THE APPLICATION OF INFRA-RED SPECTROSCOPY TO PHARMACEUTICAL ANALYSIS

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The problem of finding suitable or more satisfactory methods for the analysis of pharmaceutical preparations is an ever-growing one. The critical purity of many of the raw materials and intermediates, in addition to the examination of the finished pharmaceuticals, presents to the analyst a wide variety of problems. In order to be able to investigate such problems adequately it is essential to have available the necessary equipment for the appropriate and best method of analysis, irrespective of its specialised nature and the initial cost.

Light absorption methods in the ultra-violet and visible regions of the spectrum have proved to be of such great value for routine analytical techniques that no pharmaceutical laboratory is now complete without a spectrophotometer. The increasing complexity and multi-component nature of many medicinal preparations, however, and the need to analyse mixtures of isomers and of compounds which are difficult to separate chemically, are beginning to expose the limitations of the ultra-violet and visible regions. It is for such problems as these that the absorption in the infra-red region is potentially so useful.

The infra-red technique has for many years held an established position in most university and some industrial laboratories for investigating problems of a research nature, but in this country, apart possibly from the petroleum industry, its development for routine analytical work has in the past been proceeding extremely slowly. This is due partly to the fact that only recently has the necessary equipment become sufficiently reliable for routine industrial use on quantitative problems, and partly to the high initial cost of the equipment. The rapidly growing importance of infra-red spectroscopy as an analytical tool, however, is evidenced by the increasing number of industrial laboratories which are now beginning to apply this method of analysis to many of their problems. The field of pharmaceutical analysis, with its complex organic molecules, multi-component mixtures and structural isomers, provides abundant opportunities for the exploitation of this comparatively new technique. Our laboratory has been developing infra-red techniques for problems of a pharmaceutical nature for a little over a year. The wide variety of methods evolved for problems which, if at all, could only be investigated at length and with difficulty by other more established chemical or physical techniques, is sufficient justification for the initial expensive outlay on equipment.

The fact that every organic compound has its own characteristic infra-red spectrum is the basis of infra-red analysis, both qualitative and quantitative. Qualitative problems consist usually of a comparison of spectra, that of the sample with one or more reference spectra of

pure compounds. Such a comparison may be used to identify single compounds, mixtures, impurities present, and to establish or verify molecular structures. It is the usefulness of the technique in the quantitative analysis of mixtures of compounds which are difficult to separate chemically, however, which is rapidly making an infra-red spectrometer such an essential part of any pharmaceutical laboratory. These quantitative problems require an initial period of investigation to establish a procedure and working conditions which can be reproduced each time an analysis is required. Although all the quantitative methods used are based on Beer's Law relating absorbance and concentration, each problem presents its own difficulties and has to be treated entirely independently.

Sample Preparation

Before a spectrum may be recorded, the sample has to be prepared in a suitable manner. For a quick qualitative comparison of almost all solid samples the paraffin mull technique is by far the most popular. As little as 5 mg. of sample is ground with a repurified paraffin oil, and the paste obtained is squeezed evenly between two rock-salt plates. The ease and speed of sample preparation, the sharpness and definition of absorption bands due to the crystalline form of the sample, and apart from four absorption bands, the excellent transparency of the repurified paraffin oil over the 2μ to 15μ region are decided advantages over solution work.

When a solution spectrum is required, many difficulties have in most cases to be overcome. A solvent must be chosen which has little or no absorption in the region where the interest lies, and the sample must be sufficiently soluble in that solvent to give a satisfactory spectrum. If the solubility problem proves difficult, a thicker cell may be used, but as this also increases the solvent absorption, it may then be necessary to increase the amount of energy reaching the detector by widening the slits of the monochromator, with a consequent loss of resolution. Hence in the majority of cases, a compromise has to be reached between choice of solvent, cell thickness and slit width, and this may entail a considerable period of preliminary investigation.

An evaporated film on a rock-salt plate, or a silver chloride plate in the case of water-soluble materials, is sometimes a convenient way of preparing a sample, and a more recent development for solids is the pressed disc technique, where the sample is finely ground and mixed with potassium bromide or potassium chloride powder. When pressed under vacuum a solid and robust glass-like disc is produced[1,2].

