temperature, measure exactly 100 mL of the this solution, and transfer to a 250-mL conical flask of hard glass. Add 5 drops of bromocresol green-methyl red TS, and titrate with 0.01 mol/L sulfuric acid VS until the color of the solution changes from green through slightly grayish blue to slightly grayish red-purple. Measure accurately 100 mL of water, transfer to a 250-mL conical flask of hard glass, perform a blank determination in the same manner, and make any necessary correction. The quantity of 0.01 mol/L sulfuric acid VS consumed does not exceed 0.10 mL.

**(4)** Soluble iron test for light-resistant containers—Rinse thoroughly five or more light-resistant containers to be tested with water, and dry at 105°C for 30 minutes. Pour a volume of 0.01 mol/L hydrochloric acid VS corresponding to the labeled volume of the container into individual containers, and fuse them. In the case of containers not to be fused, cover them with small beakers of hard glass or watch glasses of hard glass. Heat them at 105°C for 1 hour. After cooling, prepare the test solution with 40 mL of this solution according to Method 1 of the Iron Limit Test, and perform the test according to Method B. Prepare the control solution with 2.0 mL of the Standard Iron Solution.

**(5)** Light transmission test for light-resistant containers—Cut five light-resistant containers to be tested, prepare test pieces with surfaces as flat as possible, and clean the surfaces. Fix a test piece in a cell-holder of a spectrophotometer to allow the light pass through the center of the test piece perpendicularly to its surface. Measure the light transmittance of the test piece with reference to air between 290 nm and 450 nm and also between 590 nm and 610 nm at intervals of 20 nm each. The percent transmissions obtained between 290 nm and 450 nm are not more than 50% and that between 590 nm and 610 nm are not less than 60%. In the case of containers not to be fused having a wall thickness over 1.0 mm, the percent transmissions between 590 nm and 610 nm are not less than 45%.

# 58. Test for Metal Particles in Ophthalmic Ointments

Test of Metal Particles in Ophthalmic Ointments is a method to test the existence of foreign metal particles in the ophthalmic ointments described in General Rules for Preparations.

**Preparation of test sample**

The test should be carried out in a clean place. Take 10 ophthalmic ointments to be tested, and extrude the contents as completely as practicable into separate flat-bottomed petri dishes 60 mm in diameter when the amount of the content is 5 g or less. Weigh 5 g of the contents when the amount of the content is more than 5 g, and proceed in the same manner as described above. Cover the dishes, and heat between 85°C and 110°C for 2 hours to dissolve bases. Allow the samples to cool to room temperature without agitation to solidify the contents.

Note: Use petri dishes with a clean bottom and free from foams and scratches, and if possible, the walls are at right angles with the bottom.

**Procedure**

Invert each dish on the stage of a suitable microscope previously adjusted to provide more than 40 times magnifications and equipped with an eyepiece micrometer disk. Each dish is illuminated from above 45° relative to the plane of the dish. Examine the entire bottom of each dish for metal particles, and record the total number of particles, measuring 50 $\mu$m or more in any dimension.

# 59. Test for Rubber Closure for Aqueous Infusions

The Rubber Closure for Aqueous Infusions means a rubber closure (containing material coated or laminated with the stuff like plastics) used for a container for aqueous infusion having a capacity of 100 mL or more, and is in direct contact with the contained aqueous infusion. The rubber closure when in use does not interact physically or chemically with the contained medicament to alter any property or quality, does not permit the invasion of microbes, does not disturb the use of the contained infusion, and meets the following requirements.

**(1)** Cadmium—Wash the rubber closures with water, dry at room temperature, cut into minute pieces, mix well, place 2.0 g of them in a crucible of platinum or quartz, moisten them with 2 mL of sulfuric acid, heat gradually to dryness, and ignite between 450°C and 500°C until the residue is incinerated. When incineration was insufficient, moisten the residue with 1 mL of sulfuric acid, heat to dryness, and ignite again. Repeat the above-mentioned procedure if necessary. Cool the crucible, moisten the residue with water, add 2 to 4 mL of hydrochloric acid, heat on a water bath to dryness, add 1 to 5 mL of hydrochloric acid, and dissolve by heating. Then add 0.5 to 1 mL of a mixture of a solution of citric acid monohydrate (1 in 2) and hydrochloric acid (1:1) and 0.5 to 1 mL of a warmed solution of ammonium acetate (2 in 5). When any insoluble residue remains, filter through a glass filter. To the solution thus obtained add 10 mL of a solution of diammonium hydrogen citrate (1 in 4), 2 drops of bromothymol blue TS and ammonium TS until the color of the solution changes from yellow to green. Then add 10 mL of ammonium sulfate solution (2 in 5) and water to make 100 mL. Next, add 20 mL of a solution of sodium *N,N*-diethyldithiocarbamate trihydrate (1 in 20), mix, allow to stand for a few minutes, add 20.0 mL of 4-methyl-2-pentanone, and mix by vigorous shaking. Allow to stand to separate the 4-methyl-2-pentanone layer from the solution, filter if necessary, and use as the sample solution. On the other hand, to 10.0 mL of Standard Cadmium Solution add 10 mL of a solution of diammonium hydrogen citrate (1 in 4) and 2 drops of bromothymol blue TS, proceed in the same manner as for the sample solution, and use this solution as the standard solution. Perform the tests according to the Atomic Absorption Spectrophotometry under the following conditions, using the sample solution and the standard solution. The absorbance of the sample solution is not more than that of the standard solution.

