the same wavelength using several optical filters for calibration of transmission rate with different transmission rates.

Procedure

After adjusting the apparatus as directed in the Apparatus and adjustment, select and set the light source, detector, mode of measurement, measuring wavelength or wavelength range, spectrum width and scanning speed.

Subsequently, allow the apparatus to stand for a certain time to confirm its stability. Then, usually adjust the apparatus so that the transmittance is 0% at measuring wavelength or over measuring wavelength range after shutting the sample side of light path. Then open the shutter and adjust the transmittance to 100% (the absorbance is zero). Adjusting the transmittance to 100% is usually done by putting cells containing the control solution in both light paths. For the control solution, unless otherwise specified, blank solvent is used.

Then perform the measurement with the cell containing the sample solution, and read the absorbance at measuring wavelength, or measure the spectrum over measuring wavelength range. Unless otherwise specified, a cell with a path length of 1 cm, made of quartz for ultraviolet range and of quartz or glass for visible range, is used. Special consideration is needed with the absorption of solvents in the ultraviolet range; use a solvent which does not disturb accurate measurement.

Specific absorbance

In the Japanese Pharmacopoeia, the absorbance, calculated on the basis that l is 1 cm and c (concentration of a medicament) is 1 w/v%, is called specific absorbance, and is expressed as $E^{1\%}_{1cm}$.

$$E^{1\%}_{1cm} = \frac{A}{c \times l}$$

l: Length of the layer of the solution (cm)
A: Absorbance value
c: Concentration of the sample in the solution (w/v%)

The description of, for example, "$E^{1\%}_{1cm}$ (241 nm): 500 – 530 (after drying, 2 mg, methanol, 200 mL)" in the monograph, indicates that observed $E^{1\%}_{1cm}$ value is between 500 and 530, when the test is performed in the following manner: The sample is dried under the conditions specified in the Test for Loss on Drying, and about 2 mg of the sample is weighed accurately with a microbalance, and dissolved in methanol to make exactly 200 mL, then the absorbance of the solution is measured as directed in the Procedure at a wavelength of 241 nm using a cell with a path length of 1 cm.

Identification

Prepare the sample solution as directed in the monograph, and test as directed in the Procedure. Usually, the test is performed by a single method or in a combination of a few methods in the following methods using the absorbance or absorption spectrum obtained from the sample solution. Subtle differences in the absorption spectrum arising from differences in the apparatus used may be neglected.

(1) Identification using Reference Spectrum

When the absorption spectrum obtained from the sample solution exhibits similar intensities of absorption at the same wavelengths as those of the Reference Spectrum, the identity of the sample and the reference may be confirmed. In this case, the range of the wavelength to be compared is the range shown on the Reference Spectrum.

Reference spectrum: Reference spectra are specified under the Ultraviolet-visual Reference Spectra, which are used as the reference for the test of identification specified in the monograph.

(2) Identification using Reference Standard

When the absorption spectrum obtained from the sample solution exhibits similar intensities of absorption at the same wavelengths as those of the spectrum obtained from the Reference Standard, the identity of the sample and the reference may be confirmed. In this case, the range of the wavelength to be compared is the range shown on the Reference Spectrum. When the relevant Reference Spectrum is not available, the range is that specified in the monograph.

(3) Identification using absorption wavelength

When maximum absorption wavelengths of the spectrum obtained from the sample solution match the wavelengths specified in the monograph, the identity of the substance may be confirmed. In this case, the range of the wavelength to be compared is the range shown on the Reference Spectrum.

(4) Identification using the ratio of the absorbances obtained at two or more wavelengths

When the ratios of absorbances at the specified wavelengths in the spectrum obtained from the sample solution meet the specifications in the monograph, the identity of the substance may be confirmed.

Assay

Prepare the control solution, the sample solution and the standard solution as directed in the monograph, measure the absorbances of the sample solution and the standard solution according to the method described in the Procedure, and determine the amount of the substance to be assayed in the sample by comparing the absorbances.

