can be obtained: chemical shifts, spin-spin coupling constants, resonance intensities (the number of nuclei) and relaxation times. These parameters are useful for the structural determination, the identification and the quantitative analysis of molecules. Spin decoupling, nuclear Overhauser effect, and two-dimensional NMR techniques are also available for the structural analysis.

Spectrometer

There are two types of spectrometers.

(1) Continuous wave NMR spectrometers.

(2) Fourier Transform NMR spectrometers.

Measurement

Prior to measurements, the sensitivity and resolution of the instrument must be adjusted to the best conditions using the standard sample (ethylbenzene, *o*-dichlorobenzene or acetaldehyde) dissolved in an appropriate NMR solvent or carbontetrachloride.

(1) The sample dissolved in a relevant solvent is transferred into an NMR tube. The reference compound can be added directly to the sample solution (internal reference), or a sealed capillary tube containing the reference compound can be inserted into the NMR tube (external reference). The sample solutions should completely be homogeneous. Particularly solid contaminants should be removed in order to obtain good spectra. Various deuterated NMR solvents and carbontetrachloride are used for the ^{1}H-NMR and the following considerations should be paid for selecting an appropriate solvent: (i) The solvent signals do not overlap with the sample signals. (ii) The sample must be soluble in the solvent selected. (iii) The solvent does not react with the sample. Furthermore it should be noted that chemical shifts can depend upon solvents employed, sample concentrations and deuterium ion concentrations and that viscous solutions usually give rather broad, poorly resolved spectra.

(2) Tetramethylsilane is usually used as the reference compound for samples dissolved in organic solvents. For samples dissolved in deuterium oxide, sodium 3-(trimethylsilyl)propane sulfonate or sodium 3-(trimethylsilyl) propionate is used.

(3) When it is required to compare the spectrum with that of the authentic sample for identification, the measurement conditions, such as the oscillator frequency, the solvent and the concentration, should be the same with those for the authentic spectra.

40. Optical Rotation Determination

The Optical Rotation Determination is a method for the measurement of the angular rotation of the sample using a polarimeter.

Generally, the vibrations of light take place on planes perpendicular to the direction of the beam. In the case of ordinary light, the directions of the planes are unrestricted. In the case of plane polarized light, commonly designated as polarized light, however, the vibrations take place on only one plane that includes the direction of the beam (plane of polarization). Some drugs in the solid state or in solution have the property of rotating the plane of the polarized light either to the right or to the left. This property is referred to as optical activity or optical rotation, and is inherently related to the chemical constitution of the substance.

The extent of the rotation, expressed in degrees of rotation of the angle of the plane of polarized light caused by the optically active substance or its solution, is measured with a polarimeter. This value is proportional to the length of the polarimeter tube, and is related to the concentration of the solution, the temperature and the wavelength. The character of the rotation is indicated by placing a plus sign (+) for that which rotates the plane of the polarized light to the right, when facing the direction of the beam, referred to as dextrorotatory, or a minus sign (−) for that which rotates the plane to the left, referred to as levorotatory, before the number indicating the degrees of rotation, as like as +20°, meaning 20° to the right, or −20°, meaning 20° to the left.

The angular rotation α_x^t is that which is measured with specific monochromatic light of x (described in terms of the wavelength or the name) at a temperature of t°C. Usually the measurement is performed at 20°C, with a polarimeter tube of 100 mm in length, and with the D line of sodium as the light source.

The specific rotation is represented by the following equation:

$$[\alpha]_x^t = \frac{100\,\alpha}{lc}$$

t: The temperature of measurement.

x: The wavelength or the name of the specific monochromatic light of the spectrum used (in the case of the D line, described as D).

α: The angle, in degrees, of rotation of the plane of the polarized light.

l: The thickness of the layer of sample solution, i.e., the length of the polarimeter tube (mm).

c: For the purpose of the Pharmacopoeia of Japan, the number of grams of a drug present in 1 mL of the solution. When an intact liquid drug is used for determination, not in solution, c represents the density. However, unless otherwise specified, the specific gravity is used instead of the density.

The description, for example, "$[\alpha]_D^{20}$: −33.0 – −36.0° (after drying, 1 g, water, 20 mL, 100 mm)," in a monograph, indicates that the $[\alpha]_D^{20}$ is between −33.0° and −36.0° in the determination in which the substance is dried under the conditions described in the test for Loss on Drying, and about 1 g of the substance is accurately weighed, and dissolved by adding water to make exactly 20 mL, then the solution is measured with a polarimeter tube 100 mm in length.

41. Osmolarity Determination

Osmolarity determination is a method for measuring the osmotic concentration of the sample solution from the extent of the freezing-point depression.

When a solution and a pure solvent are separated by a semipermeable membrane, through which the solvent can pass freely, but the solute cannot, a part of the solvent passes into the solution compartment through the membrane.

The pressure difference produced between the two compartments concomitantly with the solvent migration through the membrane, is defined as the osmotic pressure Π (Pa). The osmotic pressure is a physical quantity depending on the total of the molecular species present, including neutral molecules and ions, and does not depend on the kind of solute. A solution property, such as osmotic pressure, freezing-point depression, boiling-point elevation etc., which depends not on the kind of solute, but on the total number of all molecular species, is called a colligative property of a solution.

