhofer (1814), who, having noted their constancy of location, mapped out the positions of some five or six hundred of them. These lines are now known to be *absorption lines* caused by the selective absorption, by the elements in the sun's gaseous outer envelope, of some of the light originally emitted by the sun. The lines are still known as *Fraunhofer lines*, and are of interest in absorption spectroscopy, since they were used for many years as markers in describing the various parts of the visible spectrum. Frequently, early qualitative descriptions give the locations of absorption bands, such as those of the blood pigments, as occurring between certain Fraunhofer lines—*e.g.*, E and F, or F and G, as the case

may be. A few of the more important of these lines are tabulated below (Table 1.1).

TABLE 1.1

A few of the more important Fraunhofer lines *

Line	Due to	λ (A.)	Line	Due to	λ (A.)
A	Oxygen	7594	g	Calcium	4227
B	Oxygen	6870	h	Hydrogen	4102
C	Hydrogen	6563	H	Calcium	3969
D_1	Sodium	5896	K	Calcium	3934
D_2	Sodium	5890	L	Iron	3820
E_2	Iron	5270	M	Iron	3728
b_1	Magnesium	5184	N	Iron	3581
b_2	Magnesium	5173	O	Iron	3441
F	Hydrogen	4861	P	Titanium	3361
G′	Hydrogen	4340	Q	Iron	3287
G	Iron	4308	R	Calcium	3181
	Calcium	4308		Calcium	3179

* For wave-length values of greater precision on these and other Fraunhofer lines see *Handbook of Chemistry and Physics* (Chemical Rubber Pub. Co., 1944 and later editions).

The electromagnetic spectrum. The visible spectrum can, as its name implies, be observed visually. Invisible rays analogous to light-rays, however, exist and can be detected by other means. Thus Herschel in 1800 discovered, thermometrically, the invisible heat-rays beyond the red (long-wave-length) end of the spectrum—*i.e.*, the *infra-red* rays—and Ritter in 1801 discovered other invisible rays at shorter wave-lengths than the visible violet. These *ultra-violet* rays possess the property of darkening silver chloride (*actinic rays*), and with the advent of photographic plates and paper the ultra-violet region was readily and thoroughly investigated.

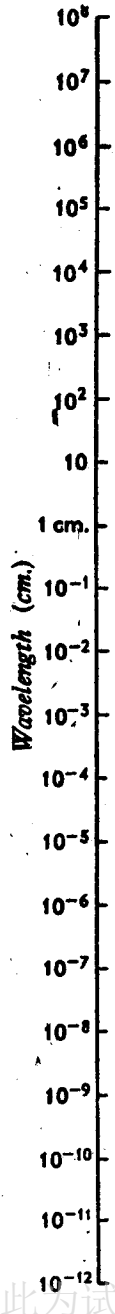
Later discoveries have revealed that the visible and adjacent infra-red and ultra-violet regions of the spectrum represent only a very small fraction of the whole range of radiations now known as the *electromagnetic spectrum*, more details of which are given in Table 1.2.

Wave-lengths and wave-numbers. The unification of the various theories of the nature of light now makes it possible to define light and the other forms of radiation of the electromagnetic spectrum in terms of wave-length or some functions thereof. Since the wave-length range which produces the physiological response in the human eye associated with each of the primary colours is now known, a precise method is available of describing any part of the visible spectrum. The other parts of the electromagnetic spectrum are more arbitrarily defined (see Table 1.2), but the position of any line in any spectrum is indicated precisely by the wave-length.

The units most commonly used for expressing the wave-lengths of the

Table 1.2

The electromagnetic spectrum



Radiation	Method of detection
Alternating-current supply.	
Wireless waves.	Production of audible telephone signals.
Hertzian waves.	Coherer.
Infra-red or heat rays.	Heating effects (thermocouples and bolometers) or Photographically on "infra-red" plates using NaCl or KBr prisms.
Visible rays { Red. Violet.	Visually or by photography. Chemical and photo-electric effects.
Ultra-violet rays.	Photography and phosphorescence. (Quartz optical parts necessary.)
X-Rays.	Photography and phosphorescence. Crystal reflection.
Gamma-rays from radio-active substances.	Similar to *X*-rays but more penetrating.
Cosmic rays.	Ionisation of gases.

visible and adjoining regions are defined in Table 1.3. "Visible light"—*i.e.*, radiations evoking the sensation of colour in the human eye—is generally taken to have wave-lengths in the region between 4000 and 8000 A., and the angstrom unit or the millimicron are thus equally convenient as units for designating the position of lines or bands in the visible spectrum. The same units are of use in studies of the near ultra-violet rays, but for the infra-red region, which is also of very great interest to the organic chemist, it is more convenient to use microns, as the wave-lengths are substantially greater.

