REVIEW ARTICLE

INFRA-RED SPECTROSCOPY AND ITS APPLICATION TO PHARMACEUTICAL ANALYSIS

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Within the last ten years infra-red spectroscopy has developed into an indispensable analytical tool of the organic chemist. In this connection therefore it has had considerable impact on processes concerned with the analysis and manufacture of drugs. The following account will deal in the first place with the fundamental principles of infra-red spectroscopy and will later describe some of its applications to the investigation and estimation of substances of pharmaceutical interest.

The infra-red region of the spectrum was discovered in 1800 by Sir William Herschel while studying the relative heating effects of light of different wavelengths by inserting the bulb of a sensitive thermometer into the different regions of the solar spectrum. Herschel found that while the heating was not very great at the blue end, it was quite considerable at the red end and persisted beyond the red well into the region where no radiation was visible. It was subsequently shown that this infra-red heating effect was due to an invisible radiation of the same nature as light whose wavelengths extended from $0{\cdot}7\mu$ to several tenths of a millimetre, a range which is much greater than that occupied by the spectrum of visible light (viz., $0{\cdot}4\mu$ to $0{\cdot}7\mu$). The exploration of the infra-red region has depended on the development of extremely sensitive detectors of thermal radiation such as vacuum thermocouples or bolometers and the use in spectrometers of prisms of alkali halide crystals such as rock salt in place of prisms of glass or quartz which absorb the longer wavelength radiation. A general study of the absorption of various materials has shown that the infra-red absorption spectra, particularly of organic molecules, consist of a large number of fairly narrow bands. The absorption bands of one compound generally differ from those belonging to another compound both in wavelength and intensity. The infra-red spectrum of a substance is therefore a physical property by which it can be identified, the pattern of its absorption bands being analogous to a fingerprint for characterising the substance. The spectrum is indeed the most specific physical property of a substance and is one which should always be recorded for any new compound. The intensity of any of its characteristic bands when introduced into suitable absorption equations permit the substance to be estimated on a quantitative basis. For the above reasons infra-red absorption spectrophotometry has come into wide use in analytical chemistry, though it should be emphasised that this has only been made possible by developments in instrumentation which have produced robust and reliable instruments capable of operating both under laboratory and plant conditions.

INSTRUMENTATION

Before reviewing some of the numerous applications of infra-red spectroscopy to pharmaceutical analysis, it is first desirable to describe briefly the types of instrument used and the techniques employed in these analyses. One of the most commonly used instruments is the recording double-beam infra-red spectrometer (Fig. 1). In this instrument, infra-red radiation from a nernst glower or carborundum rod heated to incandescence by the passage of an electric current, is split into two

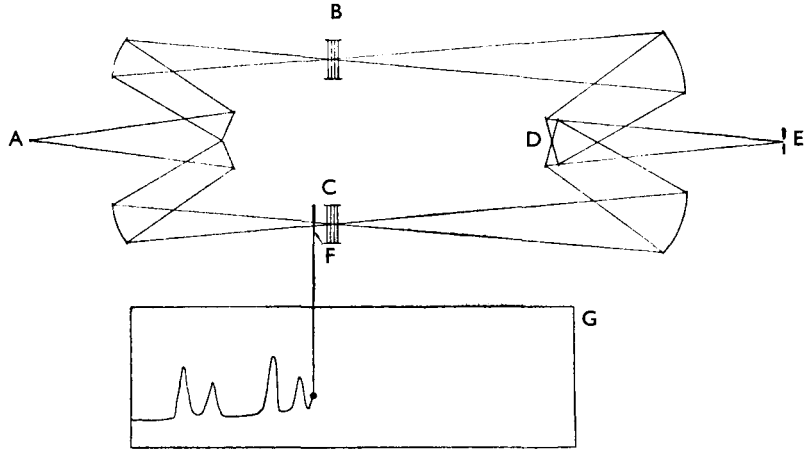


FIG. 1. Diagram of double-beam unit for the infra-red spectrometer. A is the source, B the sample cell, C the reference cell, D the alternating mirrors, E the spectrometer slit, F the attenuating shutter geared to the traverse of the recording pen and G, the record chart geared to the wavelength drive.

