

tions by viewing the tubes downward or transversely against a white background. The test solution has no more color than the control solution.

23. Infrared Spectrophotometry

Infrared Spectrophotometry is a method of measurement of the extent, at various wave numbers, of absorption of infrared radiation when it passes through a layer of a substance. In the graphic representation of infrared spectra, the plot usually shows units of wave numbers as the abscissa and units of transmittance or absorbance as the ordinate. Wave number and transmittance or absorbance at each absorption maximum may be read graphically on an absorption spectrum and/or obtained by a data-processor. Since the wave number and the respective intensity of an absorption maximum depend on the chemical structure of a substance, this measurement can be used to identify or determine a substance.

Instrument and adjustment

Several models of dispersive infrared spectrophotometers or Fourier-transform infrared spectrophotometers are available.

The instruments, adjusted according to the instruction manual of each individual instrument, should comply with the following test for resolving power, transmittance reproducibility and wave number reproducibility. When the spectrum of a polystyrene film about 0.04 mm thick is recorded, the depth of the trough from the maximum absorption at about 2851 cm^{-1} to the minimum at about 2870 cm^{-1} should be not less than 18% transmittance and that from the maximum at about 1583 cm^{-1} to the minimum at about 1589 cm^{-1} should be not less than 12% transmittance.

The wave number (cm^{-1}) scale is usually calibrated by the use of several absorption bands of a polystyrene film, shown below. The numbers in parentheses indicate the precision with which these values have been established.

3027.1 (± 0.3)	1801.6 (± 0.3)	1069.1 (± 0.3)
2924 (± 2)	1601.4 (± 0.3)	1028.0 (± 0.3)
2850.7 (± 0.3)	1583.1 (± 0.3)	906.7 (± 0.3)
1944 (± 1)	1181.4 (± 0.3)	698.9 (± 0.5)
1871.0 (± 0.3)	1154.3 (± 0.3)	

The difference of transmittance should be within 0.5% when the spectrum of a polystyrene film is measured twice at several wave numbers from 3000 to 1000 cm^{-1} , and the difference of wave number should be within 5 cm^{-1} at about 3000 cm^{-1} and within 1 cm^{-1} at about 1000 cm^{-1} .

Preparation of samples and measurement

Unless otherwise specified, when it is directed to perform the test "after drying the sample", use a sample dried under the conditions specified in the monograph. Prepare the specimen for the measurement according to one of the following procedures so that the transmittance of most of the absorption bands is in the range of 5% to 80%. Single crystals of sodium chloride, potassium bromide, etc. are available for the optical plate. Generally, the reference cell or material is placed in the reference beam for double-beam instruments, while for single-beam instruments, it is placed in the same optical path in place of the specimen and measured separately

under the same operating conditions. The composition and preparation of the reference depend on the sample preparation methods, and sometimes the background absorption of the atmosphere can be utilized.

Unless otherwise specified in the monograph, the spectrum is usually recorded between 4000 cm^{-1} and 400 cm^{-1} . The spectrum should be scanned using the same instrumental conditions as were used to ensure compliance with the requirements for the resolving power and for the precision of wave number scale and of wave numbers.

(1) *Potassium bromide disk or potassium chloride disk method*—Powder 1 to 2 mg of a solid sample in an agate mortar, triturate rapidly with 0.10 to 0.20 g of potassium bromide or potassium chloride for infrared spectrophotometry with precautions against moisture absorption, and compress the mixture with a press in a suitable die (disk-forming container) to make the sample disk. If necessary to obtain a transparent disk, press the mixture under vacuum in a die with pressure applied to the die of 50 to 100 kN per cm^2 for 5 to 8 minutes. Prepare a potassium bromide reference disk or a potassium chloride reference disk in the same manner as the sample disk.

(2) *Solution method*—Place the sample solution prepared by the method directed in each monograph in a fixed cell for liquid, and usually measure the spectrum against the reference solvent used for preparing the sample solution. The solvent used in this method should not show any interaction or chemical reaction with the specimen to be examined and should not damage the optical plate. The thickness of the fixed cell is usually 0.1 mm or 0.5 mm.

(3) *Paste method*—Powder 5 to 10 mg of a solid specimen in an agate mortar, and, unless otherwise specified, triturate the specimen with 1 to 2 drops of liquid paraffin to give a homogeneous paste. After spreading the paste to make a thin film in the center of an optical plate, place the plate upon another optical plate with precautions against intrusion of air, bubbles in the film, and examine its absorption spectrum.

(4) *Liquid film method*—Examine 1 to 2 drops of a liquid specimen as a thin film held between two optical plates. When the absorption intensity is not sufficient, place spacers of aluminum foil, etc., between the two optical plates to make a thicker liquid film.

(5) *Film method*—Examine a thin film just as it is or a prepared thin film as directed in each monograph.

(6) *Gas sampling method*—Put a sample gas in a gas cell previously evacuated under the pressure directed in the monograph, and examine its absorption spectrum. The path length of the gas cell is usually 5 cm or 10 cm, but, if necessary, may exceed 1 m.

