Prepare the solution of the substance to be examined (test solution) as prescribed in the monograph. Prepare not fewer than 3 reference solutions of the element to be determined, the concentrations of which span the expected value in the test solution. For assay purposes, optimal calibration levels are between 0.7 and 1.3 times the expected content of the element to be determined or the limit prescribed in the monograph. For purity determination, calibration levels are the limit of detection and 1.2 times the limit specified for the element to be determined. Any reagents used in the preparation of the test solution are added to the reference and blank solutions at the same concentration.

Introduce each of the solutions into the instrument using the same number of replicates for each of the solutions to obtain a steady reading.

**Calculation**. Prepare a calibration curve from the mean of the readings obtained with the reference solutions by plotting the means as a function of concentration. Determine the concentration of the element in the test solution from the curve obtained.

*METHOD II - STANDARD ADDITIONS*

Add to at least 3 similar volumetric flasks equal volumes of the solution of the substance to be examined (test solution) prepared as prescribed. Add to all but 1 of the flasks progressively larger volumes of a reference solution containing a known concentration of the element to be determined to produce a series of solutions containing steadily increasing concentrations of that element known to give responses in the linear part of the curve, if possible. Dilute the contents of each flask to volume with solvent.

Introduce each of the solutions into the instrument, using the same number of replicates for each of the solutions, to obtain a steady reading.

**Calculation**. Calculate the linear equation of the graph using a least-squares fit and derive from it the concentration of the element to be determined in the test solution.

VALIDATION OF THE METHOD

Satisfactory performance of methods prescribed in monographs is verified at suitable time intervals.

*LINEARITY*

Prepare and analyse not fewer than 4 reference solutions over the calibration range and a blank solution. Perform not fewer than 5 replicates.

The calibration curve is calculated by least-square regression from all measured data. The regression curve, the means, the measured data and the confidence interval of the calibration curve are plotted. The operating method is valid when:

- the correlation coefficient is at least 0.99,
- the residuals of each calibration level are randomly distributed around the calibration curve.

Calculate the mean and relative standard deviation for the lowest and highest calibration level.

When the ratio of the estimated standard deviation of the lowest and the highest calibration level is less than 0.5 or greater than 2.0, a more precise estimation of the calibration curve may be obtained using weighted linear regression. Both linear and quadratic weighting functions are applied to the data to find the most appropriate weighting function to be employed. If the means compared to the calibration curve show a deviation from linearity, two-dimensional linear regression is used.

*ACCURACY*

Verify the accuracy preferably by using a certified reference material (CRM). Where this is not possible, perform a test for recovery.

**Recovery**. For assay determinations a recovery of 90 per cent to 110 per cent is to be obtained. For other determinations, for example, for trace element determination the test is not valid if recovery is outside of the range 80 per cent to 120 per cent at the theoretical value. Recovery may be determined on a suitable reference solution (matrix solution) which is spiked with a known quantity of analyte (middle concentration of the calibration range).

*REPEATABILITY*

The repeatability is not greater than 3 per cent for an assay and not greater than 5 per cent for an impurity test.

*LIMIT OF QUANTIFICATION*

Verify that the limit of quantification (for example, determined using the 10 σ approach) is below the value to be measured.

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# 2.2.24. ABSORPTION SPECTROPHOTOMETRY, INFRARED

Infrared spectrophotometers are used for recording spectra in the region of 4000-650 $cm^{-1}$ (2.5-15.4 μm) or in some cases down to 200 $cm^{-1}$ (50 μm).

APPARATUS

Spectrophotometers for recording spectra consist of a suitable light source, monochromator or interferometer and detector.

Fourier transform spectrophotometers use polychromatic radiation and calculate the spectrum in the frequency domain from the original data by Fourier transformation. Spectrophotometers fitted with an optical system capable of producing monochromatic radiation in the measurement region may also be used. Normally the spectrum is given as a function of transmittance, the quotient of the intensity of the transmitted radiation and the incident radiation. It may also be given in absorbance.

The absorbance ($A$) is defined as the logarithm to base 10 of the reciprocal of the transmittance ($T$):

$$A = \log_{10}\left(\frac{1}{T}\right) = \log_{10}\left(\frac{I_0}{I}\right)$$

$T$ = $\frac{I}{I_0}$,

$I_0$ = intensity of incident radiation,

$I$ = intensity of transmitted radiation.