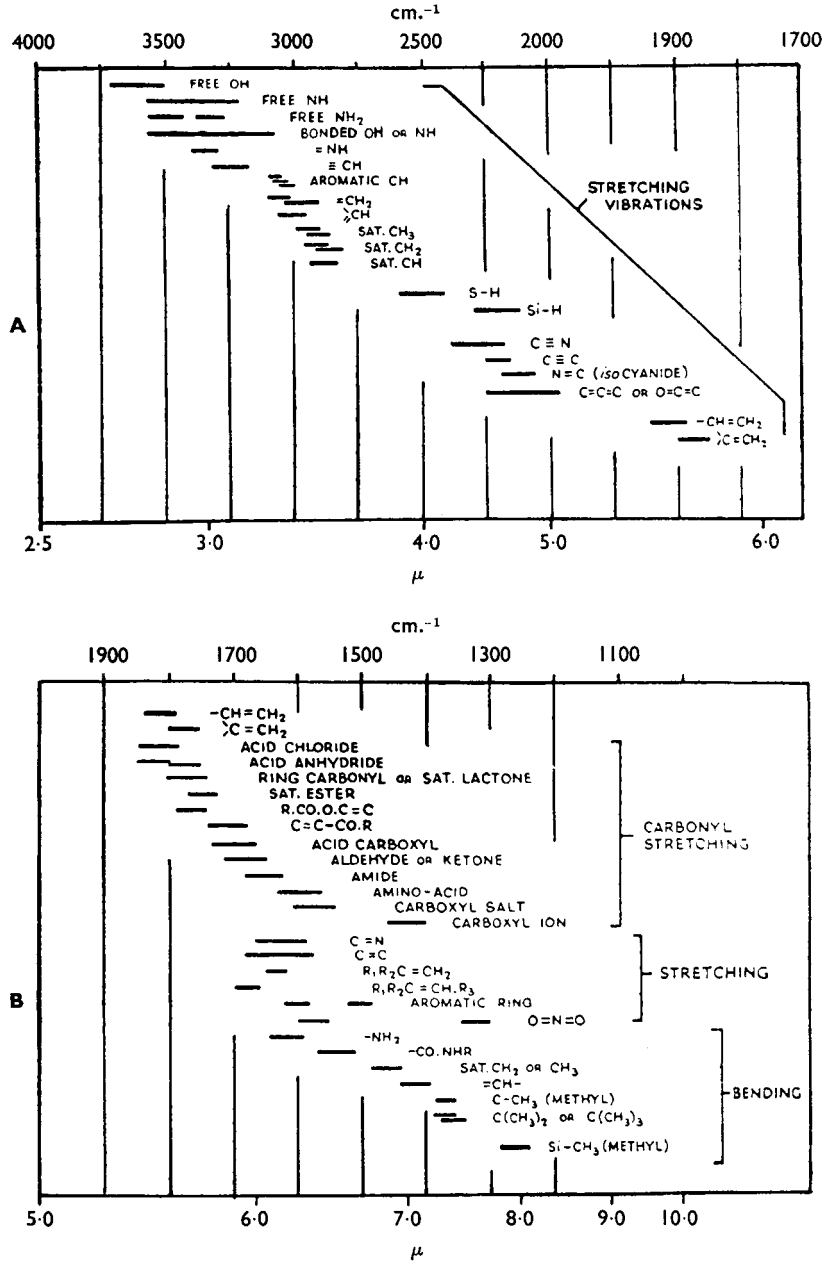
equivalent beams, the first of which passes through the sample to be analysed and the second through a comb or V-shaped shutter which can be progressively inserted into the beam by a servo-mechanism until as much radiation is cut off in this way as is absorbed by the sample in the first beam. The two beams enter the spectrometer and are dispersed by a rock salt prism so that only the radiation of the wavelength being considered passes through the exit slit and falls upon the detecting thermocouple. By a moving mirror system, the radiation from the sample and from the reference path is allowed to fall alternately on the thermocouple. Amplification of the out-of-balance alternating current of the thermocouple is used to control the insertion of the alternating shutter into the reference beam until the absorption of the sample is matched. As the wavelength transmitted by the spectrometer is changed by its scanning mechanism, the matching shutter position alters to correspond with the varying absorption of the sample with different wavelengths. The shutter is geared to the traverse and the wavelength mechanism to the scan of a pen recorder which thus automatically plots the absorption of the sample against wavelength. The absorption can either be plotted as the percentage absorption (percentage of incident radiation absorbed)

or as absorbance or optical density (the logarithm of the reciprocal of the transmittance). The latter is to be preferred since in view of the logarithmic nature of the laws of absorption the ordinates are then directly proportional to the amount of material present. In analysing a mixture quantitatively it is usual to make measurements on "key" bands These are bands which are characteristic of the substance being estimated and which as far as possible are not overlapped by strong bands belonging to other components in the mixture. For liquids, thicknesses of the order of 0·1 mm. are usually adequate for the appearance of the infra-red bands whereas for gases, cell lengths of 10 cm. and pressures of up to 1 atmosphere are common. The cell windows are usually of polished rock salt and this together with the extremely strong absorption of water itself makes the method inapplicable where large amounts of water are present. Special methods can be used for insoluble solid samples which can either be investigated as thin films or as finely ground powders mulled into liquid paraffin or another suitable substance or impregnated in a disc of an alkali halide, usually potassium bromide[1,2,3].

Theoretical Basis of Qualitative and Quantitative Analysis

The theoretical basis of the individual character of infra-red spectra rests upon the fact that the observed bands correspond to particular vibrations of the molecular framework or structure. Their wavelengths (or frequencies) thus depend upon the masses of the atoms, the strengths of the binding forces of the various bonds and the geometrical configuration of the molecule. It is for these reasons that no two different substances can ever have the same infra-red spectrum. It is possible to associate certain absorption bands in the infra-red spectrum of a molecule with vibrations in specific groupings within that molecule. Thus bands associated with vibrations in O—H, N—H, C≡C, C=C and C=O links always occur in certain characteristic wavelength regions. The presence of any of these bands in a spectrum can be taken as indicating the occurrence of the corresponding groups in the substance being investigated though it should be stressed that a considerable number of the bands in a spectrum, particularly those at wavelengths greater than 6μ, are more characteristic of the molecule as a whole than of a particular part of it. These latter are called the skeletal vibrational modes and the region in which they occur is sometimes referred to as the "fingerprint region."