Qualitative Analysis

Identification of Compounds. A positive test for the identity of a compound is the specificity of its infra-red spectrum[3]. The 8μ to 15μ region of the spectrum is commonly called the fingerprint region, and the absorption bands here may be considered as characteristic of each particular compound. For comparison purposes, it is necessary to have spectra of pure compounds, and a library of such reference spectra is an essential part of any infra-red laboratory.

For example a synthetic compound was received with a request for its identification as œstrone. Its spectrum was recorded in repurified paraffin oil over the 8μ to 15μ region and was compared with the spectrum of natural œstrone. It was found to be identical and the wavelengths of 14 characteristic absorption bands corresponded as shown in Table I.

TABLE I

ŒSTRONE ABSORPTION BANDS

Wavlength (μ)	
Natural œstrone	Synthetic œstrone
7·80	7·79
8·05	8·04
8·66	8·66
9·20	9·20
9·47	9·47
9·96	9·97
10·39	10·38
10·69	10·69
10·89	10·89
11·17	11·18
11·42	11·44
11·74	11·74
12·21	12·26
12·71	12·71

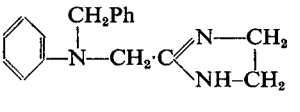
Other keto-steroids and alkaloidal plant extracts have similarly been identified by direct comparison of spectra.

Criteria of Purity. The library of spectra of pure substances may be used to establish the purity or otherwise of a particular unknown sample. The spectrum of a particular sample of *o*-chlorphenol was run over the 8μ to 15μ region and comparison with the spectrum of pure *o*-chlorphenol indicated additional absorptions at 6·67, 9·12, 9·89, 11·73 and 13·89μ. Reference to spectra of other chloro-substituted phenols showed that the 6·67, 9·12 and 9·89μ absorptions were due to *p*-chlorphenol and the 11·73 and 13·89μ absorptions were due to 2 : 4-dichlorphenol which were present as impurities.

If the impurities in the sample are of too small a concentration to show when a normal spectrum is run, then the technique of recording the spectrum of the sample with the pure substance in the blank beam of a double-beam spectrometer, will in most cases show evidence of any impurities. Concentrations of impurities of as small as 0·05 per cent. of the major constituent have been found, observed and estimated quantitatively by this technique.

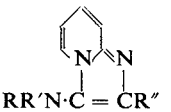
Molecular Structure. The immense volume of work which has been carried out in the past by spectroscopists on the correlation of infra-red spectra with molecular structure enables one to determine the presence or otherwise of a particular structural grouping within a molecule[4]. Stretching, bending and rocking vibrations are the most common of the many different modes of vibration which give rise to absorptions in the 2μ to 15μ region of the spectrum. A particular vibration of a specific molecular grouping has an absorption band at a characteristic wavelength, and for work of a research or investigational nature, the interpretation of a spectrum in terms of specific structural groups can often provide most valuable information.

A particular research investigation was originated to prepare analogues of the antihistamine antazoline (I)[5]. The starting point was 2-pyridyl-aminoacetonitrile (II; R = H), which on benzylation in chloroform solution afforded two monobenzyl derivatives, m.pt. 83° to 84° C. and 100° C. respectively, and a dibenzyl derivative, m.pt. 144° C.



(I)

(II)



(III)

The infra-red spectrum of the benzyl derivative, m.pt. 83° to 84° C. showed an absorption band at 4·45μ in chloroform solution, due to the $-C\equiv N$ group, but this band was absent from the spectrum of the benzyl compound, m.pt. 100° C. This was in accordance with the chemical evidence that the monobenzyl compound of lower m.pt. was *N*-benzyl-*N*-2-pyridylaminoacetonitrile (II; R = $Ph.CH_2$), and that the other monobenzyl derivative, m.pt. 100° C. was 5-benzylaminopyridino(1′:2′-1:2)glyoxaline with the structure (III; R = R″ = H, R′ = $Ph.CH_2$). This latter possibility arose, as did the dibenzyl derivative, from the capacity of 2-pyridylaminoacetonitrile to react also in the tautomeric form (III; R = R′ = R″ = H). Absence of the $-C\equiv N$ group was likewise indicated for the acetyl derivative of 2-pyridylaminoacetonitrile, 5-acetamidopyridino(1′:2′-1:2)glyoxaline (III; R = R″ = H, R′ = Ac). The presence or absence of NH or NH_2 absorptions at about 2·9μ provided additional evidence that 2-pyridylaminoacetonitrile (II; R = H) can react also as 5-aminopyridino(1′:2′-1:2)glyoxaline (III; R = R′ = R″ = H).