Gas: Combustible gas—Acetylene or hydrogen
Supporting gas—Air

Lamp: Cadmium hollow-cathode lamp
Wavelength: 228.8 nm

(2) Lead—To 1.0 mL of the Standard Lead Solution add 10 mL of a solution of diammonium hydrogen citrate (1 in 4) and 2 drops of bromothymol blue TS, proceed as directed for the sample solution under (1), and use this solution as the standard solution. Perform the tests according to the Atomic Absorption Spectrophotometry under the following conditions, using the sample solution obtained in (1) and the standard solution. The absorbance of the sample solution is not more than that of the standard solution.

Gas: Combustible gas—Acetylene or hydrogen
Supporting gas—Air
Lamp: Lead hollow-cathode lamp
Wavelength: 283.3 nm

(3) Extractable substances—Wash the rubber closures with water, and dry at room temperature. Place them in a glass container, add water exactly 10 times the mass of the test material, close with a suitable stopper, heat at 121°C for 1 hour in an autoclave, take out the glass container, allow to cool to room temperature, then take out immediately the rubber closures, and use the remaining solution as the test solution. Prepare the blank solution with water in the same manner. Perform the following tests with the test solution and the blank solution.

(i) Description: The test solution is clear and colorless. Read the transparency of the test solution at 430 nm and 650 nm (10 mm), using the blank solution as the blank. Both of them are not less than 99.0%.

(ii) Foam test: Place 5 mL of the test solution in a glass-stoppered test tube of about 15 mm in inner diameter and about 200 mm in length, and shake vigorously for 3 minutes. The foam arisen disappears almost completely within 3 minutes.

(iii) pH: To 20 mL each of the test solution and the blank solution add 1.0 mL each of potassium chloride solution, prepared by dissolving 1.0 g of potassium chloride in water to make 1000 mL. The difference of pH between the two solutions is not more than 1.0.

(iv) Zinc: To 10.0 mL of the test solution add diluted dilute nitric acid (1 in 3) to make 20 mL, and use this solution as the sample solution. Further, to 1.0 mL of Standard Zinc Solution for atomic absorption spectrophotometry add diluted nitric acid (1 in 3) to make exactly 20 mL, and use this solution as the standard solution. Perform the tests according to the Atomic Absorption Spectrophotometry, using these solutions, under the following conditions. The absorbance of the sample solution is not more than that of the standard solution.

Gas: Combustible gas—Acetylene
Supporting gas—Air
Lamp: Zinc hollow-cathode lamp
Wavelength: 213.9 nm

Standard Zinc Solution for atomic absorption spectrophotometry: Measure exactly 10 mL of the Standard Zinc Stock Solution, and add water to make exactly 1000 mL. Prepare before use. One mL of this solution contains 0.01 mg of zinc (Zn).

(v) Potassium Permanganate-reducing substances: Measure 100 mL of the test solution in a glass-stoppered, Erlenmyer flask, add 10.0 mL of 0.002 mol/L potassium permanganate VS and 5 mL of dilute sulfuric acid, and boil for 3 minutes. After cooling, add 0.10 g of potassium iodide, stopper, mix by shaking, then allow to stand for 10 minutes, and titrate with 0.01 mol/L sodium thiosulfate VS (indicator: 5 drops of starch TS). Perform the blank test in the same manner, using 100 mL of the blank solution. The difference in mL of 0.002 mol/L potassium permanganate VS required between the tests is not more than 2.0 mL.

(vi) Residue on evaporation: Measure 100 mL of the test solution, evaporate on a water bath to dryness, and dry the residue at 105°C for 1 hour. The mass of the residue is not more than 2.0 mg.

(vii) UV spectrum: Read the absorbance of the test solution between 220 nm and 350 nm against the blank solution as directed under the Ultraviolet-visible Spectrophotometry: it is not more than 0.20.

(4) Acute systemic toxicity—The test solution meets the requirements, when examined under the following conditions against the blank solution.

Preparation of the test solution and the blank solution: Wash the rubber closures with water and Water for Injection successively, and dry under clean conditions at room temperature. Transfer the rubber closures to a glass container. Add isotonic sodium chloride solution 10 times the mass of the test material, stopper adequately, heat in an autoclave at 121°C for 1 hour, take out the glass container, and allow to cool to room temperature. The solution thus obtained is used as the test solution. The blank solution is prepared in the same manner.

(i) Test procedures

Test animals: Use healthy male mice of inbred strain or from a closed colony, weighing 17 to 23 g.

Procedure: Separate the animals into two groups of 10 mice, and inject intravenously 50 mL each of the solutions per kg body mass.

(ii) Interpretation

Observe the animals for 5 days after injection: During the observation period, none of the animals treated with the test solution show any abnormality or death.

(5) Pyrogen test—The test solution specified in (4) meets the requirements of the Pyrogen Test as does the blank solution.

(6) Hemolysis test—When 0.1 mL of defibrinated blood of rabbit is added to 10 mL of the test solution specified in (4) and the mixture is allowed to stand at 37°C for 24 hours, hemolysis is not observed. Perform the blank test in the same manner, using 10 mL of the blank solution.

# 60. Test for Total Organic Carbon

The Test for Total Organic Carbon is a method for measuring the amount of organic carbon, which forms organic compounds, in water. Normally, organic carbon can be oxidized to carbon dioxide by a dry decomposition method, where organic compounds are oxidized by combustion, or by a wet decomposition method, where organic compounds are oxidized by applying ultraviolet rays or by adding oxidizing agent. The amount of carbon dioxide generated in the decomposition process is measured using an appropriate method such as infrared gas analysis, electric conductivity measurement, or resistivity measurement. The amount of or-