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 dissove 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 dihydrate 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.

$$ROCH_3 + HI = CH_3I + ROH$$
$$CH_3I + 3Br_2 + 3H_2O = CH_3Br + 5HBr + HIO_3$$
$$HIO_3 + 5HI = 3I_2 + 3H_2O$$

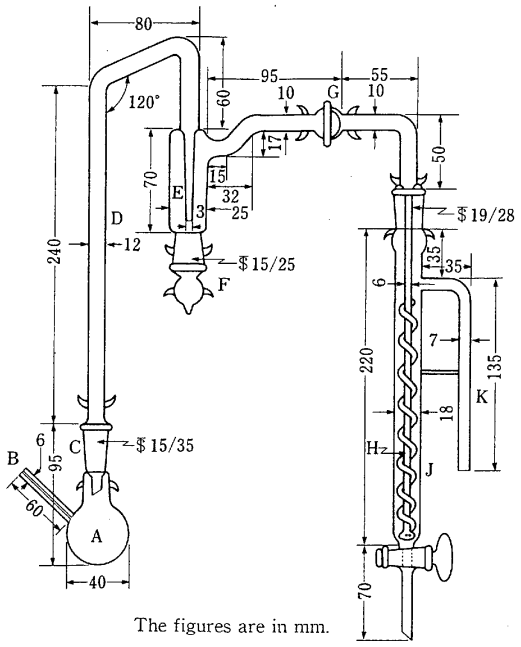
Apparatus

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.



The figures are in mm.

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

Procedure

Pour in the scrubbing solution to half the height of the gas scrubber E, and put 20 mL of the absorbing solution in the absorption tube J. Weigh accurately a sample corresponding to about 6.5 mg as methoxyl group (CH_3O: 31.03), transfer to the decomposition flask A, and add boiling tips and 6 mL of hydroiodic acid. Moisten the ground joint C of A with 1 drop of hydroiodic acid, and connect to the condenser D. Prepare the apparatus by connecting the ball joint G using a suitable silicone grease. Bubble nitrogen or carbon dioxide through the gas-introducing tube B, and adjust the bubbles appearing in E at the rate of 2 bubbles per second, using a suitable pressure-regulating device. Place A in an oil bath, heat the flask until the temperature of the bath becomes 150°C after heating for 20 or 30 minutes, and boil at the same temperature for further 60 minutes. Remove the oil bath, cool the flask to room temperature under the gas stream, cool, and separate G. Drain the contents of J into a 500 mL conical flask containing 10 mL of a solution of sodium acetate trihydrate (1 in 5), rinse the tube with water several times, adding the rinsings to the flask, and finally dilute with water to about 200 mL. Add formic acid dropwise with shaking until the red color of the bromine disappears, and add 1 mL of formic acid. Add 3 g of potassium iodide and 15 mL of dilute sulfuric acid, stopper, shake gently, allow to stand for 5 minutes, and titrate the liberated iodine with 0.1 mol/L sodium thiosulfate VS (indicator: 1 mL of starch TS). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L sodium thiosulfate VS
= 0.5172 mg of CH_3O

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.

I. 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 monograph, unless otherwise specified, select the test organism from the examples listed below.

[1] *Staphylococcus aureus* ATCC 6538 P
[2] *Staphylococcus epidermidis* ATCC 12228
[3] *Micrococcus luteus* ATCC 10240
[4] *Micrococcus luteus* ATCC 9341
[5] *Bacillus subtilis* ATCC 6633
[6] *Mycobacterium smegmatis* ATCC 607
[7] *Escherichia coli* NIHJ
[8] *Escherichia coli* ATCC 27166
[9] *Klebsiella pneumoniae* ATCC 10031
[10] *Pseudomonas aeruginosa* NCTC 10490
[11] *Comamonas terrigena* ATCC 8461
[12] *Saccharomyces cerevisiae* ATCC 9763
[13] *Candida albicans* No. Yu 1200
[14] *Penicillium chrysogenum* ATCC 10002

2. Culture media

Unless otherwise specified, use media with the following compositions. When 'peptone' is indicated as an ingredient of a medium, either meat peptone or casein peptone is applicable. Use sodium hydroxide TS or 1 mol/L hydrochloric acid TS to adjust the pH of the medium to obtain the specified value after sterilization. In the case of the medium for *Bacillus subtilis* ATCC 6633, adjust the pH using ammonia TS, potassium hydroxide TS or 1 mol/L hydrochloric acid TS. A different medium to the one specified for each test organism may be used if it has both a similar composition and an equal or better growth efficiency of the test organism in comparison with the specified medium. Sterilize the media to be used in an autoclave.

(1) Agar media for seed and base layer

1) Medium for test organism [5]

i.

Peptone	5.0 g
Meat extract	3.0 g
Agar	15.0 g
Water	1000 mL

Mix all the ingredients, and sterilize. Adjust the pH of the solution so that it will be 7.8 to 8.0 after sterilization.

ii.

Peptone	5.0 g
Meat extract	3.0 g
Trisodium citrate dihydrate	10.0 g
Agar	15.0 g
Water	1000 mL

Mix all the ingredients, and sterilize. Adjust the pH of the solution so that it will be 6.5 to 6.6 after sterilization.

2) Medium for test organism [12]

Glucose	10.0 g
Peptone	9.4 g
Meat extract	2.4 g
Yeast extract	4.7 g
Sodium chloride	10.0 g
Agar	15.0 g
Water	1000 mL

Mix all the ingredients, and sterilize. Adjust the pH of the solution so that it will be 6.0 to 6.2 after sterilization.

3) Medium for other organisms

i.

Glucose	1.0 g
Peptone	6.0 g
Meat extract	1.5 g
Yeast extract	3.0 g
Agar	15.0 g
Water	1000 mL