65. Viscosity Determination

Viscosity determination is a method to determine the viscosity of liquid samples using a viscometer.

When a liquid moves in a definite direction, and the liquid velocity has a gradient with respect to the direction rectangular to that of flow, a force of internal friction is generated along both sides of a hypothetical plane parallel to the movement. This flow property of a liquid is expressed in terms of viscosity. The internal friction per unit area on the parallel plane is called slip stress or shear stress, and the velocity gradient with respect to the direction rectangular to that of flow is called slip velocity or shear velocity. A liquid of which the slip velocity is proportional to its slip stress is called a Newtonian liquid. The proportionality constant, η, is a characteristic of a liquid at a certain temperature and is called viscosity. The viscosity is expressed in the unit of Pascal second (Pa·s), and usually milli-Pascal second (mPa·s).

A liquid whose slip velocity is not proportional to its slip stress is called a non-Newtonian liquid. Since the viscosity for a sample of a non-Newtonian liquid changes with its slip velocity, the viscosity measured at a certain slip velocity is called an apparent viscosity. In that case, the value of slip stress divided by the corresponding slip velocity is called an

apparent viscosity. Thus, the relationship between apparent viscosity and slip velocity will permit characterization of the flow properties of a given non-Newtonian liquid.

The value of the viscosity, η, divided by the density, ρ, at the same temperature is defined as a kinematic viscosity, ν, which is expressed in the unit of meters squared per second (m^2/s), and usually millimeters squared per second (mm^2/s).

The viscosity of a liquid is determined either by the following *Method I* or *Method II*.

Method I Viscosity measurement by capillary tube viscometer

For measuring the viscosity of a Newtonian liquid, a capillary tube viscometer is usually used, in which the downflowing time of a liquid, t(s), required for a definite volume of the liquid to flow through a capillary tube is measured and the kinematic viscosity, ν, is calculated according to the following equation.

$$\nu = Kt$$

Further, the viscosity, η, is calculated from the next equation:

$$\eta = \nu\rho = Kt\rho$$

where ρ (g/mL) is the density of the liquid measured at the same temperature, t (°C).

The parameter K (mm^2/s^2) represents the viscometer constant and is previously determined by using the *Standard Liquids for Calibrating Viscometers* with known kinematic viscosity. In the case of a liquid having a similar viscosity to water, water itself can be used as a reference standard liquid for the calibration. The kinematic viscosity of water is 1.0038 mm^2/s at 20°C. In the cases of liquids having a slightly higher viscosity than water, the *Standard Liquids for Calibrating Viscometers* should be used for the calibration.

The intrinsic viscosity, $[\eta]$ (dL/g), of a polymer solution is obtained by plotting the relation of viscosity versus concentration and extrapolating the obtained straight line to zero concentration. Intrinsic viscosity shows the degree of molecular expansion of a polymer substance in a given solvent (sample solution) and is also a measure of the average molecular mass of the polymer substance.

The downflowing time t (s) for a polymer solution, whose concentration is c (g/dL), and t_0 (s) for the solvent used for dissolving the polymer, are measured by using the same viscometer, and then the intrinsic viscosity of a given polymer substance, $[\eta]$, is calculated according to the following equation:

$$[\eta] = \lim_{c\to 0} \frac{\left(\frac{t}{t_0}\right) - 1}{c} \quad \text{or} \quad [\eta] = \lim_{c\to 0} \frac{\ln \frac{t}{t_0}}{c}$$

When the concentration dependency of $\{(t/t_0) - 1\}/c$ is not large, the value of $\{(t/t_0) - 1\}/c$ at a concentration directed in the respective monograph can be assumed to be the intrinsic viscosity for a given substance.