Spectra correlations have also assisted in identifying unknown extracts and in distinguishing between different crystalline forms of the same substance. An investigation into the infra-red spectrum of cortisone acetate succeeded in identifying 5 different crystalline forms. The spectra of 7 samples, A, B, C, D, E, F, G, in chloroform solution were recorded over the 5μ to 15μ region. These solution spectra were identical, and in the double-bond region, the positions of the 4 carbonyl and the C = C stretching vibration wavelengths were in agreement with the results of the work of Jones *et al.* on the side chains of C — 21 steroids[6]. (Table II.)

TABLE II

ABSORPTION BANDS OF CORTISONE ACETATE IN THE 6μ REGION

Functional group	Wavelength (μ)	
	Chloroform solution	Paraffin mull
Acetate C=O	5·73	5·73
20-ketone	5·80	5·80
11-ketone	5·86	5·87
3-ketone	6·01	5·97
		6·05
4–5 C=C	6·19	6·20

However, by grinding the samples with repurified paraffin oil, mulls were obtained and the spectra recorded were then those of the samples in their crystalline forms. In the carbonyl region they all showed 5 C = O bands; the 3-ketone band appeared to have split, and all the bands were very much sharper than the corresponding solution bands. The relative intensities of the 5 bands, however, differed considerably from sample to sample, although F and C were similar, as were also E and B.

The main difference in these solid state spectra was in the relative intensities of the two components of the 3-ketone absorption at 6·05μ and 5·97μ respectively. In the spectra of E and B, these were of approximately equal intensity, in the spectra of F, C and D the 5·97μ absorption was the more intense, the 6·05μ absorption appearing as a strong shoulder on the curve, and in the spectra of G and A the 6·05μ absorption was the stronger of the two, more so for A than for the G sample. (Table III.) Associated with the relative increase of the 6·05μ absorption was a corresponding, although less pronounced, increase in the relative intensities of the 5·73μ and 5·80μ absorptions. All absorptions were measured relative to the 11-ketone absorption at 5·87μ, which was the strongest of the 5 C = O bands.

TABLE III

THE RELATIONSHIP BETWEEN DIFFERENT CRYSTAL MODIFICATIONS OF CORTISONE ACETATE AND THE RATIO OF THE INTENSITIES OF THE TWO COMPONENTS OF THE 3-KETONE ABSORPTION

Sample of cortisone acetate	Ratio of absorbances at 5·97μ and 6·05μ
F	1·61
C	1·59
D	1·44
E	1·01
B	1·01
G	0·87
A	0·62

The differences in relative intensities of the bands suggested strongly the presence of more than one crystal modification, these probably being present in different proportions in different samples. X-ray diffraction patterns provided additional evidence of this, as also did recrystallising experiments, which produced identical modifications and spectra from originally different samples.

QUANTITATIVE ANALYSIS

Single Component Analysis. The determination of phenobarbitone provides an example of the simplest form of quantitative analysis. Tablets of an alkaloidal extract (2 mg.) and phenobarbitone (15 mg.) are examined for their phenobarbitone content. Phenobarbitone has two characteristic carbonyl absorption bands at 5·75μ and 5·84μ which occur in the form of a doublet. The procedure is to extract the phenobarbitone, and with it the alkaloidal extract, from the tablets and to compare the absorbance with that of a standard solution of phenobarbitone at 5·75μ. At the concentration used, the alkaloidal extract has no appreciable absorption at this wavelength, and a straight line calibration between absorbance and phenobarbitone concentration over the range 0 to 1 per cent. verifies Beer's law for this particular band. A direct comparison may therefore be made between the absorbances of the sample and the standard.

The standard is prepared by dissolving 75 mg. of phenobarbitone in 10 ml. of chloroform. The tablets are finely ground and the equivalent weight of 5 tablets put into a sintered funnel. 8 × 15 ml. chloroform extracts are drawn through the sinter by suction and taken to dryness. The residue is dissolved in 10 ml. of chloroform. The absorbances of both standard and sample solutions are then measured at 5·75μ, using a 0·15 mm. cell thickness and a slit width of 0·08 mm., and the phenobarbitone content of the sample solution thereby determined. The

average time for a complete phenobarbitone determination is less than one hour, and throughout the past 6 months production batches of these tablets have been examined satisfactorily by this method.

