1.7 Spectrophotometry in the infrared region

2014-01

The infrared region of the electromagnetic spectrum used in pharmaceutical analysis covers the range 4000-250 cm^{-1} (2.5-40 μm).[1]

[1] The values given in brackets are wavelengths; the preceding values are wave-numbers, which are the reciprocals of the wavelengths.

Spectrophotometric measurements in the infrared region are used mainly as an identification test. The infrared spectrum is unique for any given chemical compound with the exception of optical isomers 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 a difference in the infrared spectrum of a given compound in the solid state. The infrared spectrum is usually not greatly affected by the presence of small quantities of impurities (up to several percent) in the tested substance. For identification purposes the spectrum may be compared with that of a reference substance, concomitantly prepared or with a standard reference spectrum.

The terms *absorption spectrum, absorbance, transmittance, absorptivity* and *specific extinction* are described in *1.6 Spectrophotometry in the visible and ultraviolet regions*.

Apparatus

Spectrophotometers for the infrared region are basically similar to those used for the visible and ultraviolet regions of the spectrum but may differ as to the energy sources, optical materials and detection devices. Furthermore, in some instruments the monochromator may be located between the test substance and the detector.

Spectrophotometers suitable for use for identification tests should operate in the range 4000-670 cm^{-1} (2.5-15 μm). They should be checked frequently to ensure that they meet the standards of performance laid down by the manufacturer of the instrument, including the reliability of the wavelength scales, which should be checked by use of a polystyrene film.

For the use of the attenuated total reflectance technique the instrument should be equipped with a suitable attachment, which may be a single-reflection or a multi-reflection one. The attachment consists of a reflecting element and a suitable mounting permitting its alignment in the spectrophotometer for maximum transmission.

Use of solvents

The solvent used in infrared spectrophotometry must not affect the material, usually sodium chloride, of which the cell is made.

No solvent in appreciable thickness is completely transparent throughout the infrared spectrum. Carbon tetrachloride R is practically transparent (up to 1 mm in thickness) from 4000 to 1700 cm^{-1} (2.5 to 6 μm). Chloroform R, dichloromethane R and dibromomethane R are other useful solvents. 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} (4.2-5.0 μm) and the 1800-1300 cm^{-1} (5.5-7.5 μm) regions where it has strong absorption. Its weak absorption in the 875-845 cm^{-1} (11.4-11.8 μm) region should also be noted. Other solvents have relatively narrow regions of transparency.

Preparation of the substance to be tested

To determine the infrared absorption spectrum of a substance the latter has to be suitably prepared. Liquid substances may be tested directly or in a suitable solution. For solid substances the usual methods of preparation include dispersing the finely ground solid specimen in mineral oil or incorporating it in a transparent disc or pellet obtained by mixing it intimately with previously dried potassium halide and pressing 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.

The following procedures may be used for the preparation of the substance:

Method 1. Use a capillary film of the liquid held between two sodium chloride plates or a filled cell of suitable thickness.

Method 2. Triturate a small quantity of the substance with the minimum amount of a suitable mineral oil or other suitable liquid to give a smooth, creamy paste; 2-5 mg of the substance being tested is often sufficient to prepare a satisfactory mull which should be semi-transparent to light. Compress a portion of the mull between two flat sodium chloride or other suitable plates.

Method 3. Triturate the solid substance with dry, finely powdered potassium halide (potassium bromide IR, potassium chloride IR); the proportion of substance to the halide should be about 1 to 200 - for example, 1.5 mg in 300 mg of the halide - in the case of prism instruments, and about 1 to 300 - for example, 1.0 mg in 300 mg of the halide - in the case of grating instruments. The amount taken should be such that the weight of substance per area of the disc is about 5-15 μg per mm^2, varying with the molecular weight and to some degree with the type of apparatus used. Insert a portion of the mixture in a special dye and subject it under vacuum to a high pressure. Commercial dyes are available and the manufacturer's instructions should be followed. 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 transmission at about 2000 cm^{-1} (5 µm) in the absence of a specific absorption band is less than 60% without compensation.

Method 4. Prepare a solution of the liquid or solid substance in a suitable solvent and choose a concentration and cell thickness to give a satisfactory spectrum over a sufficiently wide wavelength range.

Identification by reference substance

Prepare the substance under examination and the reference substance by the same method and record the spectrum of each from about 4000 to 670 cm^{-1} (2.5 µm to 15 µm) on an infrared spectrophotometer. The concentration of the substance should be such that the strongest peak attributable to the substance reaches to between 5% and 25% transmittance.

If the positions and relative intensities of the absorbance maxima in the spectrum of the substance under examination are not concordant with those of the spectrum of the reference substance in the case of the curves obtained by Methods 2 or 3 this may be due to differences in crystalline form. When such difficulties are suspected the substance should, where possible, be examined in solution. If examination in solution is not practicable attempts should be made to obtain, by recrystallization, the reference substance and the substance under examination in the same crystalline form.

If the spectrum of mineral oil used in Method 2 interferes with regions of interest an additional dispersion of the substance in a medium such as a suitable fluorinated hydrocarbon oil or hexachlorobutadiene may be prepared and the spectrum recorded in the regions where the mineral oil shows strong absorption.

Identification by reference spectrum

Where, as permitted in a monograph in *The International Pharmacopoeia*, comparison is made with an infrared reference spectrum, prepare the spectrum of the substance being examined as follows, unless otherwise indicated. Prepare a disc using potassium bromide IR. Record the spectrum from about 4000 to 670 cm^{-1} (2.5 to 15 µm) using an instrument that is checked frequently to ensure that it meets the standards of performance laid down by the manufacturer.

Where a monograph in *The International Pharmacopoeia* refers to an infrared reference spectrum, this spectrum is either provided within the section on Infrared reference spectra or it is available from:

• WHO collaborating host organization for International Chemical Reference Substances: European Directorate for the Quality of Medicines & HealthCare, 7 allée Kastner, CS 30026, F-67081 Strasbourg, France; Fax: +33 (0)3 88 41 27 71

A spectrum of the test substance is considered to be concordant with a reference spectrum if the transmission minima (absorption maxima) of the principal bands in the test spectrum correspond in position, relative intensities and shape to those in the references spectrum.

When tests for infrared absorption are applied to material extracted from dosage forms, strict concordance with the specified reference spectrum may not always be possible. Nevertheless a close resemblance between the spectrum of the extracted material and the specified reference spectrum should be achieved.

The reference spectra provided within this section on International Infrared Reference Spectra (IIRS) were recorded using the indicated sample preparations techniques (phases) and instruments.

Attenuated total reflectance technique

To determine the infrared absorption spectrum of a substance by the attenuated total reflectance technique the solid substance has usually to be finely pulverized. The powder may then be either packed directly against the prism of the attachment or adhesive tape may be used to facilitate the contact. The powdered substance is spread on the adhesive side of an adhesive tape to form an almost translucent layer and the tape is pressed on the reflecting element on the side with powder in contact. Next either the backing plate is attached or moderate pressure is applied in a suitable clamp for 1-2 minutes. Finally the reflecting element is placed in the holder. The tape used in the procedure should preferably contain a natural rubber adhesive. In the case of some plastic materials it may be placed directly on the reflecting element.

Proper alignment of the attachment in the apparatus should be carefully controlled.