2. Methods of analysis

2.2.5. RELATIVE DENSITY

The relative density $d_{t_2}^{t_1}$ of a substance is the ratio of the mass of a certain volume of a substance at temperature t_1 to the mass of an equal volume of water at temperature t_2.

Unless otherwise indicated, the relative density d_{20}^{20} is used. Relative density is also commonly expressed as d_4^{20}. Density ρ_{20}, defined as the mass of a unit volume of the substance at 20 °C may also be used, expressed in kilograms per cubic metre or grams per cubic centimetre ($1\,\text{kg}\cdot\text{m}^{-3} = 10^{-3}\,\text{g}\cdot\text{cm}^{-3}$). These quantities are related by the following equations where density is expressed in grams per cubic centimetre:

$$\rho_{20} = 0.998203 \times d_{20}^{20} \ \text{ or } \ d_{20}^{20} = 1.00180 \times \rho_{20}$$

$$\rho_{20} = 0.999972 \times d_4^{20} \ \text{ or } \ d_4^{20} = 1.00003 \times \rho_{20}$$

$$d_4^{20} = 0.998230 \times d_{20}^{20}$$

Relative density or density is measured according to the number of decimals prescribed in the monograph using a density bottle (solids or liquids), a hydrostatic balance (solids), a hydrometer (liquids) or a digital density meter with an oscillating transducer (liquids and gases). When the determination is made by weighing, the buoyancy of air is disregarded, which may introduce an error of 1 unit in the 3rd decimal place. When using a density meter, the buoyancy of air has no influence.

Oscillating transducer density meter. The apparatus consists of:

– a U-shaped tube, usually of borosilicate glass, which contains the liquid to be examined;

– a magneto-electrical or piezo-electrical excitation system that causes the tube to oscillate as a cantilever oscillator at a characteristic frequency depending on the density of the liquid to be examined;

– a means of measuring the oscillation period (T), which may be converted by the apparatus to give a direct reading of density, or used to calculate density using the constants A and B described below.

The resonant frequency (f) is a function of the spring constant (c) and the mass (m) of the system:

$$f^2 = \frac{1}{T^2} = \frac{c}{m} \times \frac{1}{4\pi^2}$$

Hence:

$$T^2 = \left(\frac{M}{c} + \frac{\rho \times V}{c} \right) \times 4\pi^2$$

M = mass of the tube;

V = inner volume of the tube.

Introduction of 2 constants $A = c/(4\pi^2 \times V)$ and $B = M/V$, leads to the classical equation for the oscillating transducer:

$$\rho = A \times T^2 - B$$

The constants A and B are determined by operating the instrument with the U-tube filled with 2 different samples of known density, for example, degassed *water R* and air. Control measurements are made daily using degassed *water R*. The results displayed for the control measurement using degassed *water R* shall not deviate from the reference value ($\rho_{20} = 0.998203\,\text{g}\cdot\text{cm}^{-3}$, $d_{20}^{20} = 1.000000$) by more than its specified error. For example, an instrument specified to $\pm\,0.0001\,\text{g}\cdot\text{cm}^{-3}$ shall display $0.9982 \pm 0.0001\,\text{g}\cdot\text{cm}^{-3}$ in

order to be suitable for further measurement. Otherwise a re-adjustment is necessary. Calibration with certified reference materials is carried out regularly. Measurements are made using the same procedure as for calibration. The liquid to be examined is equilibrated in a thermostat at 20 °C before introduction into the tube, if necessary, to avoid the formation of bubbles and to reduce the time required for measurement.

Factors affecting accuracy include:

– temperature uniformity throughout the tube;

– non-linearity over a range of density;

– parasitic resonant effects;

– viscosity, whereby solutions with a higher viscosity than the calibrant have a density that is apparently higher than the true value.

The effects of non-linearity and viscosity may be avoided by using calibrants that have density and viscosity close to those of the liquid to be examined ($\pm\,5$ per cent for density, $\pm\,50$ per cent for viscosity). The density meter may have functions for automatic viscosity correction and for correction of errors arising from temperature changes and non-linearity.

Precision is a function of the repeatability and stability of the oscillator frequency, which is dependent on the stability of the volume, mass and spring constant of the cell.

Density meters are able to achieve measurements with an error of the order of $1 \times 10^{-3}\,\text{g}\cdot\text{cm}^{-3}$ to $1 \times 10^{-5}\,\text{g}\cdot\text{cm}^{-3}$ and a repeatability of $1 \times 10^{-4}\,\text{g}\cdot\text{cm}^{-3}$ to $1 \times 10^{-6}\,\text{g}\cdot\text{cm}^{-3}$.

2.2.6. REFRACTIVE INDEX

The refractive index of a medium with reference to air is equal to the ratio of the sine of the angle of incidence of a beam of light in air to the sine of the angle of refraction of the refracted beam in the given medium.

Unless otherwise prescribed, the refractive index is measured at 20 $\pm$ 0.5 °C, with reference to the wavelength of the D-line of sodium (λ = 589.3 nm); the symbol is then n_D^{20}.

Refractometers normally determine the critical angle. In such apparatus the essential part is a prism of known refractive index in contact with the liquid to be examined.

Calibrate the apparatus using certified reference materials.

When white light is used, the refractometer is provided with a compensating system. The apparatus gives readings accurate to at least the third decimal place and is provided with a means of operation at the temperature prescribed. The thermometer is graduated at intervals of 0.5 °C or less.