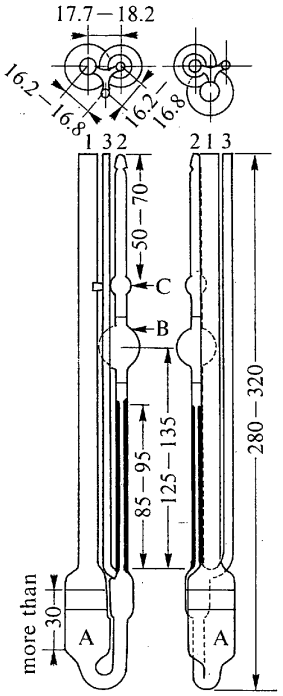
Unless otherwise specified, the viscosity of a sample solution is measured with the following apparatus and procedure.

Apparatus

For measurement of the kinematic viscosity in the range of 1 to 100,000 mm^2/s, the Ubbelohde-type viscometer illustrated in *Fig. 1* can be used. The approximate relations between kinematic viscosity range and inside diameter of the capillary tube suitable for the measurement of various liquids with different viscosity, are given in the attached *Table*. Although a capillary tube viscometer other than the Ubbelohde-type one specified in the *Table* can also be used, a viscometer should be selected in which the downflowing time, t (s), of a sample solution to be determined would be between 200 s and 1000 s.

Table Specifications of the Ubbelohde-type viscometer

Viscometer constant K (mm^2/s^2)	Inner diameter of capillary tube (mm) Permissible tolerance ± 10%	Volume of bulb B (mL) Permissible tolerance ± 10%	Measuring range of kinematic viscosity (mm^2/s)
0.005	0.46	3.0	1– 5
0.01	0.58	4.0	2 – 10
0.03	0.73	4.0	6 – 30
0.05	0.88	4.0	10 – 50
0.1	1.03	4.0	20 – 100
0.3	1.36	4.0	60 – 300
0.5	1.55	4.0	100 – 500
1.0	1.83	4.0	200 – 1000
3.0	2.43	4.0	600 – 3000
5.0	2.75	4.0	1000 – 5000
10.0	3.27	4.0	2000 – 10,000
30.0	4.32	4.0	6000 – 30,000
50.0	5.20	5.0	10,000 – 50,000
100	6.25	5.0	20,000 – 100,000



The figure are in mm.

Fig. 1 Ubbelohde-type capillary tube viscometer

Procedure

Place a sample solution in a viscometer from the upper end of *tube 1*, so that the meniscus of the solution is at a level between the two marked lines of *bulb A*. Place the viscometer vertically in a thermostatted bath maintained at a specified temperature within 0.1°C, until *bulb C* is fully immersed, and let it stand for about 20 minutes to attain the specified temperature. Close *tube 3* with a finger and pull the sample solution up to the middle part of *bulb C* by gentle suction from the top of *tube 2*, taking care not to introduce any bubbles into *tube 2*, and stop the suction. Open the end of *tube 3*, and immediately close the end of *tube 2*. After confirming that the liquid column is cut off at the lowest end of the capillary tube, open the end of *tube 2* to make the sample solution flow down through the capillary tube. Record the time, t (s), required for the meniscus of the sample solution to fall from the upper to the lower marked line of *bulb B*.

Determine the viscometer constant K previously, using the *Standard Liquids for Calibrating Viscometers* under the same conditions. The temperature at which the calibration is conducted must be identical with that specified in the monograph.

Method II Viscosity measurement by rotational viscometer

A rotational viscometer is usually used for measuring the viscosity of Newtonian or non-Newtonian liquids. The measuring principle of a rotational viscometer generally consists in the detection and determination of the force acting on a rotor (torque), when it rotates at a constant angular velocity in a liquid. The extent of torque generated by the rotation can be detected in terms of the torsion of a spring and the liquid viscosity is calculated from the scale-indicated value corresponding to the degree of torsion.

The viscosity of a sample solution is measured with the following apparatus and procedure.

Apparatus

Viscosity measurement is performed by using any one of the following three types of rotational viscometers.

(1) Coaxial double cylinder-type rotational viscometer (Couette type viscometer)

In the coaxial double cylinder-type rotational viscometer, viscosity is determined by placing a liquid in the gap between the inner and the outer cylinders, which share the same central axis and rotate separately, and the generated torque acting on one cylinder surface when the other cylinder is rotated, and the corresponding angular velocity, are measured.