TABLE 1.3

Units of wave-length (λ) *and of frequency* (ν)

Unit	Symbol	Definition
Micron	μ	One thousandth mm. (10^{-4} cm.)
Millimicron	mμ.	One millionth mm. (10^{-7} cm.)
Angstrom	A.	One tenth mμ. (10^{-8} cm.)
Wave-number	$\tilde{\nu}$	$1/\lambda$ (Dimension : cm.$^{-1}$)
Frequency	ν	$3 \times 10^{10}/\lambda$ (Dimension : sec.$^{-1}$)
Fresnel	f	$\nu \times 10^{-12}$ (Dimension : sec.$^{-1}$)

Definitions. The definitions of units of length (*e.g.*, the metre, millimetre, angstrom, micron, etc.) have now been internationally accepted in terms of the wave-length of the red cadmium line, placed at 6438·4696 A. Thus one angstrom (A.) = 10^{-10} metre = 1/6438·4696 of the wave-length of the red cadmium line.

Other important functions of the wave-length (λ) of light commonly used in spectroscopic work are defined as follows: the *wave-number* is the number of waves per centimetre (*i.e.*, $1/\lambda$ cm.$^{-1}$, or, when λ is given in A. it is $10^8/\lambda$). The *oscillation frequency* (ν in sec.$^{-1}$) is the number of complete oscillations in one second.

$$\text{Thus} \qquad \nu = \frac{\text{speed of light}}{\text{wave-length (in cm.)}} = \frac{3 \times 10^{10}}{\lambda \text{ (in cm.)}}$$

The term *fresnel* (f) is sometimes used for the unit of frequency; one fresnel = 10^{12} complete oscillations in one second.

These units are inter-related as follows:

$$\frac{1}{\text{wave-length}} = \text{wave-number} = \frac{\text{frequency}}{\text{speed of light}}$$

A simple example will best illustrate these relations: thus when λ = 2500 A. = 250 mμ., $1/\lambda$ = 40,000 cm.$^{-1}$, and $\nu = 1200 \times 10^{12}$ sec.$^{-1}$ = 1200 fresnels.

Types of spectra. The nomenclature of the types of spectra commonly encountered is, perhaps, somewhat complex at first sight, so that a few words about the classification of spectra may not be out of place here.

(*a*) *Emission Spectra.* When chemical substances are suitably excited (*e.g.*, by heat or electricity) they may emit radiations which, when passed through a prism, give an *emission spectrum* most commonly consisting of bright lines or bands on a dark background. The spectrum may be uniformly intense throughout, and is then said to be *continuous* (*e.g.*, the solar spectrum). If some parts of the spectrum are much brighter than others, however, the spectrum is said to be *discontinuous*. Discontinuous emission spectra may be due to excited atoms, which usually produce *line spectra*, or to excited molecules, which usually produce the so-called *band spectra*.

Sometimes spectra are also named from their manner of production. Thus *flame spectra* are obtained by causing substances to emit light in a suitable flame; this is done in qualitative inorganic analysis when the chlorides of metals like barium, potassium, cæsium, etc., are heated in a bunsen flame and the light is viewed through a spectroscope. *Arc* and *spark spectra* are other types of emission spectra, produced by different means of excitation; they differ from flame spectra only in the greater violence of the excitation which produces many more lines. *Gas discharge spectra* are those obtained from Geissler tubes, in which a high-tension discharge is passed through gases under low pressure.

With some gases (*e.g.*, hydrogen) it is possible to obtain a line, or a band, or a continuous spectrum at will by suitably varying the electrical conditions of excitation and the pressure of the gas.

TABLE 1.4

Types of spectra

Light from :	When passed through a prism or grating gives an emission spectrum as follows :	If light is passed through a transparent medium (solid, liquid or vapour) before going through prism
White-hot metals (*e.g.*, filament lamps)	Continuous (in the visible)	Absorption : line spectra, band spectra } gases; bands (liquids, solutions or solids)
The sun	Continuous * (in the visible)	
Carbon arc	Continuous † (in the visible)	
Glowing gases generally	Discontinuous (line spectra or band spectra)	
Glowing hydrogen (pressure of gas and voltage critical)	Continuous (in the ultra-violet	
Arcs or sparks between metal electrodes	Discontinuous (usually line spectra)	

* Except for the Fraunhofer lines.

† Sometimes has cyanogen bands superimposed due to the combination of the carbon of the electrodes with the nitrogen of the air.