The qualitative analysis of a substance is usually carried out by comparing the frequencies of the strong bands in its infra-red spectrum with the frequencies associated with standard groups by means of a chart such as that shown in Figure 2. (It is becoming more customary to use the term frequency in preference to wavelength and for this purpose the frequency is taken as the reciprocal of the wavelength in cm., the unit being termed the wave-number). The highest frequencies, e.g., those in the range 4000–2000 cm.$^{-1}$ occur for stretching vibrations in bonds containing hydrogen. This clearly is connected with the relatively low mass of the hydrogen atom. Within this group the order of the frequency is that for which we expect strongest binding, viz., OH, NH, CH, PH,



SH, SiH. The associated (H-bonded) frequencies of these bonds are somewhat lower than those of unassociated material. The CH bands can also be subdivided into aliphatic, ethylenic and aromatic types by virtue of the frequencies at which they occur. The next higher frequency

class involves stretching vibrations in triple bonds where the strength of the bond brings the frequencies into the range 2400–1950 cm.$^{-1}$ in spite of the heavier atoms involved. These are followed by double bond frequencies, particularly of the various forms of carbonyl bond which extend from 1900–1500 cm.$^{-1}$. Ethylenic stretching vibrations are strongest in the 1700–1600 cm.$^{-1}$ region with aromatic ring frequencies only slightly lower. At still lower frequencies the bands due to the stretching of single bonds occur together with certain of the deformation or bending vibrations of bonds containing hydrogen. The greater variety of possible variations giving rise to bands in the range around 1000 cm.$^{-1}$ and below make it much more difficult to assign bands in this region to

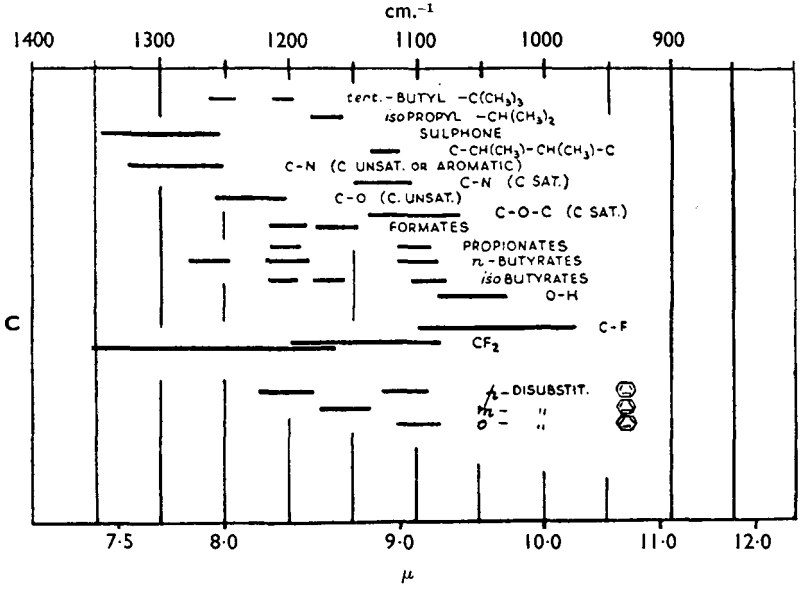


FIG. 2 A.B.C. Charts showing the frequencies of the characteristic bands associated with specific chemical bonds or groups. Frequencies are the reciprocals of the wave-length in cm., the unit being called a wave-number.

motion in any specific group in the molecule though there are certain notable exceptions. These are in particular the bending vibrations of CH bonds out of the plane of an aromatic or double bond system to which they belong. Such bands are usually very intense and their precise position, which is relatively independent of the nature of the other substituents, can be used to determine the type of the substitution about the double bond or aromatic nucleus (e.g., whether *o*, *m*, or *p*, etc.).

As an example of the qualitative analysis outlined in the previous paragraph let us consider the spectrum of *p*-methylstyrene shown in Figure 3. The bands occurring around 3000 cm.$^{-1}$ indicate the presence of ethylenic, aromatic and aliphatic CH; those around 1700–1600 ethylenic and aromatic centres. At 1460 cm.$^{-1}$ we have a band indicating the presence of CH_2, at 1375 one indicating that of CH_3 and at longer

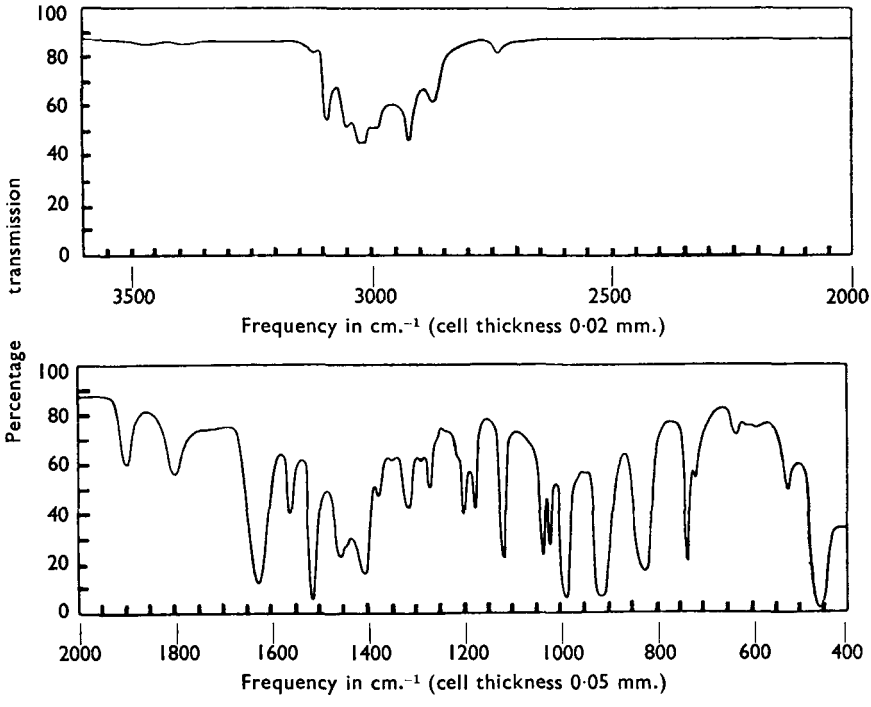


FIG. 3. The infra-red absorption spectrum of *p*-methyl styrene.

