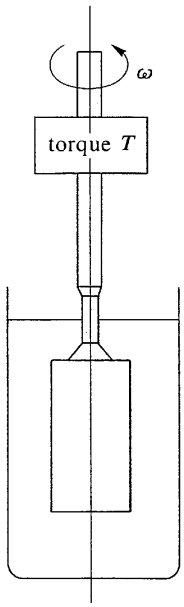

Fig. 2b Single cylinder-type rotational viscometer

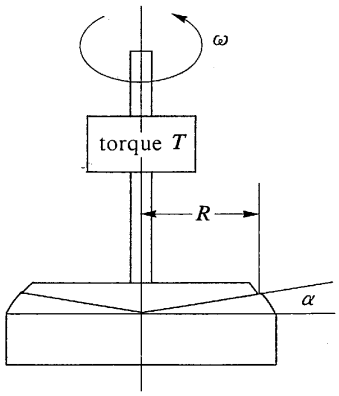

Fig. 2c Cone-flat plate-type rotational viscometer

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

nm, 320 nm, 326 nm, 330 nm, 340 nm and 350 nm, and calculate the relative extinction of each wavelength, taking the absorbance at 326 nm as 1.000.

When the wavelength of maximum absorption lies between 325 nm and 328 nm, and the relative extinction of each wavelength is within the range ±0.030 of the values in the Table, the potency of vitamin A in Unit per g of sample is calculated from the absorbance A at 326 nm.

$$\text{Units of vitamin A in 1 g} = E^{1\%}_{1\text{cm}}(326\text{ nm}) \times 1900$$

$$E^{1\%}_{1\text{cm}}(326\text{ nm}) = \frac{A}{W} \times \frac{V}{100}$$

V: Total mL of sample solution.
W: Amount (g) of sample in V mL of sample solution.

Perform the following test to identify retinol acetate and retinol palmitate.

Identification: Dissolve amounts or volumes, equivalent to 15,000 vitamin A Units, of the sample, Retinol Acetate Reference Standard for thin-layer chromatography and Retinol Palmitate Reference Standard for thin-layer chromatography in 5 mL portions of petroleum ether separately, and use these solutions as the sample solution and the standard solutions. Perform the test as directed under the Thin-layer Chromatography. Spot 5 μL each of the sample solution and the standard solutions on a plate of silica gel for thin-layer chromatography, develop it to a distance of about 10 cm with a mixture of cyclohexane and diethyl ether (12:1) as the solvent, and air-dry the plate. Spray antimony (III) chloride TS on the plate, and identify by comparing the position of the principal blue-colored spots of the sample and the standards.

When the wavelength of maximum absorption does not lie between 325 nm and 328 nm, or when the relative extinction is not within the range ±0.030 of values of the Table, proceed with the test by Method 2.

λ (nm)	Retinol acetate	Retinol palmitate
300	0.578	0.590
310	0.815	0.825
320	0.948	0.950
326	1.000	1.000
330	0.972	0.981
340	0.786	0.795
350	0.523	0.527

(2) Method 2

Unless otherwise specified, weigh accurately a sample containing not less than 500 Units of vitamin A, and not more than 1 g of fat, transfer to a flask, and add 30 mL of aldehyde-free ethanol and 1 mL of a solution of pyrogallol in ethanol (95) (1 in 10). Then add 3 mL of potassium hydroxide solution (9 in 10), attach a reflux condenser, and heat on a water bath for 30 minutes to saponify. Cool quickly to ordinary temperature, add 30 mL of water, transfer to separator A, wash the flask with 10 mL of water and 40 mL of diethyl ether, transfer the washings to separator A, shake well, and allow to stand. Transfer the water layer in separator A to separator B, wash the flask with 30 mL of diethyl ether, transfer the washing to separator B, and extract by shaking. Transfer the water layer to a flask, add the diethyl ether layer to separator A, transfer the water layer to separator B, add 30 mL of diethyl ether, and extract by shaking. Add the diethyl ether layer to separator A. Add 10 mL of water, allow to stand calmly after gentle turning upside down 2 to 3 times, and remove the water layer. Further wash with three 50-mL portions of water, and shake with increasing vigorousness as the washing proceeds. Further wash with 50-mL portions of water until the washing gives no pink color with phenolphthalein TS, and allow to stand for 10 minutes. Remove remaining water as far as possible, transfer the diethyl ether solution to an Erlenmeyer flask, wash the separator with two 10-mL portions of diethyl ether, add the washings to the flask, and add 5 g of anhydrous sodium sulfate to the diethyl ether solution, mix by shaking, and transfer the dried diethyl ether solution to a round-bottom flask by decantation. Wash the remaining sodium sulfate with two or more 10-mL portions of diethyl ether, and transfer the washings to the flask.

Evaporate the diethyl ether solution in a water bath at 45°C with swirling of the flask, using an aspirator, to about 1 mL, immediately add sufficient 2-propanol to make a solution containing 6 to 10 vitamin A Units per mL, and designate the solution as the sample solution. Read the absorbances, A_1 at 310 nm, A_2 at 325 nm, and A_3 at 334 nm with the sample solution as directed under the Ultraviolet-visible Spectrophotometry.

$$\text{Units of vitamin A in 1 g of sample} = E^{1\%}_{1\text{ cm}}(325\text{ nm}) \times 1830$$

$$E^{1\%}_{1\text{ cm}}(325\text{ nm}) = \frac{A_2}{W} \times \frac{V}{100} \times f$$

$$f = 6.815 - 2.555 \times \frac{A_1}{A_2} - 4.260 \times \frac{A_3}{A_2}$$

f: Correction factor
V: Total mL of sample solution
W: Amount (g) of sample in V mL of sample solution

67. Volatile Contaminants in Ethanol

The Volatile Contaminants in Ethanol is a method to determine the amount of methanol, acetaldehyde, acetone, 2-propanol, 1-propanol or *t*-butanol which are contained in ethanol using the Gas Chromatography.

Procedure

Take exactly 25 mL of the sample, add exactly 3 mL of the internal standard solution, and use this solution as the sample solution. Separately, weigh each accurately 5.0 g of methanol, 5.0 g of acetaldehyde for assay, 2.0 g of acetone, 1.0 g of 2-propanol, 1.3 g of 1-propanol and 1.3 g of *t*-butanol, and add ethanol (99.5) to make exactly 100 mL. Pipet 5 mL of this solution, add ethanol (99.5) to make exactly 50 mL. Pipet 5 mL of this solution, add ethanol (99.5) to make exactly 50 mL. To exactly 25 mL of this solution add 3 mL of the internal standard solution, and use this solution as the standard solution. Perform the test with 1 μL each of the sample solution and the standard solution as directed under the Gas Chromatography according to the following conditions. Determine each peak area from both solutions by the automatic integration method, and calcu-