(*b*) *Absorption spectra.* Whilst emission spectra are very suitable for the qualitative study of substances that are very stable to heat or violent electrical excitation, organic compounds are most conveniently studied by absorption spectroscopy. If the radiation from any source passes through a transparent layer of solid, liquid, solution, or gas, some of the radiation may be selectively removed and the residual rays, when passed through a prism, may yield a spectrum with gaps of some kind. Such a spectrum is called an *absorption spectrum*, and the absorption may take the form of lines (*e.g.*, the Fraunhofer lines in the solar spectrum) or of bands, complementary to the two types of emission spectra mentioned above. In absorption spectroscopic studies the danger of decomposition or alteration of the species under investigation is minimised, and experimental conditions are more readily controlled and reproduced than in emission spectroscopic work.

The laws of light absorption. Before one can consider the methods of presentation of absorption spectra, as well as the modern methods of their determination, discussion of the two principal laws of light absorption is necessary. The first of these is *Lambert's law*, which states that *the proportion of light absorbed by a transparent medium is independent of the intensity of the incident light* and *that each successive unit layer of the medium absorbs an equal fraction of the light passing through it.*

This law is readily illustrated by an example: if the intensity of light incident upon any transparent medium is unity and the absorption of each unit thickness of the absorbing medium is equal to one-tenth of the incident light, the light intensity will be diminished successively as follows, on passing through each unit layer of the medium:

$$1{\cdot}0 - 0{\cdot}90 - 0{\cdot}81 - 0{\cdot}73 - 0{\cdot}66 \ \ldots \text{ etc.}$$

Mathematically this leads to the expression

$$I = I_0 \times e^{-\alpha l} \quad \text{or} \quad \log_e (I_0/I) = \alpha l$$

where I_0 = intensity of the incident light
I = intensity of the transmitted light
l = thickness of the layer (in cm.)
α = the *absorption coefficient* of the medium
and e = the base of natural logarithms.

It is more usual and convenient, however, to use logarithms to the base 10 instead of to the base e, and on this base the absorption coefficient α is converted into the so-called Bunsen and Roscoe *extinction coefficient* K. Thus $I = I_0 \times 10^{-Kl}$ or $\log(I_0/I) = Kl$, where K is a constant depending only on the medium examined at the wave-length under consideration.

This extinction coefficient is, therefore, the reciprocal of the thickness required to weaken the light to one-tenth of the incident light. It is related to the absorption coefficient α by the factor 2·3026, relating natural to common logarithms.

Thus $\alpha = 2{\cdot}3026K$.*

It is immediately apparent that the absorption coefficients α and K contain no concentration factor, and the other important law, *Beer's law*, deals with this concentration variable. It states that *the light absorption is proportional to the number of molecules of absorbing substance through which the light passes*. Thus, if the absorbing substance is dissolved in a transparent solvent, the absorption of the solution will be proportional to its molecular concentration.

Substitution of εc for K (ε being the *molecular extinction coefficient* and c the concentration in gram-mols. per litre) in the expression already deduced from Lambert's law, $\log (I_0/I) = Kl$ yields the expression

$$I = I_0 \times 10^{-\varepsilon cl} \quad \text{or} \quad \log_{10} (I_0/I) = \varepsilon cl,$$

the various symbols having the meaning already given. This expression combines Lambert's law with Beer's law, and this is the formula on which the majority of the chemical applications of spectrophotometry is based.

An actual example of the determination of the molecular extinction coefficient ε will illustrate the arithmetical procedure. Thus, for example, a solution of purified carotene (molecular weight 536) at a concentration of 0·00050% in chloroform (*i.e.*, 5 mg. per litre) gave a value for the extinction E (= $\log I_0/I$) at the absorption maximum (463 mμ.) of 1·10 in a 1·0-cm. cell. The value of ε was therefore

$$\frac{1{\cdot}1}{0{\cdot}0005} \times \frac{536}{10} = 118{,}000$$

When the molecular weight of the absorbing substance is unknown, the expression $E^{1\%}_{1\,cm.}$, perhaps more conveniently written as E,1%,1 cm., is now frequently used for the comparison of absorption intensities. It is related to the molecular extinction coefficient ε, which may be written as $E^{molar}_{1\,cm.}$ by the expression

$$E^{1\%}_{1\,cm.} \times \frac{M.W.}{10} = \varepsilon$$

Although originally used for comparing the absorption intensity of vitamin *A* in different samples of liver oils and concentrates before the molecular weight of the vitamin was known, it has proved so valuable that it is in common use, especially in analytical work, for expressing the light absorption of any absorbing entity, whether the molecular weight is known or not. This symbol has not hitherto been assigned any generally accepted name, and it is now suggested that $E^{1\%}_{1\,cm.}$ be called simply the *E*-value, which for any given solute will, of course, only be constant for a particular wave-length and a particular solvent. When this *E*-value is known for