wavelengths there are bands which indicate $RHC{=}CH_2$ ethylenic substitution and *p*-substitution on the aromatic ring. Thus it would not require much physical or chemical information in addition to the infra-red spectrum to fix the identity of this substance.

The most valuable handbooks for the above type of work are by Bellamy[4], Randall *et al.*[5] and Barnes *et al.*[6] Of particular value in its specialised field is a compilation of spectra of steroids and related substances by Dobriner, Katz and Jones[7]. In what follows, examples will be given of the use of infra-red spectroscopy in studying substances of pharmaceutical interest. Two earlier reviews on this theme are by Carol[8] and Garratt and Marshall[9].

APPLICATIONS

Sterols and Derivatives

This class of compounds has probably been the subject of more fruitful infra-red investigations than any other. In the synthesis, identification and analysis of these important substances infra-red spectroscopy is invaluable. Figure 4 shows the infra-red spectra of the adrenocortical hormones cortisone and 17-hydroxycorticosterone, which are important in the alleviation of the symptoms of rheumatoid arthritis. In both molecules absorption bands characteristic of a hydroxyl group, at least two carbonyl groups and one double bond are indicated. At the lower

frequencies, the spectra differ greatly although the only difference in the molecular structure is the presence of a hydroxyl group at position 11 in 17-hydroxycorticosterone in place of the ketone group in cortisone[10].

Differences in the long wave (fingerprint) region of the spectrum were used by Dobriner for detecting small traces of Δ^9-etiocholenole in the presence of large amounts of etiocholanolone. He has shown that the former is present in the urine of cancer patients, but is only rarely observed in the urine of healthy individuals[11].

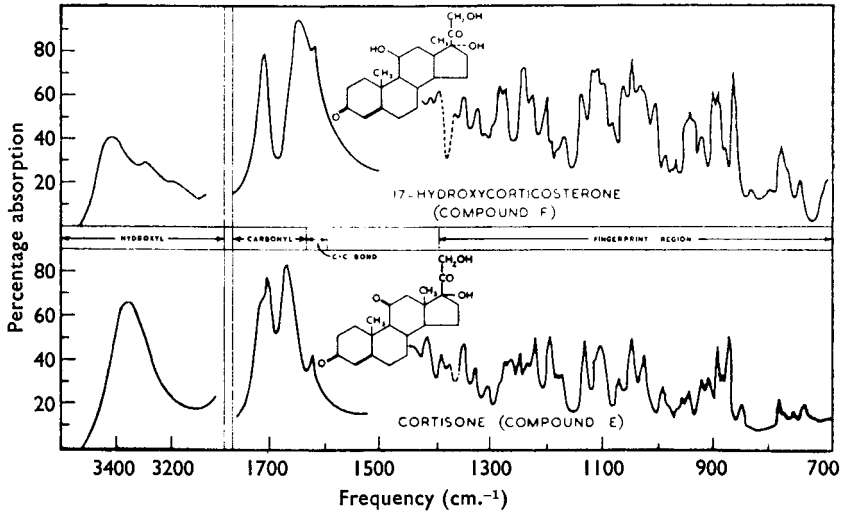


FIG. 4. The infra-red spectra of 17-hydroxycorticosterone and cortisone. Absorption bands characteristic of a hydroxyl group, at least two carbonyl groups and one double bond are indicated in both molecules.

As a simple example of the quantitative application of the technique Garratt and Marshall[9] describe the estimation of testosterone propionate in 1 per cent. solutions in arachis oil and state that it is much more satisfactory than the corresponding ultra-violet method. Carol *et al.*[13] describe general methods for the spectroscopic determination of œstrogens. The determination of methyltestosterone in tablets[13], of α-œstradiol and other œstrogens,[14] and of pregnenolone and pregnenolone acetate[15] have also been described.

One of the principal differences among the great variety of steroids which occur in nature is the presence of carbonyl groups at various positions on the ring system or on the side chain. The location of such groups profoundly influences the physiological activity. In dilute solution in carbon disulphide it has been observed that the exact position of the C=O stretching vibration in the range between 1650 and 1800 cm.$^{-1}$ is highly characteristic of the particular type of carbonyl group present and some sixty different carbonyl types have been differentiated in this manner[16]. In Figure 5 is shown the absorption in the carbonyl stretching region of the acetates of two adrenocortical hormones. The bands marked

I and II are at positions characteristic of the $-CO-CH_2-O-CO-CH_3$ group of the side chain and the band IV is at a frequency identified with the conjugated carbonyl group at position 3. The band marked III present in curve B but lacking in curve A can be identified with the carbonyl group at position 11. A catalogue of infra-red absorption spectra of a number of steroidal sapogenin acetates, has been published by Eddy, Wall and Scott[17]. They show that a sapogenin can be identified either by means of its spectrum in chloroform or by the spectrum of its acetate in carbon disulphide. Jones, Katz and Dobriner[18] give the spectra of 35 steroid sapogenins. By the use of small cells of special construction these materials can be estimated spectroscopically in μg. quantities. More recently, Haydn *et al.*[19] discuss "fingerprint" infra-red bands for steroidal *pseudo*-sapogenins.