2.2.7. OPTICAL ROTATION

PRINCIPLE

Optical rotation (also known as optical activity) is the property displayed by chiral substances of rotating the plane of polarisation of linearly polarised light.

Optical rotation is considered to be positive (+) for dextrorotatory substances (i.e. those that rotate the plane of polarisation in a clockwise direction when viewed in the direction facing the oncoming light beam) and negative (−) for laevorotatory substances (i.e. anticlockwise rotation).

The angle of optical rotation α of a liquid is the angle of rotation of the plane of polarisation, expressed in degrees (°), at the wavelength of the D-line of sodium (λ = 589.3 nm) measured at 20 °C through the liquid when using a path length of 1.00 dm.

The specific optical rotation $[\alpha]_D^{20}$ of a substance in solution is calculated from the angle of optical rotation, as defined above, with reference to a path length of 1.00 dm and a concentration of the substance to be examined of 1 g/mL. The specific optical rotation of a substance in solution is always expressed with reference to a given solvent and concentration.

As some equipment may not use sodium lamps, the wavelength of measurement is given as 589 nm instead of 589.3 nm.

In certain cases specified in the monograph, the angle of optical rotation is measured at other temperatures, other wavelengths and/or in cells with a path length other than 1.00 dm.

In the conventional system adopted by the Pharmacopoeia, the specific optical rotation is expressed by its value without units; the actual units, degree millilitres per decimetre gram $[(°)\cdot ml\cdot dm^{-1}\cdot g^{-1}]$ are understood.

EQUIPMENT

The polarimeter typically consists of:

– a light source, for example a sodium discharge lamp, a light-emitting diode (LED) or another light source capable of providing radiation at the desired wavelength (589 nm unless otherwise prescribed in the monograph); if the light source is polychromatic, a means of isolating the required wavelength is necessary, e.g. an optical filter;

– a polariser and an analyser;

– a sample cell with a path length of 1.00 dm, unless otherwise specified in the monograph;

– a detection system to measure the angle of optical rotation, which must be capable of giving readings to at least the nearest 0.01°, unless otherwise specified in the monograph;

– a temperature control system that indicates the temperature with a readability of 0.1 °C; it may be embedded in the polarimeter (e.g. a Peltier system) or be an external unit (e.g. a cycle-cryostat), and must be able to maintain the temperature of the liquid to within ± 0.5 °C of that prescribed.

EQUIPMENT PERFORMANCE

The accuracy of the scale is checked near the value to be measured or over an appropriate range, usually by means of certified quartz plates. Other certified reference materials may also be suitable (e.g. sucrose solutions).

Optical rotation measurements may be used to quantify the amount of an enantiomer or the ratio of enantiomers present in a sample. For that purpose, the linearity must be checked, for example using sucrose solutions.

PROCEDURE

Determine the zero of the polarimeter and the angle of rotation of the liquid at a wavelength of 589 nm and a temperature of 20 ± 0.5 °C, unless otherwise prescribed. The zero of the polarimeter is determined with the sample cell closed.

For neat liquids, the zero is determined with an empty sample cell.

For solutions, the zero is determined with the sample cell filled with the same solvent as that used for the solution to be examined and measured at the same temperature. The sample preparation procedure is prescribed in the monograph.

Calculate the specific optical rotation at temperature t and wavelength λ using the following formulae.

For neat liquids, the density of the liquid is taken into account:

$$[\alpha]_\lambda^t = \frac{\alpha}{l \cdot \rho_t}$$

For solutions:

$$[\alpha]_\lambda^t = \frac{1000\alpha}{l \cdot c}$$

α = angle of rotation measured at temperature t and wavelength λ, in degrees;

l = path length of the polarimeter sample cell, in decimetres;

ρ_t = density determined at the temperature of measurement t, in grams per cubic centimetre; for the purposes of the Pharmacopoeia, density is replaced by relative density (2.2.5);

c = concentration of the solution, in grams per litre.

When the limits for optical rotation or specific optical rotation are expressed as the dried substance, the anhydrous substance or the solvent-free substance, the result must be corrected for loss on drying (2.2.32), water content (2.5.12 or 2.5.32) or content of solvent as appropriate.

2.2.8. VISCOSITY

The *dynamic* viscosity or *viscosity coefficient* η is the tangential force per unit surface, known as *shearing stress* τ and expressed in pascals, necessary to move, parallel to the sliding plane, a layer of liquid of 1 square metre at a rate (v) of 1 metre per second relative to a parallel layer at a distance (x) of 1 metre.

The ratio dv/dx is a speed gradient giving the *rate of shear D* expressed in reciprocal seconds (s^{-1}), so that $\eta = \tau/D$.

The unit of dynamic viscosity is the pascal second (Pa·s). The most commonly used submultiple is the millipascal second (mPa·s).

The *kinematic viscosity* v, expressed in square metres per second, is obtained by dividing the dynamic viscosity η by the density ρ expressed in kilograms per cubic metre, of the liquid measured at the same temperature, i.e. $v = \eta/\rho$. The kinematic viscosity is usually expressed in square millimetres per second.

A capillary viscometer may be used for determining the viscosity of Newtonian liquids and a rotating viscometer for determining the viscosity of Newtonian and non-Newtonian liquids. Other viscometers may be used provided that the accuracy and precision are at least as satisfactory as those obtained with the viscometers described in the related chapters.