Further examples of this single component analysis are the determination of dieldrin[7] and aldrin[7], both insecticidal materials in agricultural and horticultural preparations. A colorimetric method has been described[8] for determining aldrin which is long and tedious, and which requires care and accurate control of critical conditions during the determination procedure. A hydrogen bromide method for determining dieldrin in formulations is similarly time-consuming, and both methods are susceptible to inteference from extraneous materials. The infra-red technique is specific for both dieldrin and for aldrin, entails a straightforward extraction procedure and a simple comparison determination.

Dieldrin is soluble in carbon disulphide and a record of its spectrum shows a band at 12·37μ suitable for an analysis. The dieldrin is extracted with ether from a sample of the preparation, the extract evaporated to dryness, and the residue dissolved in carbon disulphide. The absorbance of this solution is compared with that of a standard solution of dieldrin at 12·37μ, thereby giving the concentration of the solution and hence the amount of dieldrin in the sample. A similar method is used for aldrin, a characteristic band at 8·47μ being used in this case.

Multi-component Analysis. The simplest technique of multi-component analysis is a direct development of the comparison method described above for dieldrin and for phenobarbitone. Essentially it entails comparing the absorbances of an extract of the components with the absorbances of a multi-component standard. Each component has its characteristic key wavelength at which the other component(s) have little or no absorption. The determination of dieldrin and a commercial product of benzene hexachloride is a straightforward application of this method.

A weighed quantity of sample is shaken with ether, which extracts the dieldrin and the benzene hexachloride. After filtering and evaporating to dryness the residue is dissolved in carbon disulphide. The standard is similarly prepared from weighed amounts of dieldrin and benzene hexachloride. The concentrations of dieldrin and benzene hexachloride in the standard are approximately the same as those in the sample extract. Dieldrin has an absorption band at 9·93μ and benzene hexachloride one at 10·48μ, at each of which the other component has very little absorption. The spectra of both sample and standard solutions were recorded over the wavelength range covering these two key absorption bands using a 1·19 mm. cell and a slit width of 0·20 mm. A direct comparison of absorbances at 9·93μ for the dieldrin and at 10·48μ for the benzene hexachloride gave duplicate and triplicate results on two samples as shown in Table IV.

The alkaloids strychnine and brucine may be determined directly without the necessity of a separation procedure. In the carbonyl region, they both have a strong C = O absorption at 6·06μ, and strychnine has an additional though weaker band at 6·32μ[9]. Working curves were obtained for the absorbances of both alkaloids at 6·06μ, covering the concentration range 0 to 1 per cent., and with chloroform as solvent in a 0·13 mm. cell. They

were also obtained for strychnine as the major component and brucine as the minor component at 6·32μ, over the same concentration range 0 to 1 per cent., but using thicker cells, 1·19 mm. A base-line method[10,11] was used for measuring the absorbances at 6·32μ because of interference from the stronger absorptions at 6·06μ. At both wavelengths the curves obtained approximated very closely to straight lines, indicating the validity of Beer's law for the absorption bands used.

TABLE IV

RESULTS OF DIELDRIN AND BENZENE HEXACHLORIDE DETERMINATIONS

Sample	Dieldrin, per cent.	Benzene hexachloride, per cent.
1	12·69	8·85
	12·55	8·84
2	12·55	8·39
	12·50	8·65
	12·48	8·63

Synthetic mixtures were then prepared and the absorbances of each measured at the two key wavelengths, 6·06μ and 6·32μ, using the appropriate cells. A first approximation was obtained for the strychnine content using the working curve for 6·32μ. Passing to the working curves for the 6·06μ absorptions, the absorbance due to this amount of strychnine was obtained and subtracted from the absorbance of the mixture at this wavelength. This gave the absorbance of the brucine and a first approximation of the brucine content was then obtained from its working curve at 6·06μ. This enabled a correction and a second approximation to be obtained of the strychnine content and so on to a second approximation of the brucine content. The results on 4 synthetic mixtures were as in Table V.

