

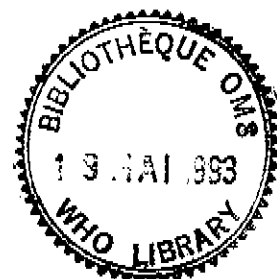
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GENERAL RECOMMENDATIONS FOR THE PREPARATION
AND USE OF
INFRARED SPECTRA IN PHARMACEUTICAL ANALYSIS

Introduction

In pharmaceutical analysis the mid-infrared region of the electromagnetic spectrum used is 4000–600 cm^{-1} (2.5–16.7 μm).

Spectrophotometric measurements in the infrared region are mainly used for identification purposes. The infrared spectrum of any given substance is unique (with the exception of enantiomers, which have identical spectra in solution). Polymorphism and other factors, such as variations in crystal size and orientation, the grinding procedure, and the possible formation of hydrates may, however, be responsible for minor, and occasionally substantial, variations in the infrared spectrum of a substance in the solid state. The infrared spectrum is usually not greatly affected by the presence of small quantities of impurities in the tested substance. For identification purposes, the spectrum may be compared with that of a reference substance, concomitantly prepared, or with a reference spectrum.

The terms *absorbance*, *transmittance*, *absorptivity* and *absorption spectrum* are described in *The international pharmacopoeia*, third edition, volume 1, pages 33–34, in the chapter "Spectrophotometry in the visible and ultraviolet regions", and the term *specific extinction* is given in the preface to volume 3, page 10.

Apparatus

Conventional infrared spectrometers disperse the infrared radiation through use of either gratings or prisms. The development of computerized laboratory equipment provides the additional option of using an interferometer coupled with a computer for reduction of the data (performing a Fourier transformation of the interferogram) to generate an infrared spectrum. These instruments are called Fourier Transform infrared spectrometers (FTIR's). Aside from small differences in the low frequency cutoff, all of the above types of infrared instruments generate comparable data and can generally be used interchangeably for qualitative analyses. However, each instrument will possess specific signal-to-noise and resolution characteristics.

Spectrophotometers suitable for use for identification tests should normally operate in the range of 4000–600 cm^{-1} (2.5–16.7 μm) or in some cases to 250 cm^{-1} (40 μm). For the use of attenuated total reflectance technique, the instrument needs to be equipped with a suitable attachment consisting of a

single or multi-reflecting element. The attachment and a suitable mounting should permit its alignment in the spectrophotometer for maximum transmission.

Method of verification of frequency scale and resolution

Record the spectrum of a polystyrene film of a suitable thickness, normally between 0.03 mm and 0.05 mm. The spectrum includes maxima at the following frequencies expressed as wavenumbers in cm^{-1} :

3027, 2851, 2924, 1944, 1871, 1802, 1601, 1583, 1181, 1154, 1069, 1028, 907, 699. Acceptable tolerances are $\pm 8 \text{ cm}^{-1}$ for the range 4000–2000 cm^{-1} and $\pm 4 \text{ cm}^{-1}$ for the range 2000–600 cm^{-1} .

The difference between the percentage transmittance of the absorption minimum at 2870 cm^{-1} and that of the absorption maximum at 2851 cm^{-1} should be greater than 18 and the difference between the percentage transmittance of the absorption minimum at 1589 cm^{-1} and that of the absorption maximum at 1583 cm^{-1} should be greater than 12.

Environment

Precautions should be taken to minimize exposure to atmospheric moisture during sample preparation. It is advisable to store the halide salts, the sodium chloride or other similar plates, and all necessary accessories in a desiccator at room temperature over silica gel. It is advisable to prepare the samples in an area of controlled heat and humidity, or alternatively, any kind of manipulation should be carried out under an infrared lamp.

Use of solvents

The solvent used in infrared spectrophotometry must not affect the cell, which usually consists of a halide salt such as sodium chloride or potassium bromide. Where possible, spectral grade solvents should be used.

No solvent exists which remains completely transparent throughout the entire infrared spectrum. Carbon tetrachloride R is practically transparent (up to 1 mm of thickness) from 4000–1700 cm^{-1} (2.5–5.9 μm). Chloroform R, dichloromethane R, and dibromomethane R are useful solvents. (Carbon tetrachloride R and chloroform R should only be used exceptionally because of their toxicity). Carbon disulfide IR (up to 1 mm in thickness) is suitable as a solvent to 250 cm^{-1} (40 μm) except in the 2400–2000 cm^{-1} (4.2–5 μm) and the 1800–1300 cm^{-1} (5.6–7.7 μm) regions where it has strong absorption. Its weak absorption in the 875–845 cm^{-1} (11.4–11.8 μm) region should be noted. Other solvents have relatively narrow regions of transparency.