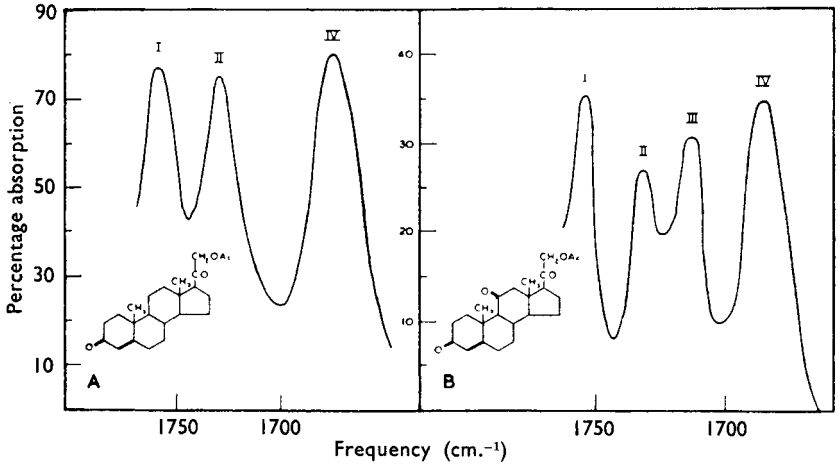


FIG. 5. Infra-red absorption spectra of the acetates of two adrenocortical hormones. Absorption in the carbonyl stretching regions are shown.

Jones and his collaborators have done a considerable amount of correlation work between the structure and spectra of different steroids. Most of this has been published in various articles in the *Journal of the American Chemical Society* in recent years. For example, they have been able to characterise the methyl and methylene groups in steroids by means of their infra-red bands occurring near 1350 and 1500 cm.$^{-1}$ respectively[20]. They found (*a*) that the cyclic methylene groups of the steroid ring system absorb at a different frequency from the linear methylene groups in the side chain, (*b*) methylene groups adjacent to carbonyl groups and to ethylenic linkages absorb at characteristic positions. In steroids containing the groups $-CH_2-CO-$ the frequency of the α-methylene bending vibration is determined by the location of the carbonyl group and serves to supplement the C=O stretching frequency for the characterisation of the carbonyl position. It was further shown (*c*) that the angular methyl groups, the terminal side chain methyl groups and the methyl group in the acetoxy radical of steroid acetates, absorb

at different frequencies and can be distinguished by this fact. Studies of steroids in which the methylene groups have been deuterated confirm the above statements[21].

Antibiotics

Infra-red spectroscopy played a valuable part in determining the lactam ring structure of penicillin[22] and it often forms the best method of characterising the material and its derivatives as well as estimating it quantitatively. An early quantitative method of spectroscopic analysis for crystalline penicillin employed an internal standard which was added to the sample, the whole being then made into a mull whose spectrum was obtained[23]. The penicillin was estimated by the comparison of the absorption of one of its key bands with that of the internal standard which was chosen to have bands conveniently located for this purpose. More recently, a method for the estimation of benzylpenicillin by the pressed potassium bromide disk method has been given by Jensen[24]. A method of spectroscopic estimation involving an isotope dilution principle using deuterium has been used to determine the best time to harvest penicillin brews[25,26]. This is similar to the procedure described later for the γ-isomer of benzene hexachloride. The principle of the procedure depends on the introduction of a known amount of deuterobenzyl penicillinic acid into a known quantity of the brew, which is then concentrated in the usual manner. The ratio of the intensities of the corresponding bands of the ordinary benzylpenicillinic acid to that of the deutero-analogue together with the weight of the latter introduced into the original sample of the brew enables the concentration of penicillin in the brew to be calculated. In practice, the infra-red spectrum of deuterobenzyl penicillinic acid was not sufficiently different from that of the natural product to form the basis of a precise determination. This difficulty was overcome by subjecting the isotopically mixed benzylpenicillinic acid to an alkaline hydrolysis isolating qualitatively (but in pure form) the phenylacetic acid and determining by infra-red spectroscopy the ratio of the two isotope analogues of phenylacetic acid.

Analytical procedures for erythromycin are given by Washburn[27]. Spectra have been reported of mycomycin and isomycomycin[28], amicetin, a new antituberculosis antibiotic[24], oxytetracycline[30], streptolin[31] and many others. Recent interesting examples of spectral structure determination are the proof of the presence of the conjugated allene structure in magnamycin[32,33] and the existence of the diazo group in the new tumour inhibiting antibiotic azarin[34].

Insecticides

The dependence of the infra-red spectrum on the geometrical configuration of a molecule makes it extremely valuable for detecting its different isomers and estimating the relative amounts present in any product containing the substance. For this reason the method has been extensively used for the determination of the γ-isomer of benzene hexachloride in commercial preparations[35,36]. Trenner *et al.*[37] apply the isotope

dilution method for this purpose. This method depends upon the use of γ-hexachlorohexadeutero-*cyclo*-hexane as a tracer. A known weight of the latter is added to a known amount of the crude mixture, which is dissolved in acetone, evaporated and the ordinary crystallisation procedure for isolation of the γ-isomer followed. The relative amount of deutero to normal γ-benzene hexachloride can then be obtained by comparing the absorbances of corresponding bands of these materials and knowing the former the latter can be calculated.

