RECENT ADVANCES IN PHARMACEUTICAL ANALYSIS*

INFRA-RED SPECTROSCOPY

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Methods based on the measurement of the absorption of infra-red radiation by substances have up till now been used comparatively little in pharmacy. The reasons are many. Firstly, the equipment needed is expensive and available only in large laboratories or in big manufacturing units. Secondly, the work which must precede a determination is often rather tedious. Pharmacy with its broad spectrum of substances and complicated mixtures is, however, a natural field for the application of the infra-red technique. If one looks through some of the scientific pharmaceutical journals, one notices also in recent years a distinct trend to increased use of infra-red spectroscopy.

The fundamental advantages of the technique are two: (1) it is possible to obtain a fool-proof identification of a substance, and (2) it is usually possible to construct assay methods for quite complex mixtures. Where only a single substance is involved in a quantitative determination infra-red spectroscopy seldom has any special advantages over visible or ultra-violet spectrophotometry.

Infra-red technique is especially useful in basic work with a new substance, for example in the control of methods of syntheses. Another application which often is well worth investigating is the application of the technique to routine assays. In the first example the method often gives the best criteria of identity, and in the second example it is often the cheapest way to solve difficult or tedious analytical problems.

No attempt has been made in this survey of infra-red spectroscopy to cover the literature completely. On the contrary, only some special applications have been singled out; perhaps too many according to personal opinion and experience. Extensive literature reviews have been published during the last few years. Those of Gore[1–4], Price[5], Carol[6] and Larsson[7] deserve a mention. Recently some new books have appeared, for example, Bellamy[8], Brügel[9], Dobriner[10]. Older standard texts are by Randall[11] and by Barnes[12].

The modern instrumentation is well covered in the above cited literature. The instruments may be either single or double beam instruments. All commercial instruments are to-day based on some form of recording, usually coupled with a servo mechanism and are sturdy enough to allow robust use. The interpretation of the data, however, remains the difficult part of the technique. Good schemes covering typical group absorptions have been published and may be found in the literature cited above.

On the following pages some recent applications of infra-red spectroscopy to pharmaceutical problems will be discussed.

* Papers read at the University (Scientific) Section of the London meeting of the Fédération Internationale Pharmaceutique on Wednesday, September 21, 1955.

IDENTIFICATION OF UNKNOWN SUBSTANCES

One of the most embarrassing problems for an analyst is to identify a single member in a large group of similar substances, for example from among the barbituric acids, antihistamines, derivatives of isoniazid or derivatives of cyanoacetic acid hydrazides.

The usual way to solve such a problem is to record a number of spectra of known substances and to compare the spectrum of the unknown

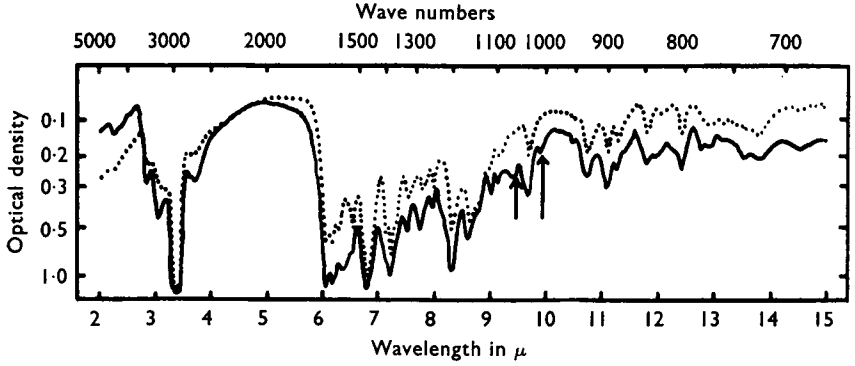


FIG. 1. Infra-red spectra of flavaspidic acids in paraffin mull. Note the difference at 9·44 μ (short arrow) and 9·92 μ (long arrow).

compound with the spectra of known compounds. If identical spectra can be found, identification is obtained. However, in practice the problem usually is a little more difficult. Often the commercial substances are not pure enough to give an unquestionable identification. A barbituric acid may contain a small quantity of an isomer. A very useful method in such cases is to record a differential spectrum. The method is well-known from ultra-violet spectroscopy, but not until recently has it been applied to infra-red spectroscopy by McDonald[13]. The method requires a double-beam instrument with sufficient energy from the source to allow the instrument to operate on a favourable signal-to-noise ratio. The method consists essentially in adding to the compensating cell the known compound in a concentration equivalent to that in the sample cell. The spectrum is recorded and, if