As shown in *Fig. 2a*, the inner cylinder is hung by a wire whose twist constant is designated as k. In *Fig. 2a*, half the outer diameter of the inner cylinder and inner diameter of the outer cylinder are designated as R_i and R_o, respectively, and the length of the inner cylinder immersed in a liquid is designated as l. When a liquid is introduced into the gap between the two cylinders, and the outer cylinder is made to rotate at a constant angular velocity, ω, the inner cylinder is also forced to rotate due to the viscosity of the liquid. Consequently, torque, T, is generated by the forced rotation in a viscous liquid, and in the steady state the torque is balanced by the torsion of the wire, as indicated by the degree of rotation θ. Then, the relationship can be expressed by $T = k\theta$ and the viscosity of a liquid, η, is determined from the following equation by measuring the relationship between ω and θ. Conversely, viscosity measurement can also be performed by rotating the inner cylinder, and the same relationship holds.

$$\eta = \frac{100T}{4\pi l\omega}\left[\frac{1}{R_i^2} - \frac{1}{R_o^2}\right]$$

where, η: viscosity of a liquid (mPa·s)
π: circumference/diameter ratio
l: length of the inner cylinder (cm)
ω: angular velocity (rad/s)
T: torque acting on cylinder surface (10^{-7} N·m)
R_i: 1/2 of outer diameter of the inner cylinder (cm)
R_o: 1/2 of inner diameter of the outer cylinder (cm)

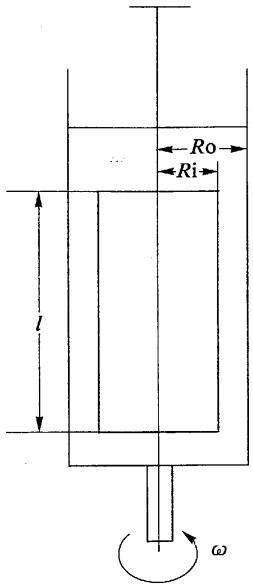


Fig. 2a Coaxial double cylinder-type rotational viscometer

(2) Single cylinder-type rotational viscometer (Brookfield type viscometer)

In the single cylinder-type rotational viscometer, viscosity is determined by measuring the torque acting on the cylinder surface when the cylinder immersed in a liquid is rotated at a given angular velocity. Use an apparatus of the type illustrated in *Fig. 2b*. If the apparatus constant K_B is previously determined experimentally by using the *Standard Liquids for Calibrating Viscometers*, the viscosity of a liquid, η, can be obtained from the following equation.

$$\eta = K_B\frac{T}{\omega}$$

where, η: viscosity of a liquid (mPa·s)
K_B: apparatus constant of viscometer (rad/cm^3)
ω: angular velocity (rad/s)
T: torque acting on cylinder surface (10^{-7} N·m)

(3) Cone-flat plate-type rotational viscometer (Cone-plate type viscometer)

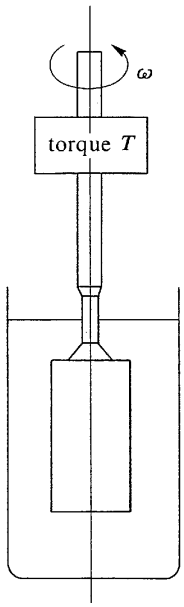


Fig. 2b Single cylinder-type rotational viscometer

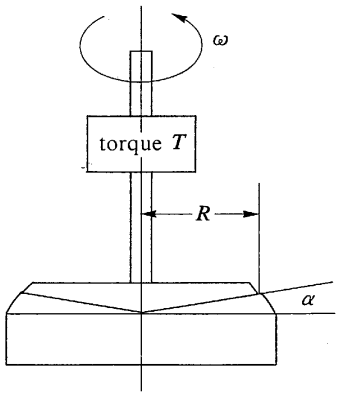


Fig. 2c Cone-flat plate-type rotational viscometer

In the cone-flat plate-type rotational viscometer, viscosity is determined by placing a liquid in the gap between a flat disc and a cone with a large vertical angle sharing the same rotational axis, and the torque and the corresponding angular velocity are measured, when either the disc or the cone is rotated in a viscous liquid.

