ble, and, taking care to prevent bubbles, draw it into a capillary tube (one as used in Method 1 and which is left open at both ends) to a depth of about 10 mm. Allow the charged tube to stand for 24 hours at a temperature below 10°C, or for at least 1 hour in contact with ice, holding the tube so as not to allow loss of the sample from it. Then attach the tube to the thermometer by means of a rubber band so that the sample is on a level with the middle part of the mercury bulb. Adjust the tube in a water-containing beaker to such a position that the lower edge of the sample is 30 mm below the water surface. Heat the beaker with constant stirring until the temperature rises to 5°C below the expected melting point. Then regulate the rate of increase to 1°C per minute. The temperature at which the sample is observed to rise in the capillary tube is taken as the melting point.

**Method 3** This method is applied to petrolatums.

**Procedure**
Melt the sample slowly, with thorough stirring, until it reaches a temperature between 90°C and 92°C. Discontinue the heating, and allow the sample to cool to a temperature between 8°C and 10°C above the expected melting point. Chill the bulb of the thermometer to 5°C, wipe, dry, and, while still cold, thrust into the molten sample to such a depth that approximately the lower half of the bulb is submerged. Withdraw it immediately, hold vertically, cool until the attached sample becomes dull, then dip for 5 minutes in water having a temperature not higher than 16°C. Fix the thermometer securely in a test tube by means of a cork stopper so that the lower end is 15 mm above the bottom of the test tube. Suspend the tube in water contained in a beaker at a temperature of about 16°C, and raise the temperature of the bath to 30°C at a rate of 2°C per minute, then at a rate of 1°C per minute until it reaches the melting point. Read the temperature at which the first drop leaves the thermometer. If the variations between each of three determinations are not more than 1°C, take the average of the three. If any of the variations is greater than 1°C, make two additional determinations, and take the average of the five as the melting point.

### 32. Methanol Test

The Methanol Test is a method to determine methanol adhering in ethanol.

**Reagents**

1. **Standard Methanol Solution**—To 1.0 g of methanol, accurately measured, add water to make exactly 1000 mL. To 5 mL of this solution, exactly measured, add 2.5 mL of methanol-free ethanol and water to make exactly 50 mL.
2. **Solution A**—To 75 mL of phosphoric acid add water to make 500 mL, then dissolve 15 g of potassium permanganate in this solution.
3. **Solution B**—Add sulfuric acid carefully to an equal volume of water, cool, and dissolve 25 g of oxalic acid hydrate in 500 mL of this dilute sulfuric acid.

**Procedure**

Pipet 1 mL of the sample, and add water to make exactly 20 mL. Use this solution as the sample solution. Transfer 5 mL each of the sample solution and the Standard Methanol Solution, accurately measured, to test tubes, add 2 mL of Solution A to each solution, and allow to stand for 15 minutes. Decolorize these solutions by adding 2 mL of Solution B, and mix with 5 mL of fuchsin-sulfurous acid TS. Allow to stand for 30 minutes at ordinary temperature. The sample solution has no more color than the Standard Methanol Solution.

### 33. Methoxyl Assay

The Methoxyl Assay is a method to determine methoxyl groups, in which the sample is heated with hydroiodic acid, the produced iodomethane is oxidized with bromine to give iodic acid, potassium iodide and dilute sulfuric acid are added, and the liberated iodine is titrated with sodium thiosulfate VS.

$$ROCH_2 + HI = CH_3I + ROH$$
$$CH_3I + 3Br_2 + 3H_2O = CH_3Br + 5HBr + HIO_3$$
$$HIO_3 + 5HI = I_2 + 3H_2O$$

**Apparatus**

Use the apparatus illustrated in the figure.

**Reagents**

1. **Scrubbing solution**—Prepare a suspension by mixing 1 g of red phosphorus with 100 mL of water.
2. **Absorbing solution**—Dissolve 15 g of potassium acetate in 150 mL of a mixture of acetic acid (100) and acetic anhydride (9:1), and to 145 mL of the solution add 5 mL of bromine. Prepare the absorbing solution before use.

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![Diagram of Methanol Test Apparatus]

The figures are in mm.

A: Decomposition flask  F: Glass stopper
B: Gas-introducing tube  G: Ball joint
C: Ground joint  H: Gas duct
D: Air condenser  J: Absorption tube
E: Gas scrubber  K: Gas-expelling tube
34. Microbial Assay for Antibiotics

The Microbial Assay for Antibiotics is a method that uses microorganisms to determine the antimicrobial potency of antibiotics contained in medicines. There are three methods for this test: the cylinder-plate, perforated plate, and turbidimetric methods. The former two are based on the measurement of the size of the zones of microbial growth inhibition in a nutrient agar medium, and the turbidimetric method is based on the measurement of the inhibition of turbidity development in a fluid medium with microbial growth. Unless otherwise specified in the individual monograph, tests specified to be carried out by the cylinder-plate method may be conducted under the same test conditions using the perforated plate method instead. If necessary, first sterilize water, isotonic sodium chloride solution, buffer solutions, reagents, test solutions and essential parts of measuring instruments and appliances to be used for the test.

1. Cylinder-plate method

The cylinder-plate method is a method to determine the antimicrobial potency of the antibiotic to be tested, and is based on the measurement of the size of the zone of growth inhibition of a test organism by the use of cylinder-agar plates.

1. Test organisms

According to the specification of the individual mono-