PREPARATION OF THE SAMPLE

*FOR RECORDING BY TRANSMISSION OR ABSORPTION*

Prepare the substance by one of the following methods.

**Liquids**. Examine a liquid either in the form of a film between 2 plates transparent to infrared radiation, or in a cell of suitable path length, also transparent to infrared radiation.

**Liquids or solids in solution**. Prepare a solution in a suitable solvent. Choose a concentration and a path length of the cell which give a satisfactory spectrum. Generally, good results are obtained with concentrations of 10-100 g/L for a path length of 0.5-0.1 mm. Absorption due to the solvent is compensated by placing in the reference beam a similar cell containing the solvent used. If an FT-IR instrument is used, the absorption is compensated by recording the spectra for the solvent and the sample successively. The solvent absorbance, corrected by a compensation factor, is subtracted using calculation software.

**Solids**. Examine solids dispersed in a suitable liquid (mull) or in a solid (halide disc), as appropriate. If prescribed in the monograph, make a film of a molten mass between 2 plates transparent to infrared radiation.

A. Mull

Triturate a small quantity of the substance to be examined with the minimum quantity of *liquid paraffin R* or other suitable liquid; 5-10 mg of the substance to be examined is

usually sufficient to make an adequate mull using one drop of *liquid paraffin R*. Compress the mull between 2 plates transparent to infrared radiation.

B. Disc

Triturate 1-2 mg of the substance to be examined with 300-400 mg, unless otherwise specified, of finely powdered and dried *potassium bromide R* or *potassium chloride R*. These quantities are usually sufficient to give a disc of 10-15 mm diameter and a spectrum of suitable intensity. If the substance is a hydrochloride, it is recommended to use *potassium chloride R*. Carefully grind the mixture, spread it uniformly in a suitable die, and submit it to a pressure of about 800 MPa (8 t·cm$^{-2}$). For substances that are unstable under normal atmospheric conditions or are hygroscopic, the disc is pressed *in vacuo*. Several factors may cause the formation of faulty discs, such as insufficient or excessive grinding, humidity or other impurities in the dispersion medium or an insufficient reduction of particle size. A disc is rejected if visual examination shows lack of uniform transparency or when transmittance at about 2000 cm$^{-1}$ (5 μm) in the absence of a specific absorption band is less than 60 per cent without compensation, unless otherwise prescribed.

**Gases**. Examine gases in a cell 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 gas 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* and *argon R*). To avoid absorption interferences due to water, carbon dioxide or other atmospheric gases, place in the reference beam, if possible, an identical cell that is either evacuated or filled with the gas transparent to infrared radiation.

*FOR RECORDING BY DIFFUSE REFLECTANCE*

**Solids**. Triturate a mixture of the substance to be examined with finely powdered and dried *potassium bromide R* or *potassium chloride R*. Use a mixture containing approximately 5 per cent of the substance, unless otherwise specified. Grind the mixture, place it in a sample cup and examine the reflectance spectrum.

The spectrum of the sample in absorbance mode may be obtained after mathematical treatment of the spectra by the Kubelka-Munk function.

*FOR RECORDING BY ATTENUATED TOTAL REFLECTION*

Attenuated total reflection (including multiple reflection) involves light being reflected internally by a transmitting medium, typically for a number of reflections. However, several accessories exist where only one reflection occurs.

Prepare the substance as follows. Place the substance to be examined in close contact with an internal reflection element (IRE) such as diamond, germanium, zinc selenide, thallium bromide-thallium iodide (KRS-5) or another suitable material of high refractive index. Ensure close and uniform contact between the substance and the whole crystal surface of the internal reflection element, either by applying pressure or by dissolving the substance in an appropriate solvent, then covering the IRE with the obtained solution and evaporating to dryness. Examine the attenuated total reflectance (ATR) spectrum.

## IDENTIFICATION USING REFERENCE SUBSTANCES

Prepare the substance to be examined and the reference substance by the same procedure and record the spectra between 4000-650 cm$^{-1}$ (2.5-15.4 μm) under the same operational conditions. The transmission minima (absorption maxima) in the spectrum obtained with the substance to be examined correspond in position and relative size to those in the spectrum obtained with the reference substance (*CRS*).