Preparation of the substance to be examined

To obtain a suitable infrared absorption spectrum, it is necessary to follow the instructions below for the preparation of the substance. Liquid substances may be tested directly or in a suitable solution. The usual methods of preparation for solid substances include dispersing the finely ground solid specimen in mineral oil, or incorporating it in a transparent disc or pellet obtained by mixing it thoroughly with previously-dried potassium halide and compressing the mixture in a die, or preparing a solution in a suitable solvent. Preparation of the substance for the attenuated total reflectance technique is described separately.

Method 1

Triturate the solid substance with dry, finely powdered potassium halide (normally potassium bromide). When hydrochlorides are being examined, potassium chloride should be employed to avoid the risk of halide exchange.

The proportion of substance to the halide salt should be about 1 to 200-300; for example, 1.5 mg in 300 mg of the halide salt in the case of prism instruments, or about 1.0 mg in 300 mg of the halide salt for grating or Fourier transform instruments. Carefully grind the mixture using an agate mortar and pestle for 1 minute. In exceptional cases the use of a ball mill may be indicated, but generally, the risk of obtaining polymorphic changes outweighs any improvement in resolution. Spread the triturate uniformly in a suitable die and compress it, under vacuum, at a pressure of about 800 MPa. As an alternative, potassium halide discs can be prepared using a hand-held minipress. Mount the resultant disc in a suitable holder

Several factors, for example, inadequate or excessive grinding, moisture or other impurities in the halide carrier, may give rise to unsatisfactory discs. Unless its preparation presents particular difficulties, a disc should be rejected if visual inspection shows lack of uniformity or, if the transmittance at about 2000 cm^{-1} ($5\text{ }\mu\text{m}$), in the absence of a specific absorption band, is less than 75% without compensation.

Improving the quality of a spectrum is often achieved by placing a blank disc of the appropriate potassium halide, of similar thickness to that of the sample disc, of the appropriate potassium halide in the reference beam.

Method 2

Triturate a small quantity of the finely ground substance with the minimum amount of a suitable mineral oil (such as Nujol) or other suitable liquid to give a smooth creamy paste; 10 mg of the substance to be examined combined with 1-2 drops of mineral oil is often sufficient to prepare a satisfactory mull. The prepared mull should appear opaque. Compress a portion of the mull between two flat sodium chloride or other suitable halide salt plates.

Method 3

Use a capillary film of the liquid held between two sodium chloride plates or a filled cell of suitable thickness.

Method 4

Prepare a solution in a suitable solvent and choose concentration and cell thickness which give a satisfactory spectrum over a sufficiently wide wave number range. Generally, good spectra are obtained with concentrations of 1-10% w/v for a cell thickness of 0.1-0.5 mm. To compensate for the absorption of the solvent, place in the reference beam of matched pathlength cell containing the solvent used or obtain a spectrum of the solvent to allow for differentiation between solvent and sample absorptions. Alternatively, the solvent absorbance spectrum versus air may be subtracted from the solution spectrum versus air to obtain the absorbance spectrum of the solute. (When using an FTIR instrument the spectrum of the solvent recorded under identical conditions can be subtracted digitally).

Method 5

Examine gases in a cell with windows which are transparent to infrared radiation and having an optical path length of about 100 mm. Evacuate the cell and fill to the desired pressure through a stopcock or needle valve using a suitable gas transfer line between the cell and the container of the substance to be examined. If necessary, adjust the pressure in the cell to atmospheric pressure using a gas transparent to infrared radiation (for example, nitrogen R or argon R). To avoid absorption interferences due to water, carbon dioxide or other atmospheric gases, place in the reference beam an identical cell that is either evacuated or filled with the gas transparent to infrared radiation.

Identification by reference substance

Prepare substance to be examined and the reference substance using the same method and record the spectrum of each from about $4000\text{--}600\text{ cm}^{-1}$ ($2.5\text{--}16.7\text{ }\mu\text{m}$). The concentration of the substance should be such that the strongest peak attributable to the substance reaches about 10% transmittance.

