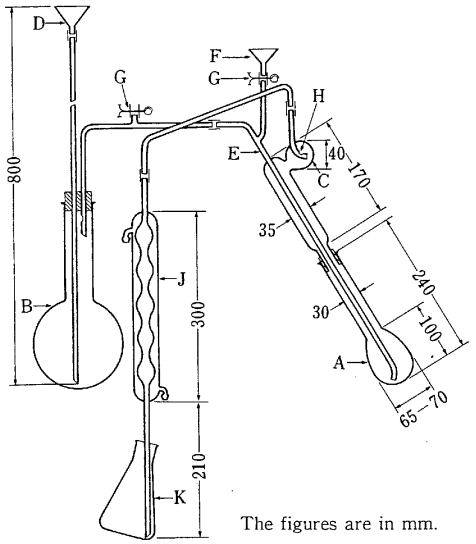


The figures are in mm.

A: Kjeldahl flask
B: Steam generator, containing water, to which 2 to 3 drops of sulfuric acid and fragments of boiling tips for preventing bumping have been added
C: Spray trap
D: Water supply funnel
E: Steam tube
F: Funnel for addition of alkali solution to flask A
G: Rubber tubing with a clamp
H: A small hole having a diameter approximately equal to that of the delivery tube
J: Condenser, the lower end of which is beveled
K: Absorption flask

Then, while shaking the flask, add cautiously 1 mL of hydrogen peroxide (30) drop by drop along the inside wall of the flask. Heat the flask gradually, then heat so strong that the vapor of sulfuric acid is condensed at the neck of the flask, until the solution changes through a blue and clear to a vivid green and clear, and the inside wall of the flask is free from a carbonaceous material. If necessary, add a small quantity of hydrogen peroxide (30) after cooling, and heat again. After cooling, add cautiously 20 mL of water, cool the solution, and connect the flask to the distillation apparatus washed beforehand by passing steam through it. To the absorption flask K add 15 mL of boric acid solution (1 in 25), 3 drops of bromocresol green-methyl red TS and sufficient water to immerse the lower end of the condenser tube J. Add 30 mL of sodium hydroxide solution (2 in 5) through the funnel F, rinse cautiously the funnel with 10 mL of water, immediately close the clamp attached to the rubber tubing G, then begin the distillation with steam, and continue until the distillate measures 80 to 100 mL. Remove the absorption flask from the lower end of the condenser tube J, rinsing the end part with a small quantity of water, and titrate the distillate with 0.005 mol/L sulfuric acid VS until the color of the solution changes from green through pale grayish blue to pale grayish red-purple. Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.005 mol/L sulfuric acid VS
= 0.14007 mg of N



# 39. Nuclear Magnetic Resonance Spectroscopy ($^1$H)

Nuclear magnetic resonance (NMR) spectroscopy is based on the phenomenon that specific radio frequency radiations are absorbed by magnetic nuclei in a sample placed in a magnetic field. These nuclei have intrinsic spin angular momentum, of which magnitude is given by $I(I+1)/h/2\pi$, where $I$ is the spin quantum number and is integral or half-integral ($I = 1/2$ for $^1$H). When the magnetic nuclei are placed in a magnetic field, they are oriented similarly to a bar magnet in $2I+1$ possible orientations corresponding to $2I+1$ energy levels equally spaced (two energy levels for $^1$H). The transition between two successive quantized energy levels corresponding to the adjacent orientations can be induced by electromagnetic radiation with a suitable frequency. The precise relation between the field strength and the resonant frequency $\upsilon$ is given by

$$\upsilon = \gamma \cdot \frac{H_0}{2\pi}$$

where $H_0$ is the strength of the applied external magnetic field and $\gamma$ is the gyromagnetic ratio, a constant characterizing a particular isotope. The absorption of radiation (NMR signal) can occur only when the irradiating radio frequency satisfies the resonance condition. Since this absorption coefficient (the transition probability) does not depend on the environments where the nuclei are located, the intensity of an absorption line is proportional to the number of nuclei involving in the absorption. The excess spins shifted to the higher energy levels by the transition process return to the thermal equilibrium state at various rates determined by characteristic time constants (known as relaxation time).

A nucleus is shielded from the applied magnetic field by the electrons belonging to its own atom and to the molecule. Therefore nuclei in different environments are shielded to different extents and resonate at different frequencies. The difference in resonance frequencies is defined as chemical shift ($\delta$), which is given by

$$\delta \text{ ppm} = \frac{\upsilon_S - \upsilon_R}{\omega} \times 10^6,$$

where,

$\omega$: The oscillator frequency in MHz (60 MHz, 100 MHz, etc.),
$\upsilon_S$: The resonance frequency of the observed signal,
$\upsilon_R$: The resonance frequency of the reference signal.

The chemical shifts are normally expressed in ppm, a dimensionless unit, by assuming the chemical shift of the reference compound as 0 ppm.

In addition to the shielding due to electrons, the nucleus is subjected to effects due to the spin orientations of other magnetic nuclei, resulting in an additional splitting of the signal. The spacing between two adjacent components of the signal is known as the spin-spin coupling constant ($J$). Coupling constants are measured in Herz and independent of the strength of the external magnetic field. The increased number of interacting nuclei will make the multiplet pattern more complex.

From the NMR spectrum the following four parameters

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 $\alpha_x^t$ 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).

$\alpha$: 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.