(7) *ATR method*—Place a specimen in close contact with an attenuated total reflectance (ATR) prism, and examine its reflectance spectrum.

(8) *Diffuse reflectance method*—Powder 1 to 3 mg of a solid specimen into a fine powder of not more than about $50\text{ }\mu\text{m}$ particle size in an agate mortar, and triturate rapidly with 0.05 to 0.10 g of potassium bromide or potassium chloride for infrared spectrophotometry with precautions against moisture absorption. Place the mixture in a sample cup, and examine its reflectance spectrum.

Identification

When the spectrum of a specimen and the Reference Spec-

trum of the substance expected to be found or the spectrum of the Reference Standard exhibit similar intensities of absorption at the same wave numbers, the specimen can be identified as being the substance expected to be found. Furthermore, when several specific absorption wave numbers are specified in the monograph, the identification of a specimen with the substance expected to be found can be confirmed by the appearance of absorption bands at the specified wave numbers.

(1) Identification by the use of a Reference Standard

When the spectra of a specimen and the Reference Standard exhibit similar intensities of absorption at the same wave numbers, the specimen can be identified as being the same substance as the Reference Standard. When a sample treatment method for a solid specimen is indicated in the monograph in the case of nonconformity of the spectrum with that of the Reference Standard, treat the specimen being examined and the Reference Standard in the same manner as directed in the monograph, then repeat the measurement.

(2) Identification by the use of a Reference Spectrum

When the spectra of a specimen and the Reference Spectrum exhibit similar intensities of absorption at the same wave numbers, the specimen can be identified as being the same substance associated with the Reference Spectrum. When a sample treatment method for a solid specimen is indicated in the monograph in the case of nonconformity of the spectrum with the Reference Spectrum, treat the specimen being examined as directed in the monograph, then repeat the measurement.

(3) Identification by the use of absorption wave number

When several specific absorption wave numbers of the substance being examined are specified in the monograph, a specimen can be identified as being the same substance as the expected substance by confirmation of clear appearance of the absorption bands at all the specified wave numbers.

Reference spectra

Infrared Reference Spectra, in the range between 4000 cm^{-1} and 400 cm^{-1} , are shown at the end of this book for the monographs requiring the identification test by *Infrared Spectrophotometry*, except for monographs in which "Identification by absorption wave number" is specified.

24. Insoluble Particulate Matter Test for Injections

The test is to examine the size and the number of insoluble particulate matters in injections. When Method 1 is not applicable (as in the case where the preparation of a 25 mL sample solution is impossible or in another case of a protein formulation where observed data by Method 1 has exceeded the limit specified in Method I), the test should be carried out according to Method 2. The test specified herein is not applied to emulsion type or suspension type injections.

Method 1. Light Obscuration Particle Count Test

Instrument Standardization

Apparatus

The apparatus is an electronic particle counting system that uses a light-obscuration sensor with a suitable sample feeding device. Commercially available sensors employ a tungsten lamp, LED, or laser as light source, showing a differ-

ence in light wavelength for detection and consequently in sensitivity. Moreover, the sensor concentration limits (the maximum rated particle concentration) is difference of the mechanism of particulate-detecting unit.

The sample feeding device is different by its types such as the compression type or suction type, then, the standardization of the automatic light obscuration particle counter should be the basis for evaluating the performance of apparatus to be used. It is necessary to perform calibration, as well as to demonstrate the sample volume accuracy, sample flow rate, particle size response curve, sensor resolution, and counting accuracy, at least once a year.

Calibration

Particles to be used for calibration should be subject to particle-size sensitivity measurement, using spherical polystyrene particles having at least 5, 10 and 25 μm in diameter (PSL particles) in mono-dispersed suspension. The PSL particles should have either a domestic or international traceability in terms of length, with a level of uncertainty at not greater than 3%.

Manual method

The particle size response of the system to be applied should be determined using at least 3 channels for threshold-voltage setting, according to the half counting method of window moving type. The threshold-voltage window should be $\pm 20\%$ of the measuring particle size. After measuring the sensitivity of response for the designated particle size, the size response curve is prepared by the method indicated by the manufacturer from particle-response measuring point, and threshold-voltage of 5, 10 and 25 μm of the apparatus is obtained.

Electronic method

In the use of multichannel peak height analyzer, the particle size response is measured by half-count method of moving window system same as the manual method, and the particle size response curve is prepared by the method designated by the instrument manufacturer, then, the threshold voltage of 5, 10 and 25 μm of the apparatus is obtained. In this case, the instrument manufacturer or the user should validate the obtainability of the same result as that of the manual method.

Automated method

The particle size response curve of the apparatus may be obtained by using the software developed by the user or supplied by the instrument manufacturer, whereas, the manufacturer or the user should validate the obtainability of the same result as that of the manual method.

Sample volume accuracy

Sample volume accuracy should fall within 5% of the measuring value in case measuring the decrease of test solution by the mass method after measuring the test solution of 10 mL.

Sample flow rate

The flow rate of the sample indicated into the sensor should be calculated from the observed sample volume and time, and should be conformed within the range of the manufacturer's specification for sensor used.

Sensor

There is a possibility of changes of particle size resolution