* Some continental workers in describing absorption intensities use the term absorption coefficient (a), but symbolise it by κ (Greek kappa). This leads to confusion, because the English letter K and kappa are superficially alike in print. The values for the extinction coefficient are related to the English values by $\kappa = 2{\cdot}303$ K.

the pure substance at the same wave-length and in the same solvent, the percentage of the absorbing substance present in any particular sample is directly proportional to its observed E-value. Thus

$$100 \times E^{1\%}_{1\,cm.}\text{(observed)}/E^{1\%}_{1\,cm.}\text{(pure substance)} = \%\text{ of absorbing substance present.}$$

Although the E-value represents the absorption in terms of the optical density for a 1% solution in a 1-cm. layer, it is well-established practice to determine the extinction at the desired wave-length for any convenient concentration and thickness of cell that will bring it into the range of values measured by the particular instrument in use, and then to calculate the E-value. In this procedure it is assumed, of course, that Beer's law holds over the range of concentrations used. In cases where this is not so or where there is any doubt, the original suggestion of Gillam and Morton [1] out of which the $E^{1\%}_{1\,cm.}$ nomenclature grew can be followed. In this method the absorption in terms of either ε or E is placed in square brackets with the *actual* concentration and thickness of cell (in cm.) stated outside the bracket. Thus the *observed* value of the optical density at the particular wave-length can be calculated since all the necessary data are given. The following example of Gillam and Morton [1] illustrates the point:

$$\varepsilon^{\mathrm{Br}_2}_{\mathrm{CCl}_4}\text{ (417 m}\mu\text{.) for bromine in CCl}_4 = [208]^{0\cdot00157\text{-m.}}_{1\,cm.}$$

The concentration can, of course, be given either in molarity or as a percentage. The method facilitates the recording in a simple manner of all the experimental conditions, including concentration and layer thickness, which may be necessary for subsequent reference.

The validity of Beer's law. From Beer's law (absorption is proportional to the number of molecules in the light path) it follows that, when concentration of solution and layer thickness are varied in such a manner that their product remains constant, the intensity of absorption of the solution should also remain constant. Thus a 10-cm. layer of 1% solution should absorb exactly the same amount of light as does a 1-cm. layer of a 10% solution.

While Lambert's law holds rigidly in all cases so far examined, there are many exceptions to Beer's law; usually, however, the anomalies can be explained by a change in composition with change of concentration. Many acids, bases, and salts in solution do not obey Beer's law, simply because they are more completely ionised with increasing dilution, and because in most cases the light-absorption of the ions differs from that of the non-ionised molecules. Thus concentrated nitric acid shows the absorption of HNO_3 molecules, while the dilute acid shows the characteristic absorption band of the nitrate ion. For a similar reason concentrated cupric chloride solution is green (colour of $CuCl_2$), although the dilute solution is blue (colour of Cu^{++} ions).

The absorption of azobenzene in benzene solution illustrates a case where Beer's law is followed approximately within the reproducibility (±2–3%) of the photographic method of recording (see Table 1.5).

TABLE 1.5

Light absorption of azobenzene (in benzene)

Wave-length (mμ.)	Molecular extinction coefficient (ε)		
	Concn. of solution		
	0·0001-M.	0·001-M.	0·002-M.
546	17	16	15
486	320	310	305
436	650	665	645
404	350	343	—

It is an accepted principle in absorption spectrophotometry that Beer's law cannot be assumed to hold for any substance without confirmation; most non-ionising organic compounds obey Beer's law, however, at least approximately. When a spectrophotometric method is being developed for any substance, however, it is essential that the validity of Beer's law should be established for the substance in the particular solvent and over the range of concentrations likely to be used. To illustrate the importance of this point, the data of Scheibe [2] for the light absorption of acetone in hexane are reproduced in Table 1.6. Although acetone in *n*-hexane might

TABLE 1.6

Light absorption of acetone in hexane [2]

λ (A.)	Molecular extinction coefficient (ε)	
	Concn. of solution	
	0·146-M.	1·82-M.
3085	4·71	3·12
3065	5·38	3·63
3045	6·25	4·39
2615	10·78	8·33
2570	8·34	6·51
2480	5·38	3·63

reasonably be expected to obey Beer's law, it is obvious from the Table that the molar extinction coefficient varies very considerably with the concentration of the acetone, and that, in fact, Beer's law is invalid for this system. Whilst this could be explained quite readily for aqueous solutions of acetone (*e.g.*, by assuming hydration and consequent disappearance of the light-absorbing carbonyl group), the only likely explanation for the deviation from Beer's law in an "inert" solvent such as