Samples of azobenzene used in pesticidal smoke formulations have been analysed for *cis*- and *trans*-azobenzene and hydrazobenzene[38]. The key bands of dicophane have been used to determine this material in the presence of any combination of the common impurities present[39,40,41]. The alkali-stable insecticides aldrin and dieldrin used in agricultural and horticultural preparations can be estimated in micro quantities on their key bands at 8·47 and 12·37μ[42]. Samples are extracted with ether, the extract evaporated to dryness, the residue then redissolved in carbon disulphide and the absorbance compared with standard solutions. As an example of a multicomponent analysis Garratt and Marshall[9] describe the analysis of mixtures of dieldrin and benzene hexachloride using a band at 9·93μ for dieldrin and one at 10·48μ for benzene hexachloride. At these positions there is little overlapping of the band systems of the two materials. For best accuracy comparison is made with standard synthetic mixtures. For a qualitative example in the field mention should be made of the work of Harper and Crombie[43] on the synthesis of the pyrethrins which depends at many stages on infra-red confirmation of structural features. The analysis of the pyrethrum insecticide rotenone has been described by Cupples[44]. The spectrum of dihydrorotenone has also been given[45].

Vitamins

Infra-red spectra of vitamin B_{12} and B_{12b} have been used in the isolation of vitamin B_{12} from neomycin fermentations[46]. Spectra of vitamins A_2 and A_1 and retinene 2 and retinene 1 have been given[47]. Jones[10] has shown that the vitamins D_2 and D_3 can be distinguished although they differ in structure only by a hydrocarbon side chain, which is saturated in the latter but has only one double bond in the former case. The quantitative estimation of nicotinic acid using the isotope dilution method has been described by Trenner and his collaborators[48].

Alkaloids

Much spectroscopic work on alkaloids, terpenes and other plant products is to be found in the literature, mostly in connection with structural problems. Spectroscopy is providing a rapid and easy method of identification of these complex substances. A simple example of the use of the fingerprint region for purposes of identification occurs for the substances rhombine, monolupine and anagyrine[10]. These three alkaloids isolated from different plants were suspected to be identical from their melting points and empirical formulæ. Their identity was confirmed by

the fact that all three had the same infra-red spectrum which consisted of a complicated pattern of some 50 bands in the region 5 to 15μ. An example is also given of the ease of estimation of the two isomers of citronellol[10] where *iso*propenyl and *iso*propylidine structures may arise[49]. About 1 per cent. of the α-isomer can be detected in admixture with the β-isomer but not less than 10 per cent. of the β-isomer in admixture with the α-isomer[50]. Spectra are also reported of citronellol, geraniol, geranyl acetate, geranamide citral, linalol, dihydromyrcene (α and β). The analysis of aspirin, phenacetin, caffeine and codeine has been reported[51] in which the first three are determined in chloroform solution at 9·27, 8·99 and 10·26μ and the last by ultra-violet absorption. A more recent paper[52] reports the same analysis using the 5 to 7μ region for the first three and for the codeine a band at 10·62μ in carbon disulphide solution.

The spectroscopic determination of quinine and strychnine in elixirs has been reported by Washburn and Krueger[53]. After a preliminary solvent exchange purification, quinine is estimated on its band at 6·20μ and strychnine at 6·06μ. Data for the estimation of piperine, nicotine, hydrastine, piperidine and strychnine are given by Pleat, Harley and Wilsley[54]. The infra-red spectra of 47 alkaloids in the 2·8 to 6·5μ region have been reported by Marion, Ramsay and Jones[55], a testimony to the recognition of the spectrum as being the most specific physical property of the compounds.

While perfumes are not strictly pharmaceuticals, there are many common problems and infra-red spectrometry is increasingly being used not only for the study of natural odorous substances, but also for the analysis of mixtures of these and for the technical control of the manufacture of synthetic products[56]. Spectroscopic studies of the isomers of the aliphatic terpenes and of the structures of ionones and irones and their derivatives have been carried out by Naves and others[57,58]. Even in the cosmetic industry infra-red spectrometry is used to identify and estimate such ingredients as oils, waxes, wetting agents, resins, solvents and more recently for the analysis of mixtures of sugars, sulphonic acids, drugs, surface agents and other materials employed in this industry[57].

Barbiturates

Phenobarbitone can be conveniently estimated by its absorption bands around 5·75μ, a direct comparison being made by Beer's law between the absorbancies of a standard solution and the sample, chloroform being used as solvent[9]. A method for estimating total barbituric acids has been given by Ribley, Kennedy, Hilty and Parke[59]. Characteristic absorption bands of the purified compounds in chloroform solution are given by Umberger and Adams[60] for allobarbitone, amylobarbitone, barbitone, bromvaletone, butobarbitone, carbromal, cyclobarbitone, hexobarbitone, methylphenobarbitone, pentobarbitone, phenobarbitone, quinalbarbitone, thiopentone, aprobarbital, butobarbital, hexethal, vinbarbital, Cyclopal, Sandoptal, Sedormid; a concentration of 20 $\pm$ 5 mg. of compound per ml. of chloroform being used. In view of the increasing

use of barbiturates for criminal and suicidal purposes the analysis of the small quantities recovered in these instances assumes considerable forensic importance. Infra-red spectroscopy is being successfully used for this purpose[60].