When the spectra recorded in the solid state show differences in the positions of the transmission minima (absorption maxima), treat the substance to be examined and the reference substance in the same manner so that they crystallise or are produced in the same form, or proceed as prescribed in the monograph, then record the spectra.

## IDENTIFICATION USING REFERENCE SPECTRA

**Control of resolution performance**. For instruments having a monochromator, record the spectrum of a polystyrene film approximately 35 μm in thickness. The difference $x$ (see Figure 2.2.24.-1) between the percentage transmittance at the transmission maximum $A$ at 2870 cm$^{-1}$ (3.48 μm) and that at the transmission minimum $B$ at 2849.5 cm$^{-1}$ (3.51 μm) must be greater than 18. The difference $y$ between the percentage transmittance at the transmission maximum $C$ at 1589 cm$^{-1}$ (6.29 μm) and that at the transmission minimum $D$ at 1583 cm$^{-1}$ (6.32 μm) must be greater than 10.

For Fourier-transform instruments, use suitable instrument resolution with the appropriate apodisation prescribed by the manufacturer. The resolution is checked by suitable means, for example by recording the spectrum of a polystyrene film approximately 35 μm in thickness. The difference between the absorbances at the absorption minimum at 2870 cm$^{-1}$ and the absorption maximum at 2849.5 cm$^{-1}$ is greater than 0.33. The difference between the absorbances at the absorption minimum at 1589 cm$^{-1}$ and the absorption maximum at 1583 cm$^{-1}$ is greater than 0.08.

**Verification of the wave-number scale**. The wave-number scale may be verified using a polystyrene film, which has transmission minima (absorption maxima) at the wave numbers (in cm$^{-1}$) shown in Table 2.2.24.-1.

Table 2.2.24.-1. – *Transmission minima and acceptable tolerances of a polystyrene film*

| Transmission minima (cm$^{-1}$) | Acceptable tolerance (cm$^{-1}$) | |
|---|---|---|
| | Monochromator instruments | Fourier-transform instruments |
| 3060.0 | ± 1.5 | ± 1.0 |
| 2849.5 | ± 2.0 | ± 1.0 |
| 1942.9 | ± 1.5 | ± 1.0 |
| 1601.2 | ± 1.0 | ± 1.0 |
| 1583.0 | ± 1.0 | ± 1.0 |
| 1154.5 | ± 1.0 | ± 1.0 |
| 1028.3 | ± 1.0 | ± 1.0 |

**Method**. Prepare the substance to be examined according to the instructions accompanying the reference spectrum/reference substance. Using the operating conditions that were used to obtain the reference spectrum, which will usually be the same as those for verifying the resolution performance, record the spectrum of the substance to be examined.

The positions and the relative sizes of the bands in the spectrum of the substance to be examined and the reference spectrum are concordant in the 2 spectra.

**Compensation for water vapour and atmospheric carbon dioxide**. For Fourier-transform instruments, spectral interference from water vapour and carbon dioxide is compensated using suitable algorithms according to the manufacturer's instructions. Alternatively, spectra can be

acquired using suitable purged instruments or ensuring that sample and background single beam spectra are acquired under exactly the same conditions.

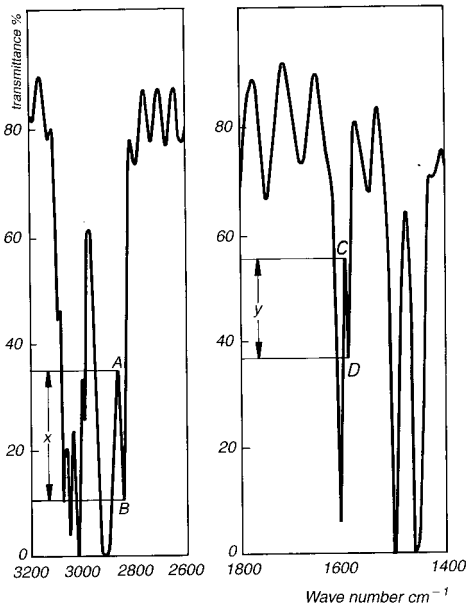


Figure 2.2.24.-1. – *Typical spectrum of polystyrene used to verify the resolution performance*

IMPURITIES IN GASES

For the analysis of impurities, use a cell transparent to infrared radiation and of suitable optical path length (for example, 1-20 m). Fill the cell as prescribed under Gases. For detection and quantification of the impurities, proceed as prescribed in the monograph.