If the positions and relative intensities of the absorbance maxima in the spectrum of substance to be examined are not concordant with those of the spectrum of the reference substance for spectra obtained by methods 1 or 2, it may be caused by differences in crystalline form. To avoid such difficulty, use one of the following procedures:

Prepare both the substance to be examined and the reference substance as follows:

- a) The concentration of the solution of the reference substance and the sample are similar as described in method 4.
- b) Place 2 or 3 drops of a concentrated solution in a volatile organic solvent on a blank disc of potassium halide and evaporate to dryness in an oven at $105\text{ }^{\circ}\text{C}$. Treat both the reference substance and the substance to be examined in the same manner.
- c) Mix 2 or 3 drops of concentrated solution in a volatile organic solvent with 300 mg of potassium halide and evaporate to dryness in an oven at $105\text{ }^{\circ}\text{C}$. Treat both the reference substance and the substance to be examined in the same manner and prepare doses as described in method 1.
- d) Recrystallize the reference substance and substance to be examined from a suitable solvent.

If the spectrum of mineral oil used in method 2 interferes with regions of interest, prepare an additional dispersion of the substance in a medium such as a suitable fluorinated hydrocarbon oil or hexachlorobutadiene R and record the spectrum in the regions where the mineral oil shows strong absorption.

Identification by reference spectrum

Prepare substance to be examined exactly as described in the note accompanying the International Infrared Reference Spectrum and record the spectrum from about $4000\text{--}600\text{ cm}^{-1}$ ($2.5\text{--}16.7\text{ }\mu\text{m}$) using an instrument that is checked frequently to ensure that it meets the standards of performance required. Reference maxima of a polystyrene film should be superimposed on the spectrum of the substance to be examined at about 2851 cm^{-1} ($3.5\text{ }\mu\text{m}$), 1601 cm^{-1} ($6.25\text{ }\mu\text{m}$) and 1028 cm^{-1} ($9.73\text{ }\mu\text{m}$). Taking these polystyrene maxima into account, the identification is considered to be positive if the principal absorbance maxima in the spectrum of the substance to be examined is concordant with the corresponding maxima in the relevant International Infrared Reference Spectrum. When comparing the two spectra, care should be taken to allow for the possibility of differences in resolving power between the instrument on which the International Infrared Reference Spectrum was prepared and the instrument being used to examine the substance. An International Infrared Reference Spectrum of polystyrene that was recorded on the same instrument as the collection of the reference spectra

should be used for assessing these differences. The greatest variation due to differences in resolving power is likely to occur in the region between 4000 and 2000 cm^{-1} (2.5 and 5 μm). However, if the positions and relative intensities of the absorbance maxima in the spectrum of substance to be examined are not concordant with those of the reference spectrum when using methods 1 or 2, this may be due to differences in crystalline form. In such cases, apply the another procedure as described above under "Identification by reference substance" will be indicated in the note accompanying the reference spectrum.

Reflectance techniques

a) Attenuated total reflectance technique (ATR)

The attenuated total reflectance technique is best adapted to smooth, flexible surfaces, such as various plastics or to strongly absorbing liquids and solutions, but can also be employed to determine infrared absorption spectra of solid substances. It is usually necessary to reduce the solid substance to a fine powder. Pack the powder directly against the reflecting element of the attachment or use an adhesive tape to facilitate the contact. Spread the powdered substance on the adhesive side of the tape to form an almost translucent layer and press the powdered side of the tape onto the reflecting element. Next, either attach the backing plate or apply moderate pressure using a suitable clamp for 1-2 minutes. Finally, place the reflecting element in the holder. The tape used in the procedure should preferably contain a natural rubber adhesive. Some plastic materials may be placed directly onto the reflecting element.

Reflective elements are usually constructed of zinc selenide (refractive index = 2.3) or germanium (refractive index = 4.0). The correct alignment of the attachment in the apparatus should be carefully controlled.

b) Diffuse reflectance

In this technique it signifies that the surface of a sample reflects light in many different directions. Reduce the solid substance to a fine powder with a non-absorbing matrix (potassium bromide or chloride are suitable). Place the mixture directly into the sample cup holder of the diffuse reflectance instrument. The spectrum of the matrix recorded under identical conditions should be subtracted digitally. Some plastic materials can be placed directly into the sample cup holder of the diffuse reflectance accessory.

These provisional recommendations are intended to accompany the International Infrared Reference Spectra. Any comments arising from the practicable application of this guide are most welcome.

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