The spectra in the 6μ region of a number of barbiturates have been examined to see if any chemical differences in the barbiturate nucleus, which could be inferred from spectral shifts, could be correlated with the duration of drug activity[61]. Minor shifts were found indicating minor chemical changes in the barbiturate nucleus itself. It thus appears that the main effect is probably due to differences in the fat or water solubility of the different substituents. It is possible that spectroscopy will be one of the helpful factors in explaining the varied activities of different steroids which also show small shifts of the carbonyl bands similar to those occurring in barbiturates.

Antihistamines

Frediani[62] reports the use of infra-red for the analysis of pyrimaline and ephedrine mixtures. Garrett and Marshall[9] describe the use of infra-red spectroscopy in an attempt to prepare analogues of the antihistamine antazoline[63].

Bacteria

Different strains of bacteria can be identified by their infra-red spectra. The various chemical groupings present and their relative proportions are comparatively invariant for any particular strain hence the infra-red spectrum is characteristic of that strain. Randall and Smith[64] give the spectra of several different strains of tuberculi bacilli. It is shown that there are spectral differences between virulent and avirulent strains of the standard laboratory strain H37, between human and bovine varieties of tubercle bacilli and between strains isolated from different tuberculous patients.

Miscellaneous

The spectra of DL-thyroxine and diiodothyronine are given by Wang, Hummel and Winnick[65] and shown to be identical with DL-thyroxine-1-^{14}C and DL-diiodothyronine-1-^{14}C synthesised by these authors.

The analysis for barbiturates in forensic laboratories has already been mentioned. Another example of the use of infra-red in criminology is the identification of a fraction of a drop of oily liquid which had been recovered from the organs of a victim, with the poison methyl salicylate[66].

Assaying khellin and visnagin, two similar new drugs derived from crude extracts of seeds of *Ammi visnaga* was tackled by Bailey, Geary and de Wald[67,68] by infra-red methods. Both materials have been synthesised in the laboratory. Infra-red studies proved to be an accurate and rapid means of assaying their presence in plant fractions and pharmaceutical mixtures. The crude extract is dissolved in chloroform and using key bands in the 8 to 9μ region the time of assay is only 20 minutes.

The determination of triple bonds C≡N or C≡C, usually so difficult by ordinary methods, is one which is accomplished with great ease and certainty by infra-red methods because the corresponding key frequencies lie in a spectral region well clear from any other bands.

Conclusion

The present survey is not by any means a comprehensive summary of the position of the subject to date. Much has been neglected in a rapidly expanding field but it is hoped that enough has been recorded to illustrate the value of the method and to indicate the important future which the technique has in the study of pharmaceuticals. Even the type of analysis which has been described here, which is mainly the analysis of end products, may not be the major use to which infra-red is put. In fact, the analysis of organic intermediates such as, for example, various isomeric nitroparaffins for the introduction of different alkyl groups in attempts to produce superior drugs, may form the larger part of the work of an infra-red spectrometer in a pharmaceutical laboratory. It is significant that nowadays all the large organic laboratories have installed infra-red spectrometers, sometimes two or three. These are constantly being used by the research worker even in following the course of his reactions. He quickly realises the value of the instrument and has no difficulty in acquiring the technique provided normal servicing of the equipment can be arranged. It is not to be understood from what has been said that infra-red methods will replace in accuracy, economy and speed any of the simpler pharmaceutical assays. It is largely for the more difficult analyses which cannot be done any other way and also for the structural information that can be obtained from it that the spectroscopic method will find its proper use. Already the technique is being employed on a wide variety of problems, especially on the American continent, and it is time that infra-red methods were accepted by the British Pharmacopœia as alternatives to long and tedious chemical analyses in cases where the accuracies and reliabilities are comparable. As an example of this a comparison of the analyses of atropine sulphate tablets by infra-red spectrometry, and by U.S.P. assay has been made by Carol[8] and the results indicate that the proposed spectroscopic method has an accuracy equal to the U.S.P. XIV assay, and in addition offers qualitative proof of identity by comparison of standard and sample.

References

1. Williams, *Rev. Sci. Inst.*, 1948, **19**, 135.
2. Lord, McDonald and Miller, *J. Opt. Soc. Amer.*, 1952, **42**, 149.
3. Ford and Wilkinson, *J. Sci. Inst.*, 1954, **31**, 338.
4. Bellamy, *The Infra-red Spectra of Complex Molecules*, Methuen, London, 1954.
5. Randall, Fowler, Fuson and Dangl, *The Infra-red Determination of Organic Structures*, Van Nostrand, New York, 1949.
6. Barnes, Gore, Liddell and Williams, *Infra-red Spectroscopy*, Rheinhold, New York, 1944.
7. Dobriner, Katz and Jones, *Infra-red Absorption Spectra of Steroids—An Atlas*, Interscience Publishers, New York, 1953.
8. Carol, *J. Ass. off. agric. Chem. Wash.*, 1954, **37**, 692.
9. Garratt and Marshall, *J. Pharm. Pharmacol.*, 1954, **6**, 950.