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# 2.2.25. ABSORPTION SPECTROPHOTOMETRY, ULTRAVIOLET AND VISIBLE

**Determination of absorbance**. The absorbance (*A*) of a solution is defined as the logarithm to base 10 of the reciprocal of the transmittance (*T*) for monochromatic radiation:

$$A = \log_{10}\left(\frac{1}{T}\right) = \log_{10}\left(\frac{I_0}{I}\right)$$

$T$ = $I/I_0$;

$I_0$ = intensity of incident monochromatic radiation;

$I$ = intensity of transmitted monochromatic radiation.

In the absence of other physico-chemical factors, the absorbance (*A*) is proportional to the path length (*b*) through which the radiation passes and to the concentration (*c*) of the substance in solution in accordance with the equation:

$$A = \varepsilon cb$$

$\epsilon$ = molar absorptivity, if *b* is expressed in centimetres and *c* in moles per litre.

The expression $A_{1\ \mathrm{cm}}^{1\ \mathrm{per\ cent}}$ representing the specific absorbance of a dissolved substance refers to the absorbance of a 10 g/L solution in a 1 cm cell and measured at a defined wavelength so that:

$$A_{1\ \mathrm{cm}}^{1\ \mathrm{per\ cent}} = \frac{10\varepsilon}{M_\mathrm{r}}$$

Unless otherwise prescribed, measure the absorbance at the prescribed wavelength using a path length of 1 cm. Unless otherwise prescribed, the measurements are carried out with reference to the same solvent or the same mixture of solvents. The absorbance of the solvent measured against air and at the prescribed wavelength shall not exceed 0.4 and is preferably less than 0.2. Plot the absorption spectrum with absorbance or function of absorbance as ordinate against wavelength or function of wavelength as abscissa.

Where a monograph gives a single value for the position of an absorption maximum, it is understood that the value obtained may differ by not more than ± 2 nm.

**Apparatus**. Spectrophotometers suitable for measuring in the ultraviolet and visible range of the spectrum consist of an optical system capable of producing monochromatic radiation in the range of 200-800 nm and a device suitable for measuring the absorbance.

**Control of wavelengths**. Verify the wavelength scale using the absorption maxima of *holmium perchlorate solution R*, the line of a hydrogen or deuterium discharge lamp or the lines of a mercury vapour arc shown in Table 2.2.25.-1. The permitted tolerance is ± 1 nm for the ultraviolet range and ± 3 nm for the visible range. Suitable certified reference materials may also be used.

Table 2.2.25.-1. – *Absorption maxima for control of wavelength scale*

| | |
|---|---|
| 241.15 nm (Ho) | 404.66 nm (Hg) |
| 253.7 nm (Hg) | 435.83 nm (Hg) |
| 287.15 nm (Ho) | 486.0 nm (Dβ) |
| 302.25 nm (Hg) | 486.1 nm (Hβ) |
| 313.16 nm (Hg) | 536.3 nm (Ho) |
| 334.15 nm (Hg) | 546.07 nm (Hg) |
| 361.5 nm (Ho) | 576.96 nm (Hg) |
| 365.48 nm (Hg) | 579.07 nm (Hg) |

**Control of absorbance**. Check the absorbance using suitable filters or a solution of *potassium dichromate R* at the wavelengths indicated in Table 2.2.25.-2, which gives for each wavelength the exact value and the permitted limits of the specific absorbance. The table is based on a tolerance for the absorbance of ± 0.01.

For the control of absorbance, use solutions of *potassium dichromate R* that has been previously dried to constant mass at 130 °C. For the control of absorbance at 235 nm, 257 nm, 313 nm and 350 nm, dissolve 57.0-63.0 mg of *potassium dichromate R* in *0.005 M sulfuric acid* and dilute to 1000.0 mL with the same acid. For the control of absorbance at 430 nm, dissolve 57.0-63.0 mg of *potassium dichromate R* in *0.005 M sulfuric acid* and dilute to 100.0 mL with the same acid. Suitable certified reference materials may also be used.