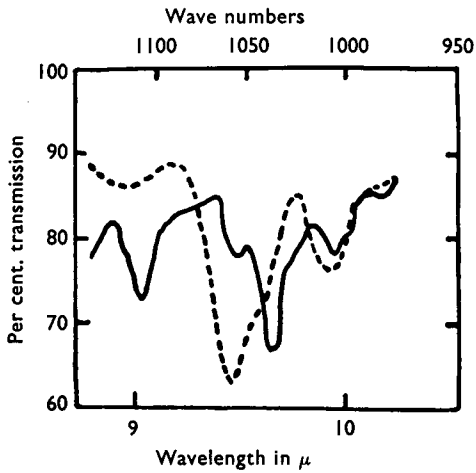


FIG. 2. Broken line = Differential spectrum of the two flavaspidic acids in Fig. 1. Unbroken line = Amyl alcohol.

nothing but the same compound is present in both cells and in the same concentration a straight line is obtained. If an impurity is present in the unknown substance a rough spectrum of that compound is recorded. As an example I have included the infra-red spectrum of pure flavaspidic acid in a paraffin mull together with the spectrum of an impure acid. (Fig. 1.)

As seen, the spectra are very alike but some minor differences may be noticed at 9·44 and 9·92 μ. The differential spectrum in carbon disulphide, however, unveils immediately the presence of a second compound. (Fig. 2.)

If you compare that spectrum with the spectrum of amyl alcohol the impurity is identified. This method is very useful, especially when the purification of a new compound is being followed.

As a second example I have included a new morphine antagonist, recently introduced to medicine by Shaw *et al.*[14,15], 2:4-diamino-5-phenylthiazole (amiphenazole). (Fig. 3.)

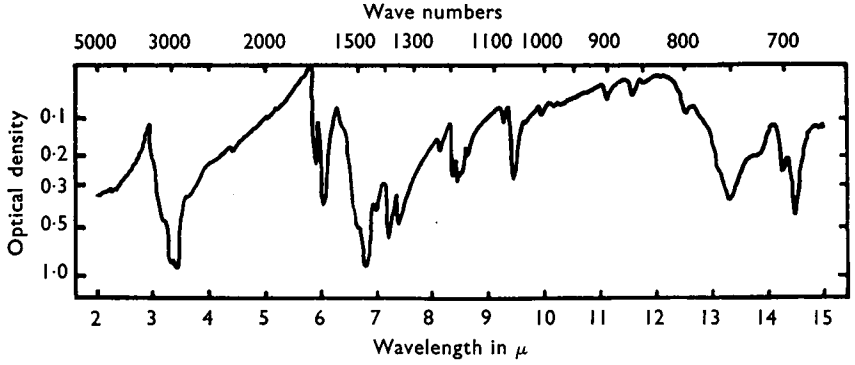
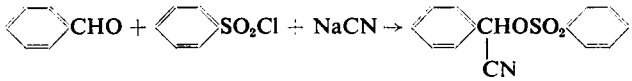
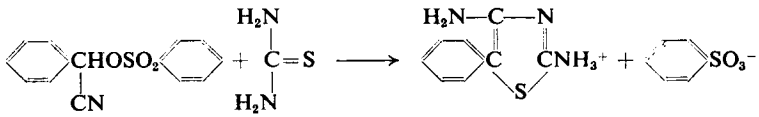


FIG. 3. Infra-red spectrum of 2:4-diamino-5-phenylthiazole hydrochloride in paraffin mull.

The substance can be synthesized by different routes. One way is to react benzaldehyde, benzene sulphochloride and sodium cyanide to give α-cyanobenzyl benzenesulphonate.

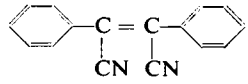


This compound reacts with thiourea:—



which can be transformed into the hydrochloride in the usual way. The reaction is in practice not as simple as the above scheme indicates. There are several possibilities for side-reactions. After running a few test syntheses we discovered that our final product was not a single substance. A small quantity of an unknown compound was present.

After some discussion we made a rough guess and the expected stilbene compound was shown to be the impurity:



The compound was synthesized. It was soluble in chloroform. Our impure 2:4-diamino-5-phenylthiazole hydrochloride was extracted with chloroform and the extracted compound was shown to be identical with the expected one. (Fig. 4.)

