

(2) Procedure

Weigh a defined amount of a specimen in a beaker, and add an indicated quantity of solvent to dissolve the specimen, as directed in the individual monograph. Next, after washing the two indicator electrodes with water, immerse both electrodes in the solution to be examined, apply a constant voltage suitable for measurement across two electrodes by using an appropriate device, and titrate the solution with a standard solution for volumetric analysis. During the titration, the tip of the buret should be dipped into the solution to be examined. The endpoint of titration is determined by following the changes of microcurrent between the two electrodes as a function of the quantity of titrant added. In the vicinity of the endpoint, the amounts of the titrant added should be 0.1 mL or less for adequate titrimetry. Plot the obtained current values along the ordinate and the quantity of the titrant added V (mL) along the abscissa to draw a titration curve, and usually take the inflection point of the titration curve (the point of intersection given by the extrapolation of two straight lines before and after the inflection) as the endpoint in amperometric titration.

The blank test in this titration is usually performed as follows: Take a volume of the solvent specified in the individual monograph or in the "Standard Solution for Volumetric Analysis", and use this as the sample solution. Determine the amount of the volumetric standard solution needed for giving the endpoint, and use this volume as the blank. If this volume is too small to determine accurately, the blank may be considered as 0 (mL).

Unless otherwise specified, the endpoint in this titration is decided by either of the following methods.

(i) Drawing method

Usually, extrapolate the two straight lines before and after the inflection, and obtain the inflection point of the titration curve. Next, read the quantity of titrant added at the inflection point, and assume this point to be the endpoint.

(ii) Automatic detection method

In the case of amperometric titration using an automatic titration system, the endpoint can be determined by following the instrumental indications. The endpoint is decided by following the variation of the indicator current during the course of a titration, and the quantity of titrant added is assumed to be that at which the current has reached the endpoint current set previously for the individual titration system.

When atmospheric carbon dioxide or oxygen is expected to influence the titration, a beaker with a lid should be used, and the procedure should be carried out in a stream of an inert gas, such as nitrogen gas. Further, when a specimen is expected to be influenced by light, use a light-resistant container to avoid exposure of the specimen to direct sunlight.

17. Fats and Fatty Oils Test

The Fats and Fatty Oils Test is a method applied to fats, fatty oils, waxes, fatty acids, higher alcohols, and related substances.

Preparation of test sample

For a solid sample, melt with care, and, if necessary, filter the melted sample with a dry filter paper by warming. For a turbid liquid sample, heat at about 50°C. If it is still turbid,

filter it with a dry filter paper while warm. In either case, mix the sample to make it homogeneous.

Melting point

Proceed by the method described in Method 2 of the test for Melting Point.

Congeaing point of fatty acids

(1) Preparation of fatty acids—Dissolve 25 g of potassium hydroxide in 100 g of glycerin. Transfer 75 g of this solution into a 1-L beaker, and heat at 150°C. Add 50 g of the sample to this solution, and heat at a temperature not higher than 150°C for 15 minutes under frequent stirring to saponify completely. Cool the solution to 100°C, dissolve by addition of 500 mL of hot water, and add slowly 50 mL of diluted sulfuric acid (1 in 4). Heat the solution under frequent stirring until the clear layer of fatty acid is separated distinctly. Separate the fatty acid layer, and wash the fatty acid with hot water until the washing shows no acidity to methyl orange TS. Transfer the fatty acid phase to a small beaker, and heat on a water bath until the fatty acid becomes clear owing to the separation of water. Filter the warm solution, and complete the evaporation of water by carefully heating the filtered solution to 130°C.

(2) Measurement of congealing point—Proceed by the method described in the test for Congeaing Point.

Specific gravity**(1) Liquid sample at ordinary temperature**

Proceed by the method described in the test for Specific Gravity and Density.