TABLE V

RESULTS OF STRYCHNINE AND BRUCINE DETERMINATIONS

Mixture number	Concentration as per cent. w/v in chloroform			
	Strychnine		Brucine	
	Taken	Found	Taken	Found
1	0·51	0·50	0·50	0·50
2	0·41	0·39	0·61	0·61
3	0·26	0·26	0·25	0·26
4	0·31	0·30	0·20	0·22

The increasing tendency to combine 2 or more drugs in medicinal preparations has complicated analytical methods and provided an excellent opening for infra-red techniques. The determination of aminophylline and phenobarbitone in tablets is an example of this. Various methods for determining the 2 compounds individually are reviewed in a paper by Bartilucci and Discher[12], and preliminary investigations into a potentiometric titration method for determining both compounds in a mixture are described. The results obtained were not considered satisfactory, although further work was in progress. The infra-red method, besides being specific for aminophylline and for phenobarbitone is simple and time-saving.

Aminophylline B.P. contains between 75·0 per cent. and 82·0 per cent. of anhydrous theophylline and between 12·3 per cent. and 13·8 per cent. of ethylenediamine, and it is the infra-red absorption of the theophylline, together with that of the phenobarbitone, which is made use of in the analysis. Theophylline has 2 characteristic absorption bands at 5·87μ and 6·03μ, and phenobarbitone two at 5·75μ and 5·84μ. The theophylline and the phenobarbitone are both extracted from the tablets with a 3 : 1 mixture

of chloroform and *iso*propanol, and are estimated by comparing the absorption of the extract with that of a standard. The standard is itself an extract from a mixture of phenobarbitone and that batch of aminophylline which was used in the tablet manufacture. Because of the relatively small concentration of phenobarbitone in the particular tablets for which this determination was designed, each substance has to be estimated separately. The spectra of the sample and standard extracts in chloroform solution are each run in cells of thickness 0·15 mm., against a blank of chloroform. Comparison of the spectra at 6·03μ gives an estimation of the aminophylline content, the phenobarbitone concentration being insufficient to interfere at this cell thickness and at this wavelength. The spectra of the two extracts are then run in thicker cells, 1·21 mm., against a standard blank of theophylline. This blank balances out the strong theophylline absorption, leaving the 5·75μ phenobarbitone band of sufficient intensity to permit a direct comparison. Because of the neighbouring strong theophylline absorption at 5·87μ, the light transmission at the other phenobarbitone wavelength of 5·84μ is too small to permit a further comparison check.

The equivalent weight of one tablet, finely-ground, is weighed into a sintered funnel and extracted with 8 quantities, each of 15 ml., of a 3 : 1 mixture of chloroform and *iso*propanol. The combined extracts are taken to dryness and the residue dissolved in 50 ml. of chloroform. The standard is prepared by shaking for 30 minutes 170 mg. of aminophylline and 8 mg. of phenobarbitone with 50 ml. of chloroform, and then filtering. To prepare the blank, 170 mg. of aminophylline is shaken with 50 ml. of chloroform for 30 minutes and filtered. The absorbances of both sample and standard solution are determined at 6·03μ using a 0·15 mm. cell with a slit width of 0·08 mm. Using a 1·21 mm. cell and having the theophylline blank in the blank beam, the absorbances of both sample and standard solutions are then determined at 5·75μ with the same slit conditions. A direct comparison of absorbances then gives the concentrations and hence the amounts of phenobarbitone and aminophylline in the tablets. A batch of tablets was examined as above with the following results:—

Aminophylline	173 mg. ≡ 2·67 grains/tab.
Phenobarbitone	7·7 mg. ≡ 0·12 grains/tab.
Total ..	2·79 grains/tab.

This is to be compared with a chemical assay result of 2·65 grains/tab. for the combined aminophylline and phenobarbitone content, in which the B.P. extraction procedure described for theophylline was used.

Major Component Compensation. This technique is most useful when one is more interested in estimating the amounts of constituents of the order of 1 per cent., such as impurities, than in estimating the major components. An example of this was the estimation of impurities present in a sample of γ-picoline, reputed to be about 98 per cent. pure. The method of Coulson and Hales[13] for the determination of β-picoline, γ-picoline,

2:6-lutidine and 2-ethylpyridine in mixtures appears to be quite satisfactory for mixtures containing the different components in amounts varying from 5 per cent, to 90 per cent., but the small absorptions of the impurities, when these are present in amounts less than 1 per cent., are completely masked by the intense absorption of the γ-picoline, and a different technique has to be used. As in the qualitative identification of impurities already referred to, a solution of pure γ-picoline is used in the blank cell of the double-beam spectrometer to balance out this intense absorption, thereby uncovering the comparatively weak absorptions of the impurities. This technique also makes it possible to use stronger concentrations of sample so that the absorption of the impurities are sufficiently intense to permit their accurate measurement.