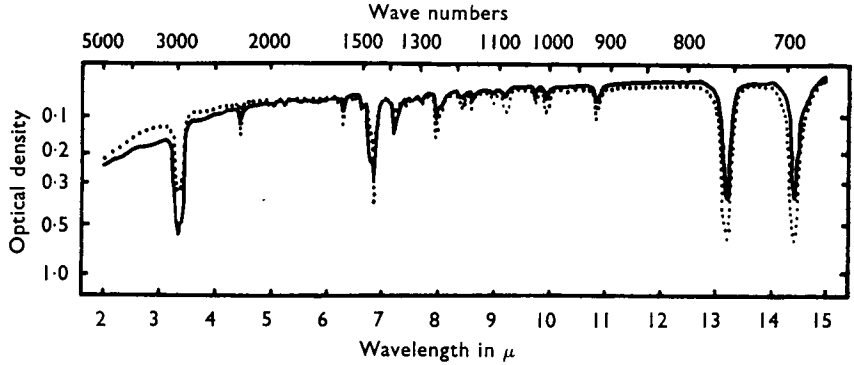


FIG. 4. Infra-red spectrum of αα′-dicyanostilbene.

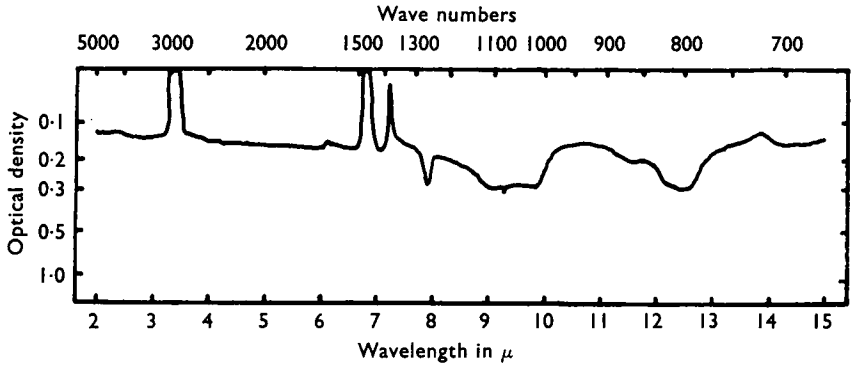


FIG. 5. Infra-red spectrum of extracted silcone-grease.

With this rather tedious method we were able to show the presence of side-reactions in the selected method of synthesis. One might have expected that the stilbene compound would have given a pronounced peak in the infra-red spectrum at 4·48 μ where the CN group has a strong absorption. This is not the case. However, a differential spectrum between the impure compound and the purified compound shows the presence of the stilbene compound.

When substances have been extracted in separators by solvents some caution has to be exercised in the interpretation of the recorded spectrum.

One particular spectrum was a peculiar one which introduced some unexpected difficulties until it was identified as the infra-red spectrum of the silicon-grease used on the stopcocks. (Fig. 5.)

An example may illustrate the usefulness of infra-red methods in stability tests. 2:4-Diamino-5-phenylthiazole is in aqueous solution very sensitive to hydrolysis. The amino group in the 4-position is easily replaced by an OH-group. If an aqueous solution is autoclaved, the resulting bases extracted and the infra-red spectrum of the mixture recorded, the spectrum is only slightly different from that of the parent compound. The spectrum of the pure 2-amino-4-hydroxy-5-phenylthiazole is given in Figure 6.

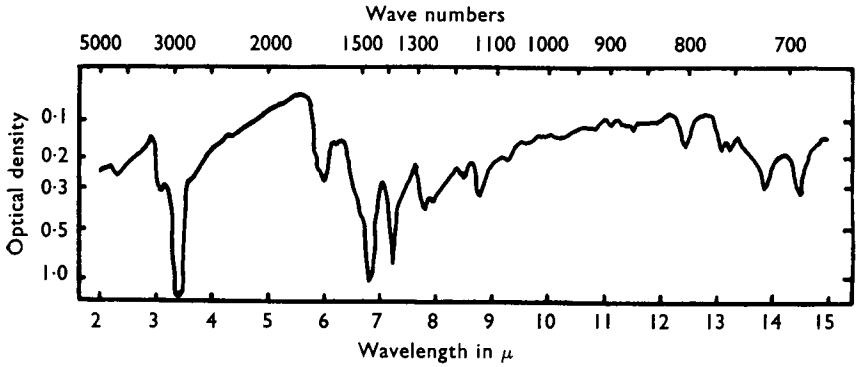


FIG. 6. Infra-red spectrum of 2-amino-4-hydroxy-5-phenylthiazole.