(2) Solid sample at ordinary temperature

Unless otherwise specified, fill a pycnometer with water at 20°C. Weigh accurately the pycnometer, and, after discarding the water and drying, weigh accurately the empty pycnometer. Then, fill the pycnometer with the melted sample to about three-fourths of the depth, and allow to stand at a temperature a little higher than the melting temperature of the sample for 1 hour to drive off the air in the sample. After keeping at the specified temperature, weigh accurately the pycnometer. Fill up the pycnometer with water over the sample at 20°C, and weigh accurately again.

The other procedure is the same as described in Method 1 of the test for Specific Gravity and Density.

$$d = \frac{W_1 - W}{(W_2 - W) - (W_3 - W_1)}$$

W : Mass (g) of the empty pycnometer.

W_1 : Mass (g) of the pycnometer filled with the sample.

W_2 : Mass (g) of the pycnometer filled with water.

W_3 : Mass (g) of the pycnometer filled with the sample and water.

Acid value

The acid value is the number of milligrams of potassium hydroxide (KOH) required to neutralize the free acids in 1 g of sample.

Procedure: Unless otherwise specified, weigh accurately the amount of sample shown in Table 1, according to the expected acid value of the sample, in a glass-stoppered, 250-mL flask, add 100 mL of a mixture of diethyl ether and ethanol (95) (1:1 or 2:1) as the solvent, and dissolve the sample by warming, if necessary. Then, add a few drops of phenolphthalein TS, and titrate with 0.1 mol/L potassium hydroxide-ethanol VS until the solution develops a light red

color which persists for 30 seconds. If the sample solutions is turbid at lower temperature, titration should be done while warm. To the solvent used add phenolphthalein TS as an indicator, and add 0.1 mol/L potassium hydroxide-ethanol VS before use, until the solvent remains light red for 30 seconds.

$$\text{Acid value} = \frac{\text{consumed volume (mL) of 0.1 mol/L potassium hydroxide-ethanol VS}}{\text{amount (g) of sample}} \times 5.611$$

Table 1

Acid value	Amount (g) of sample
Less than 5	20
5 to 15	10
15 to 30	5
30 to 100	2.5
More than 100	1.0

Saponification value

The saponification value is the number of milligrams of potassium hydroxide (KOH) required to saponify the esters and to neutralize the free acids in 1 g of the sample.

Procedure: Unless otherwise specified, weigh accurately 1 to 2 g of the sample, transfer to a 200-mL flask, and add exactly 25 mL of 0.5 mol/L potassium hydroxide-ethanol VS. Attach a short reflux condenser or an air condenser 750 mm in length and 6 mm in diameter to the neck of the flask, and heat gently in a water bath for 1 hour with frequent shaking. Cool the solution, add 1 mL of phenolphthalein TS, and titrate immediately the excess potassium hydroxide with 0.5 mol/L hydrochloric acid VS. If the sample solution is turbid at lower temperature, titration should be done while warm. Perform a blank determination.

$$\text{Saponification value} = \frac{(a - b) \times 28.05}{\text{amount (g) of sample}}$$

a: Volume (mL) of 0.5 mol/L hydrochloric acid VS consumed in the blank determination.

b: Volume (mL) of 0.5 mol/L hydrochloric acid VS consumed for titration of the sample.

Ester value

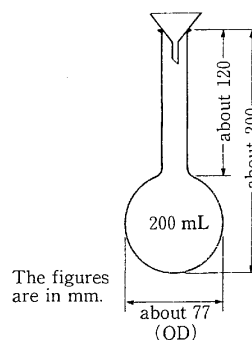
The ester value is the number of milligrams of potassium hydroxide (KOH) required to saponify the esters in 1 g of sample.

Procedure: Unless otherwise specified, designate the difference between the saponification value and the acid value determined as the ester value.

Hydroxyl value

The hydroxyl value is the number of milligrams of potassium hydroxide (KOH) required to neutralize acetic acid combined with hydroxyl groups, when 1 g of the sample is acetylated by the following procedure.