Using highly purified samples of the impurities α-picoline, β-picoline, 2:6-lutidine and *o*-xylene, obtained from the Chemical Research Laboratory, Teddington, working curves for each were prepared in *cyclo*hexane solution at their respective characteristic wavelengths: α-picoline, 13·38μ; β-picoline, 14·09μ; 2:6-lutidine 12·99μ; *o*-xylene 13·49μ. Each absorption band is at a wavelength where there is little or no interference from the absorptions of the other 3 components, providing all are of the same order of concentration. The spectrum of a solution of the sample in *cyclo*hexane against a solution of pure γ-picoline is then recorded, and the absorbances and concentrations of the 4 impurities are measured and calculated. Two samples were examined in triplicate with the results as shown in Table VI.

TABLE VI

RESULTS OF DETERMINATIONS OF IMPURITIES IN γ-PICOLINE

Sample	Impurities, per cent.				
	α-picoline	β-picoline	2:6-lutidine	*o*-xylene	Total
1	0·45	0·05	0·37	0·29	1·16
	0·43	0·05	0·37	0·27	1·12
	0·41	0·05	0·36	0·28	1·10
2	0·56	0·04	0·38	0·29	1·27
	0·58	0·06	0·37	0·30	1·31
	0·57	0·10	0·41	0·30	1·38

Solutions of compounds in oils from which they cannot be easily extracted provide a further example of an application of this method. Injection of testosterone propionate is a 10 mg./ml. solution of testosterone propionate in arachis oil. An infra-red technique enables a determination of the testosterone propionate to be made with much greater certainty than is possible by the ultra-violet method, which invariably gives rise to inaccuracies due to the strong absorption of arachis oil in the region of the testosterone propionate maximum at about 230 mμ, and the consequent low intensity of transmitted light by the arachis oil solution. Testosterone propionate has a characteristic infra-red absorption band at 5·95μ, and the absorbance of the sample at this wavelength is compared with that of a standard solution of testosterone propionate in arachis oil. Arachis oil has

a C = O absorption at about 5·75μ, and the 5·95μ testosterone propionate absorption falls on the side of this band. At a concentration of 10 mg./ml. it occurs as a shoulder, but by putting arachis oil of the same batch as that used in the manufacture of the injection in the blank beam, the C = O absorption is balanced out, leaving the testosterone band resolved and of sufficient intensity to permit a direct quantitative measurement.

The standard is prepared by weighing 100 mg. of testosterone propionate and dissolving in 10 ml. of arachis oil. Gentle heating is required. The testosterone propionate and arachis oil should be of the same batch as those used in making up the injection. The absorbances of both standard and sample are then determined at 5·95μ, using a 0·15 mm. cell and having arachis oil in the blank beam. A constant slit width of 0·14 mm. is used.

To ensure that a direct comparison between the absorbance of standard and sample may be permitted, i.e., to verify that Beer's law is obeyed by this particular absorption band, standards of 5, 10 and 15 mg./ml. were made up and their absorbances obtained as above. A plot of absorbance against concentration gave a curve approximating very closely to a straight line. A sample was examined as above in triplicate with the following results:—10·2, 10·0, 10·1 mg./ml. testosterone propionate (theory 10·0 mg./ml.).

Summary

1. A general outline of infra-red techniques is presented for the purpose of indicating the potential range of applications in the routine examination of pharmaceutical materials.

2. Examples of qualitative investigations include the identification of compounds, the observation of impurities and spectra-structure correlations.

3. Quantitative analyses of single compounds and of multi-component mixtures are described which are impossible, more difficult or time-consuming to perform by chemical or other physical methods.

4. Advantages of the analytical techniques described are the ease of sample preparation, the straightforward nature of extraction procedures, and the fundamental fact that every organic compound has its own specific infra-red spectrum.