The osmotic pressure of a polymer solution can be measured directly as the hydrostatic pressure difference between two compartments separated by a semipermeable membrane, such as a cellulose membrane. However, this is not applicable to a solution containing low molecular species, which can pass through a semipermeable membrane. Though the osmotic pressure of such a solution cannot be measured directly, the direction and extent of solvent migration through biological membranes can be predicted from the total number of all molecular species present when the solution is placed under physiological conditions. Other colligative properties of a solution such as freezing-point depression, boiling-point elevation, vapor-pressure depression, etc. can be directly obtained by observing changes of temperature and/or pressure, etc. These solution properties depend on the total number of ionic and neutral species in the solution in the same way as the osmotic pressure, and the molecular particle concentration is defined as the osmotic concentration. The osmotic concentration can be defined in two ways, one being mass-based concentration (osmolarity, mol/kg) and the other, volume-based concentration (osmolarity, mol/L). In practice, the latter is more convenient.

Unless otherwise specified, the freezing-point depression method is used for measuring the osmotic concentration. The method is based on the linear dependency of the freezing-point depression ΔT (°C) upon the osmolarity m (mol/kg), as expressed in the following equation,

$$\Delta T = K \cdot m$$

In this equation, K is the molal freezing-point depression constant, and it is known to be 1.86°C kg/mol for water. Since the constant K is defined on the basis of molarity, the molar osmotic concentration can be obtained from the above equation. In the dilute osmotic concentration range, osmolarity m (mol/kg) can be assumed to be numerically equal to osmolarity c (mol/L). Thus, the conventional osmolarity (mol/L) and the unit of osmole (Osm) are adopted in this test method. One Osm means that the Avogadro number (6.022×10^{23}/mol) of species is contained in 1 L of solution. Usually the osmotic concentration is expressed as the submultiple milliosmole (mOsm, mosmol/L) in the Pharmacopoeia.

Apparatus

Usually, the osmotic concentration of a solution can be obtained by measuring the extent of the freezing-point depression. The apparatus (osmometer) is composed of a sample cell for a fixed volume of sample solution and a cell holder, a cooling unit and bath with a temperature regulator, and a thermistor for detecting temperature.

Procedure

A fixed volume of the test solution is introduced into the sample cell, as indicated for the individual apparatus.

The apparatus must first be calibrated by the two-point calibration method by using osmolal standard solutions. For the calibration, select two different standard solutions just covering the expected osmolar concentration of a sample solution. Other than the indicated osmolal standard solutions in the *Table* below, water can also be used as a standard solution (0 mOsm) for measuring low osmolar sample solutions (0 – 100 mOsm). Next, after washing the sample cell and the thermistor as indicated for the individual apparatus, measure the degree of the freezing-point depression caused by a sample solution. Using the above-mentioned relation of osmolar concentration m and ΔT, the osmolarity of a sample solution can be obtained, and it is assumed to be numerically equal to the osmolarity.

In the case of higher osmolar solutions over 1000 mOsm, dilute the sample by adding distilled water and prepare n times diluted sample solution (1 in n). Measure the osmolarity of the diluted solution, as described above. In this case, it is necessary to state that the calculated osmolarity for the sample (see below) is an apparent osmolarity obtained by the dilution method. When the dilution method is applied, the dilution number should be selected so that the expected osmolarity is nearly equal to that of physiological saline solution.

In the case of solid samples, such as freeze-dried medicines, prepare a sample solution by dissolving the solid using the indicated solution for dissolution.

Suitability of the apparatus

After the calibration of the apparatus, a suitability test must be done by repeating the measurement of osmolarity for one of the standard solutions not less than 6 times. In performing the test, it is advisable that the osmolarity of a sample solution and the selected standard solution are similar to each other. In this test, the repeatability of measured values and the deviation of the average from the indicated value should be less than 2.0% and 3.0%, respectively.

Preparation of the osmolar standard solutions

Weigh exactly an amount indicated in the *Table* below of sodium chloride (standard reagent), previously dried between 500°C and 650°C for 40 to 50 minutes and allowed to cool in a desiccator (silica gel). Dissolve the weighed sodium chloride in exactly 100 g of water to make the corresponding osmolar standard solution.