Procedure: Place about 1 g of the sample, weighed accurately, in a 200-mL round-bottom flask (shown in the figure), and add exactly 5 mL of pyridine-acetic anhydride TS. Place a small funnel on the neck of the flask, and heat by immersing the flask up to 1 cm from the bottom in an oil bath between 95°C and 100°C. Put a thick, round paper with a round hole on the joint of the neck of the flask to protect the neck from the heat of the oil bath. After heating for 1



hour, take the flask from the oil bath, and cool by standing. Add 1 mL of water to the flask, and shake to decompose acetic anhydride. Heat the flask in the oil bath for 10 minutes again. After cooling, wash the funnel and neck with 5 mL of neutralized ethanol down into the flask, and titrate with 0.5 mol/L potassium hydroxide-ethanol VS (indicator: 1 mL of phenolphthalein TS). Perform a blank determination.

$$\text{Hydroxyl value} = \frac{(a - b) \times 28.05}{\text{amount (g) of sample}} + \text{acid value}$$

a: Volume (mL) of 0.5 mol/L potassium hydroxide-ethanol VS consumed in the blank determination.

b: Volume (mL) of 0.5 mol/L potassium hydroxide-ethanol VS consumed for titration of the sample.

Unsapifiable matter

Unsapifiable matter is calculated as the difference between the amount of materials, which are unsapifiable by the procedure described below, soluble in diethyl ether and insoluble in water, and the amount of fatty acids expressed in terms of the amount of oleic acid. Its limit is expressed as a percentage in the monograph.

Procedure: Transfer about 5 g of the sample, accurately weighed, to a 250-mL flask. Add 50 mL of potassium hydroxide-ethanol TS, attach a reflux condenser to the flask, boil gently on a water bath for 1 hour with frequent shaking, and then transfer to the first separator. Wash the flask with 100 mL of warm water, and transfer the washing to the separator. Further, add 50 mL of water to the separator, and cool to room temperature. Wash the flask with 100 mL of diethyl ether, add the washing to the separator, extract by vigorous shaking for 1 minute, and allow to stand until both layers are separated clearly. Transfer the water layer to the second separator, add 50 mL of diethyl ether, shake, and allow to stand in the same manner. Transfer the water layer in the second separator to the third separator, add 50 mL of diethyl ether, and extract by shaking again in the same manner. Combine the diethyl ether extracts in the second and third separators into the first separator, wash each separator with a small amount of diethyl ether, and combine the washings into the first separator. Wash the combined extracts in the first separator with 30 mL portions of water successively, until the washing does not develop a light red color with 2 drops of phenolphthalein TS. Add a small amount of anhydrous sodium sulfate to the diethyl ether extracts, and allow to stand for 1 hour. Filter the diethyl ether extracts with dry filter paper, and collect the filtrates into a tared flask. Wash well the first separator with diethyl ether, and add the washing to the flask through the above filter paper. After evaporation of the

filtrate and washing almost to dryness on a water bath, add 3 mL of acetone, and evaporate again to dryness on a water bath. Complete the drying between 70°C and 80°C under reduced pressure (about 2.67 kPa) for 30 minutes, allow to stand for cooling in a desiccator (reduced pressure, silica gel) for 30 minutes, and then weigh. After weighing, add 2 mL of diethyl ether and 10 mL of neutralized ethanol, and dissolve the residue by shaking well. Add a few drops of phenolphthalein TS, and titrate the remaining fatty acids in the residue with 0.1 mol/L potassium hydroxide-ethanol VS until the solution develops a light red color which persists for 30 seconds.

$$\text{Unsaponifiable matter (\%)} = \frac{a - (b \times 0.0282)}{\text{amount (g) of sample}} \times 100$$

a: Amount (g) of the extracts.

b: Volume (mL) of 0.1 mol/L potassium hydroxide-ethanol VS consumed for titration.

Iodine value

The iodine value, when measured under the following conditions, is the number of grams of iodine (I), representing the corresponding amount of halogen, which combines with 100 g of sample.