10. Jones, *Chemistry in Canada*, June, 1950, p. 3.
11. Dobriner, *Açta de l'Union Contre le Cancer*, 1948, **6**, 315.
12. Carol, Molitor and Haenni, *J. Amer. pharm. Ass., Sci. Ed.*, 1948, **37**, 173.
13. Carol, *J. Ass. off. agric. Chem. Wash.*, 1951, **34**, 572.
14. Carol, *J. Amer. pharm. Ass., Sci. Ed.*, 1950, **39**, 425.
15. Papineau-Couture and Burley, *ibid.*, 1950, **39**, 683.
16. Jones and Dobriner, *Vitamins and Hormones*, 1949, **7**, 293.
17. Eddy, Wall and Scott, *Analyt. Chem.*, 1953, **25**, 266.
18. Jones, Katzenellenbogen and Dobriner, *J. Amer. chem. Soc.*, 1953, **75**, 158.
19. Hayden, *Analyt. Chem.*, 1954, **26**, 550.
20. Jones and Cole, *J. Amer. chem. Soc.*, 1952, **74**, 5648.
21. Jones, Cole and Martin, *ibid.*, 1952, **74**, 5668.
22. *The Chemistry of Penicillin*, Princeton University Press, 1949, p. 404.
23. Barnes, *et al.*, *Analyt. Chem.*, 1947, **19**, 620.
24. Jensen, *Acta chem. scand.*, 1954, **8**, 393.
25. Trenner, *Perkin Elmer Inst. News.*, 1951, **2**, (4), 1.
26. Trenner, Arison and Walker, *Applied Spectroscopy*, 1953, **7**, 166.
27. Washburn, *J. Amer. pharm. Ass., Sci. Ed.*, 1954, **43**, 48.
28. Celmer and Solomons, *J. Amer. chem. Soc.*, 1952, **74**, 2245 and 3838.
29. Hinman, Caron and DeBoer, *ibid.*, 1953, **75**, 5864.
30. Hochstein, *et al.*, *ibid.*, 1953, **75**, 5455.
31. Larson, Sternberg and Peterson, *ibid.*, 1953, **75**, 2036.
32. Oroshnik, Mebane and Karmas, *ibid.*, 1953, **75**, 1050.
33. Celmer and Solomons, *ibid.*, 1953, **75**, 1372.
34. Fusari, *et al.*, *ibid.*, 1954, **76**, 2878 and 2881.
35. Marrison, *J. Soc. chem. Ind.*, 1949, **68**, 192.
36. Mecke and Mutter, *Z. Electrochem.*, 1954, **58**, 1.
37. Trenner, Walker, Arison and Buhs, *Analyt. Chem.*, 1949, **21**, 285.
38. Tetlow, *Research*, 1950, **3** (4), 187.
39. Downing, Freed, Walker and Patterson, *Industr. Engng Chem. (Anal.)*, 1946, **18**, 461.
40. Henry, Colas and Pratt, *Chim. et Ind.*, 1954, **71**, 919.
41. *U.S. Public Health Report*, 1946, **61**, 450.
42. Garhart, Witmer and Tajima, *Analyt. Chem.*, 1952, **24**, 851.
43. Harper, Crombie, *et al.*, *J. chem. Soc.*, 1950, 971, 1152, 3552; 1951, 2445, 2906; 1952, 869.
44. Cupples, *Analyt. Chem.*, 1952, **24**, 1657.
45. Cupples and Hornstein, *J. Amer. chem. Soc.*, 1951, **73**, 4023.
46. Jackson, Whitfield, De Vries, Nelson and Evans, *ibid.*, 1951, **73**, 337.
47. Farrar, Hamlet, Henbest and Jones, *J. chem. Soc.*, 1952, 2657.
48. Trenner, Walker, Arison and Trumbauer, *Analyt. Chem.*, 1951, **23**, 487.
49. Thompson and Whiffen, *J. chem. Soc.*, 1948, 412.
50. Werner and Sutherland, *J. Amer. chem. Soc.*, 1952, **74**, 2688.
51. Washburn and Krueger, *J. Amer. pharm. Ass., Sci. Ed.*, 1950, **39**, 473.
52. Parke, Ridley, Kennedy and Hilty, *Analyt. Chem.*, 1951, **23**, 953.
53. Washburn and Krueger, *J. Amer. pharm. Ass., Sci. Ed.*, 1951, **40**, 291.
54. Pleat, Harley and Wiberley, *ibid.*, 1951, **40**, 107.
55. Marion, Ramsay and Jones, *J. Amer. chem. Soc.*, 1951, **73**, 305.
56. Naves, *Perkin Elmer Instrument News*, 1952, **3** (3), 3.
57. Naves, *Perfum. essent. Oil Rec.*, 1954, **45**, 123.
58. Naves and Lecomte, *Bull. Soc. Chim., Fr.*, 1953, **20**, 112.
59. Ribley, Kennedy, Hilty and Parke, *J. Amer. pharm. Ass., Sci. Ed.*, 1951, **40**, 572.
60. Umberger and Adams, *Analyt. Chem.*, 1952, **24**, 1309.
61. Price, Bradley, Fraser and Quilliam, *J. Pharm. and Pharmacol.*, 1954, **6**, 522.
62. Frediani, *Ann. Chim. Roma.*, 1952, **42**, 129.
63. Bristow, Charlton, Peak and Short, *J. chem. Soc.*, 1954, 616.
64. Randall and Smith, *J. opt. Soc. Amer.*, 1953, **43**, 1086.
65. Wang, Hummel and Winnick, *J. Amer. chem. Soc.*, 1952, **74**, 2445.
66. Hoover, *Perkin Elmer Inst. News*, 1954, **5** (3), 1.
67. Bailey, Geary and de Wald, *J. Amer. pharm. Ass., Sci. Ed.*, 1951, **40**, 280.
68. *Perkin Elmer Inst. News*, 1952, **3**, (4), 7.