Usually not less than one mol. per cent. of an impurity can be seen on an infra-red spectrum. However, if an appropriate amount of the parent compound is added to the reference cell, it is possible to evaluate the degree of decomposition from the differential spectrum.

In a similar way it is possible to evaluate the decomposition of barbituric acids in alkaline solution.

QUANTITATIVE MEASUREMENTS

As previously mentioned, one of the greatest advantages of the technique is its application to assays of multicomponent systems. The first step is to choose a suitable technique for the preparation of the sample. The main problem is to choose between recording the spectrum of the solid substance (or mixture) or that of a solution of the substance (or mixture). When working with solids one can choose between the mull technique and the pressed potassium bromide disk technique. The latter is a recent contribution which eliminates some of the difficulties of the mull technique. The concentration in the pressed disk is fairly easy to estimate and disturbing absorption is not present as when working with paraffin mull. The pressed disk technique is elegant but requires a new tool and a heavy press.

The main part of the quantitative measurements on multi-component systems will in the future be made on solutions. One is not so much

limited by the choice of solvents as by the recording of suitable curves for identification. In the latter instance an essential point is to record as broad a spectrum as possible, while in the former, one can select narrow ranges where suitable peaks are located. The most used solvents for the recording of identification spectra are carbon tetrachloride, carbon disulphide and chloroform, quantitative measurements can, however, be made in polar solvents such as acetone and dioxane. Aided by the numerous tables of infra-red spectra of solvents, the analyst has little difficulty in choosing a suitable one. In this connexion it may be mentioned that acids like phenylacetic acid in carbon disulphide have a pronounced tendency to attack the amalgamated lead spacer in fixed-thickness cells, with the result that lead phenyl acetate is precipitated on the sodium chloride plates. The precipitate has a strong absorption and as a consequence the cell must be dismounted and cleaned.

When the solvent has been chosen, absorption curves of the substance or substances under test must be recorded and suitable bands selected for measurement. If only one substance is involved, the task is comparatively easy. However, if more than two, and as many as six substances are present, the problem of locating test points is much more complicated. Seldom is it possible to find test points for three substances all of which are free from interference of the other compounds and to which the Beer-Lambert equation may be applied. If polar solvents like dioxane or acetone are used, hydrogen bridges may introduce considerable difficulties. Usually it is necessary to introduce the old technique of correcting the values first obtained, then to start again with the corrected value, and go on until a repetition of the calculation gives values which are close or identical. The evaluation of a four component system can involve a remarkable amount of calculation. Special machines for this type of calculation have been constructed.

In recent years the isotope dilution principle using deuterium has been applied to a number of problems. The best known method is that of determination of penicillins in brews. The determination depends on the specific absorption of proteophenylacetic acid at 14·37 μ which deuterophenylacetic acid lacks. The technique presupposes deuterobenzylpenicillin. This compound is produced by fermentation, deuterophenylacetic acid being used as precursor. The latter is obtained from proteophenylacetic acid which is dissolved in deuterosulphuric acid which in turn is produced from SO_3 and D_2O. One obtains a compound with the approximate composition $C_6D_5CH_2COOH$.

Similar methods have been constructed for the determination of the γ-isomer of benzene hexachloride. A recent application is to tropic acid derivatives[16].

As mentioned above, it is usually not possible to estimate less than one mol. per cent. of an impurity by means of infra-red spectroscopy. A recent technique enables, however, such small amounts as 0·1 to 0·01 per cent. of an impurity to be determined. The method depends on the use of fractional crystallization[17]. Certain requirements must be fulfilled. The solubility of the main substance in the solvent chosen

should be of the same order of magnitude at or below room-temperature as the impurity. The solubility of the main substance shall increase with temperature, and further, the impurity shall not have a tendency to be occluded by the main substance during crystallization.

Further to increase the sensitivity of the method the differential method discussed above was used. The method was used for the determination of small quantities of catechol and resorcinol in hydroquinone. The substance to be tested was crystallized from acetonitrile, and the mother liquid which contained the impurities was worked up. The method seems to be generally applicable.

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