Procedure: Unless otherwise specified, weigh accurately the amount of sample shown in Table 2, according to the expected iodine value of the sample, in a small glass container. In a 500-mL glass-stoppered flask place the container containing the sample, add 20 mL of cyclohexane to dissolve the sample, then add exactly 25 mL of Wijs' TS, and mix well. Stopper the flask, and allow to stand, protecting against light, between 20°C and 30°C for 30 minutes (when the expected iodine value is more than 100, for 1 hour) with occasional shaking. Add 20 mL of potassium iodide solution (1 in 10) and 100 mL of water, and shake. Then, titrate the liberated iodine with 0.1 mol/L sodium thiosulfate VS (indicator: 1 mL of starch TS). Perform a blank determination.

$$\text{Iodine value} = \frac{(a - b) \times 1.269}{\text{amount (g) of sample}}$$

a: Volume (mL) of 0.1 mol/L sodium thiosulfate VS consumed in the blank determination.

b: Volume (mL) of 0.1 mol/L sodium thiosulfate VS consumed for titration of the sample.

Table 2

Iodine value	Amount (g) of sample
Less than 30	1.0
30 to 50	0.6
50 to 100	0.3
More than 100	0.2

18. Flame Coloration Test

The Flame Coloration Test is a method to detect an element, by means of the property that the element changes the colorless flame of a Bunsen burner to its characteristic color.

(1) Salt of metal—The platinum wire used for this test is about 0.8 mm in diameter, and the end part of it is straight. In the case of a solid sample, make the sample into a gruel by

adding a small quantity of hydrochloric acid, apply a little of the gruel to the 5-mm end of the platinum wire, and test by putting the end part in a colorless flame, keeping the platinum wire horizontal. In the case of a liquid sample, immerse the end of the platinum wire into the sample to about 5 mm in length, remove from the sample gently, and perform the test in the same manner as for the solid sample.

(2) Halide—Cut a copper net, 0.25 mm in opening and 0.174 mm in wire diameter, into a strip 1.5 cm in width and 5 cm in length, and wind in round one end of a copper wire. Heat the copper net strongly in the colorless flame of Bunsen burner until the flame no longer shows a green or blue color, and then cool it. Repeat this procedure several times, and coat the net completely with cupric oxide. After cooling, unless otherwise specified, apply about 1 mg of the sample to the copper net, ignite, and burn it. Repeat this procedure three times, and then test by putting the copper net in the colorless flame.

The description, "Flame coloration persists", in a monograph, indicates that the reaction persists for 4 seconds.

19. Fluorometry

The Fluorometry is a method to measure the intensity of fluorescence emitted from a solution of fluorescent substance irradiated with an exciting light in a certain wavelength range. The Fluorometry is also applied to the phosphorescent substances.

Fluorescence intensity *F* in a dilute solution is proportional to the concentration *c* in mol per liter of the solution and the pathlength *l* of light through the solution in centimeter.

$$F = kI_0\phi\epsilon cl$$

k: Constant

*I*₀: Intensity of exciting light

φ: Quantum yield of fluorescence or phosphorescence

$$\phi = \frac{\text{number of quanta emitted as fluorescence or phosphorescence}}{\text{number of quanta absorbed}}$$

ε: Molar extinction coefficient of the substance at the excitation wavelength

Apparatus

Spectrofluorometer is usually used. Generally, a xenon lamp, laser, an alkaline halide lamp, etc. which provide stable exciting light are used as the light source. Usually, a nonfluorescent quartz cell (1 cm × 1 cm) with four transparent sides is used as the container for sample solution.

Procedure

Excitation spectrum is obtained by measuring fluorescence intensities of sample solution with varying excitation wavelengths at a fixed emission wavelength (in the vicinity of the fluorescence maximum) and drawing a curve showing the relationship between the excitation wavelength and the fluorescence intensity. Fluorescence spectrum is obtained by measuring fluorescence intensities of sample solution with varying emission wavelengths at a fixed excitation wavelength (in the vicinity of the excitation maximum) and drawing the same curve as described for the excitation spectrum. If necessary, the spectra are corrected with regard to the optical characteristics of the apparatus.