References

1. Stimson, *J. Amer. chem. Soc.*, 1952, **74**, 1805.
2. Schiedt, *Applied Spectroscopy*, 1953, **7**, 75.
3. Barnes, Gore, Stafford and Williams, *Analyt. Chem.*, 1948, **20**, 402.
4. Colthup, *J. Optical Soc. Am.*, 1950, **40**, 397.
5. Bristow, Charlton, Peak and Short, *J. chem. Soc.*, 1954, 616.
6. Jones, Humphries, Herling and Dobriner, *J. Amer. chem. Soc.*, 1952, **74**, 2820
7. Lidov, Bluestone, Soloway and Kearns, *Advances in Chemistry Series*, 1950, No. **1**, 175.
8. Danish and Lidov, *Analyt. Chem.*, 1950, **22**, 702.
9. Washburn and Krueger, *J. Amer. pharm. Ass., Sci. Ed.*, 1951, **40**, 291.
10. Wright, *Industr. Engng Chem. (Anal.)*, 1941, **13**, 1.
11. Heigl, Bell and White, *Analyt. Chem.*, 1947, **19**, 293.
12. Bartilucci and Discher, *J. Amer. pharm. Ass., Sci. Ed.*, 1950, **39**, 641.
13. Coulson and Hales, *Analyst*, 1953, **78**, 923.

DISCUSSION

The paper was presented by DR. D. C. GARRATT.

The CHAIRMAN asked the cost of the apparatus and was informed that it was between £3000 and £4000, which Dr. Garratt claimed was not expensive when one considered the resulting saving of labour.

DR. F. HARTLEY (London) said that Dr. Garratt had rightly pointed out that the capital cost ought not to be the paramount consideration; rather, it ought to be the saving which might ultimately be achieved. Unfortunately, there were always "teething" troubles with new equipment. In his reference to the examination of steroid hormones in oily solutions the only example given was a solution containing 10 mg./ml. He understood that the British instruments available normally required about 10 mg. of material for a successful examination. Micromethods were available in American instruments. Having regard to the low concentrations of testosterone which were often used did the authors consider that they could get an accuracy of $\pm$ 5 to 10 per cent. when dealing with solutions of a strength of 2 or 5 mg./ml.?

DR. W. MITCHELL (London) asked if the authors had any experience of the analysis of essential oils by infra-red spectroscopy. Could the method pick out the numerous individual components in an essential oil or did overlapping of bands tend to make analysis impossible? Could infra-red spectroscopy distinguish between *Mentha piperita* and *M. arvensis* oils; if so, could it detect the latter as an adulterant in the former?

DR. R. E. STUCKEY (London) asked for further details of the type of instrument used. When the instrument was properly adjusted did it continue to work satisfactorily for prolonged periods without readjustment? In the estimation of testosterone in arachis oil could the authors give the relative values for the absorption of the oil and the dissolved testosterone at different concentrations?

MR. S. G. E. STEVENS (London) said it was interesting to learn that the instrument was being used for quantitative work. Could the quantitative application of the infra-red spectrometer be used for complex preparations containing two or more compounds with closely related chemical structures?

MR. P. G. MARSHALL, in reply, said the steroid solution submitted for analysis contained approximately 10 mg./ml. and they based their analysis on concentrations of that order, but he did not see why similar orders of accuracy should not be obtained with lower concentrations by using thicker cells. Replying to Dr. Stuckey, he said that they had used a Hilger double beam instrument. The arachis oil absorption was larger than that of the testosterone propionate, but that was immaterial in that the absorption was balanced out. Tests on different concentrations in the arachis oil gave a good straight line result relating absorption to concentration; and the range was large enough to give an accuracy of $\pm$ 1 per cent. As for distinguishing between essential oils, the advantage of the infra-red spectrometer was that the absorption was specific for a particular material,

and with a mixture depending on the relative concentrations the different oils could be detected. Regarding complex mixtures, he said that whether the different materials present could be distinguished depended on the relative amounts present. Their instrument had been in operation for 18 months. For 3 or 4 months they had had trouble setting it up, but since then there had been no difficulty, and for the past year there had been no need to re-set any of the controls. Reproducibility of results from day to day was good, although for work of the highest accuracy it was advisable to use standards rather than to compare an absorption with another made the previous month.

DR. GARRATT said that work on essential oils of the kind suggested by Dr. Mitchell would take a considerable time.