Standard solution for osmometer calibration (milliosmoles)	Amount of sodium chloride (g)
100	0.309
200	0.626
300	0.946
400	1.270
500	1.593
700	2.238
1000	3.223

Osmolar ratio

In this test method the osmolar ratio is defined as the ratio of osmolarity of a sample solution to that of the isotonic sodium chloride solution. The ratio can be used as a measure of isotonicity of sample solution. Since the osmolarity of the isotonic sodium chloride solution (NaCl 0.900 g/100 mL) c_S (mOsm) is assumed to be constant (286 mOsm), the osmolar ratio of a sample solution, of which the osmolarity

is c_T (mOsm), can be calculated by means of the following equation,

$$\text{Osmolar ratio} = \frac{c_T}{c_S}$$

c_S: 286 mOsm

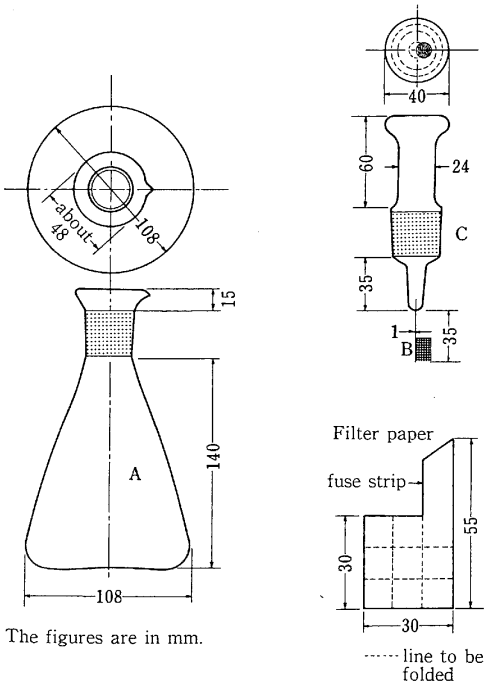
When the measurement is done by the dilution method, because the sample has an osmolarity over 1000 mOsm, the apparent osmolarity of the sample solution c_T can be calculated as $n \cdot c'_T = c_T$, in which n is the dilution number and c'_T is the measured osmolarity for the diluted solution. In this calculation, a linear relation between osmolarity and solute concentration is assumed. Thus when the dilution measurement is performed, the dilution number must be stated as (1 in n).

42. Oxygen Flask Combustion Method

The Oxygen Flask Combustion Method is a method for the identification or the determination of halogens or sulfur produced by combusting organic compounds, which contain chlorine, bromine, iodine, fluorine or sulfur, in a flask filled with oxygen.

Apparatus

Use the apparatus shown in the figure.



The figures are in mm.

A: Colorless, thick-walled (about 2 m), 500-mL hard glass flask, the upper part of which is made like a saucer. A flask made of quartz should be used for the determination of fluorine.

B: Platinum basket or cylinder made of platinum woven gauge. (It is hung at the end of the stopper C with platinum wire.)

C: Ground stopper made of hard glass. A stopper made of quartz should be used for the detemination of fluorine.

Preparation of test solution and blank solution

Unless otherwise specified, prepare them by the following method.

(1) Preparation of sample

(i) For solid samples: Place the quantity of the sample specified in the monograph on the center of the filter illustrated in the figure, weigh accurately, wrap the sample carefully along the dotted line without scattering, and place the parcel in a platinum basket or cylinder B, leaving its fuse-strip on the outside.

(ii) For liquid samples: Roll a suitable amount of absorbent cotton with filter paper, 50 mm in length and 5 mm in width, so that the end part of the paper is left to a length of about 20 mm as a fuse-strip, and place the parcel in a platinum basket or cylinder B. Place the sample in a suitable glass tube, weigh accurately, and moisten the cotton with the quantity of the sample specified in the monograph, bringing the edge of the sample in contact with the cotton.

(2) Method of combustion

Place the absorbing liquid specified in the monograph in flask A, fill it with oxygen, moisten the ground part of the stopper C with water, then ignite the fuse-strip, immediatey transfer it to the flask, and keep the flask airtight until the combustion is completed. Shake the flask occasionally until the white smoke in A vanishes completely, allow to stand for 15 to 30 minutes, and designate the resulting solution as the test solution. Prepare the blank solution in the same manner, without sample.

Procedure of determination

Unless otherwise specified in the monograph, perform the test as follows.

(1) Chlorine and bromine

Apply a small amount of water to the upper part of A, pull out C carefully, and transfer the test solution to a beaker. Wash C, B and the inner side of A with 15 mL of 2-propanol, and combine the washings with the test solution. To this solution add 1 drop of bromophenol blue TS, add dilute nitric acid dropwise until a yellow color develops, then add 25 mL of 2-propanol, and titrate with 0.005 mol/L silver nitrate VS according to the potentiometric titration under the Electrometric titration. Perform the test with the blank solution in the same manner, and make any necessary correction.

Each mL of 0.005 mol/L silver nitrate VS
= 0.17727 mg of Cl
Each mL of 0.005 mol/L silver nitrate VS
= 0.39952 mg of Br

(2) Iodine

Apply a small amount of water to the upper part of A, pull out C carefully, add 2 drops of hydrazine hydrate to the test solution, put C on A, and decolorize the solution by vigorous shaking. Transfer the content of A to a beaker, wash C, B and the inner side of A with 25 mL of 2-propanol, and transfer the washings to the above beaker. To this solution add 1 drop of bromophenol blue TS, then add dilute nitric acid dropwise until a yellow color develops, and titrate with 0.005 mol/L silver nitrate VS according to the Potentiometric tiration under the Electrometric Titration. Perform the test with the blank solution in the same manner, and make any necessary correction.