As shown in *Fig. 2c*, a liquid is introduced to fill the gap between a flat disc and a cone forming an angle α(rad). When either the flat disc or the cone is rotated at a constant angular velocity or a constant torque, the torque acting on the disc or cone surface rotated by the viscous flow and the corresponding angular velocity in the steady state, are measured. The viscosity of the liquid, η, can be calculated from the following equation.

$$\eta = 100 \times \frac{3\alpha}{2\pi R^3} \cdot \frac{T}{\omega}$$

where, η: viscosity of a liquid (mPa·s)
π: circumference/diameter ratio
R: radius of cone (cm)
α: angle between flat disc and cone (rad)
ω: angular velocity (rad/s)
T: torque acting on flat disc or cone surface (10^{-7} N·m)

Procedure

Set up the viscometer so that its rotational axis is perpendicular to the horizontal plane. Place a sufficient quantity of a sample solution in the viscometer, and allow the measuring system to stand until a specified temperature is attained, as directed in the monograph. Where it is desired to measure the viscosity within a precision of 1%, measuring temperature should be controlled within 0.1°C. Next, after confirming that the sample solution is at the designated temperature, start operating the rotational viscometer. After the forced rotation induced by the viscous flow has reached a steady state and the indicated value on the scale, which corresponds to the rotational frequency or the torque, has become constant, read the value on the scale. Then, calculate the viscosity η by using the respective equation appropriate to the type of viscometer being used. Determination or confirmation of the apparatus constant should be conducted beforehand by using the *Standard Liquids for Calibrating Viscometers*, and the validation of the apparatus and operating procedure should also be performed by using those standard liquids.

In the case of a non-Newtonian liquid, repeat the procedure for measuring the viscosity of the liquid with variation of the rotation velocity or torque from one measurement to another. From a series of such viscosity measurements, the relationship between the slip velocity and the slip stress of a non-Newtonian liquid, *i.e.*, the flow characteristics of a non-Newtonian liquid, can be obtained.

Calibration of a rotational viscometer is conducted by using water and the *Standard Liquids for Calibrating Viscometers*. These standard liquids are used for the determination or confirmation of the apparatus constant of the rotational viscometer. They are also used for periodic recalibration of the viscometer to confirm maintenance of a specified precision.

66. Vitamin A Assay

The Vitamin A Assay is a method to determine vitamin A by ultraviolet absorption spectrophotometry in Retinol Acetate, Retinol Palmitate, Vitamin A Oil, Cod Liver Oil and other preparations. However, proper pretreatments are generally necessary depending on the kind of preparations or on the existence of substances which disturb the assay.

One Vitamin A Unit (equal to 1 vitamin A I.U.) is equivalent to 0.3 μg of vitamin A (all-trans vitamin A alcohol).

Reagents

2-propanol and diethyl ether used in the assay meet the following requirements.

2-propanol: Read the absorbance as directed under the Ultraviolet-visible Spectrophotometry, using water as the blank: the absorbance at 300 nm is not more than 0.05, and the absorbance between 320 nm and 350 nm is not more than 0.01. If necessary, it should be purified by distillation.

Diethyl ether: Freshly distil, discarding the first and last 10% portions.

Procedure

All procedures should be carried out quickly, care must be taken as far as possible to avoid exposure to air and to oxidizing agents, and light-resistant containers are used.

Unless otherwise specified in the monograph, proceed by Method 1, but apply Method 2 when the assay conditions required for Method 1 are not available.

(1) Method 1

Weigh accurately about 0.5 g of the sample, and dissolve in 2-propanol to make exactly 250 mL. Dilute this solution with 2-propanol to make a solution having an absorbance of about 0.5 at 326 nm when it is determined as directed under the Ultraviolet-visible Spectrophotometry, and use this solution as the sample solution. Determine the wavelength of maximum absorption and the absorbance at 300 nm, 310