Each mL of 0.005 mol/L silver nitrate VS
= 0.6345 mg of I

(3) Fluorine

Apply a small amount of water to the upper part of A, pull out C carefully, transfer the test solution and the blank solution to 50 mL volumetric flasks separately, wash C, B and the inner side of A with water, add the washings and water to make 50 mL, and use these solutions as the test solution and the correction solution. Pipet the test solution (V mL) equivalent to about 0.03 mg of fluorine, V mL of the correction solution and 5 mL of standard fluorine solution, transfer to 50-mL volumetric flasks separately, add 30 mL of a mixture of alizarin complexone TS, acetic acid-potassium acetate buffer solution, pH 4.3 and cerium (III) nitrate TS (1:1:1), add water to make 50 mL, and allow to stand for 1 hour. Perform the test with these solutions as directed under the Ultraviolet-visible Spectrophotometry, using a blank prepared with 5 mL of water in the same manner. Determine the absorbances, A_T, A_C and A_S, of the subsequent solutions of the test solution, the correction solution and the standard solution at 600 nm.

Amount (mg) of fluorine (F) in the test solution
= amount (mg) of fluorine in 5 mL of

$$\text{the standard solution} \times \frac{A_T - A_C}{A_S} \times \frac{50}{V}$$

Standard Fluorine Solution: Dry sodium fluoride (standard reagent) in a platinum crucible between 500°C and 550°C for 1 hour, cool it in a desiccator (silica gel), weigh accuraly about 66.3 mg of it, and dissolve in water to make exactly 500 mL. Pipet 10 mL of this solution, and dilute with sufficient water to make exactly 100 mL.

(4) Sulfur

Apply a small amount of water to the upper part of A, pull out C carefully, and wash C, B and the inner side of A with 15 mL of methanol. To this solution add 40 mL of methanol, then add exactly 25 mL of 0.005 mol/L barium perchlorate VS, allow to stand for 10 minutes, add 0.15 mL of arsenazo III TS with a measuring pipet, and titrate with 0.005 mol/L sulfuric acid VS. Perfrom the test with the blank solution in the same manner.

Each mL of 0.005 mol/L barium perchlorate VS
= 0.1603 mg of S

43. Paper Chromatography

Paper Chromatography is a method to separate each ingredient by developing a mixture in a mobile phase, using a sheet of filter paper, and is used for identification, purity test, etc, of substances.

Procedure

Unless otherwise specified, proceed by the following method.

Designate a line about 50 mm distant from the bottom of a sheet of filter paper, 20 to 30 mm wide and 400 mm long, as the starting line, spot the directed amount of the sample solution in the monograph with a micropipet or capillary tube on the center of the starting line, and air-dry. Then, suspend the paper in a container for developing of about 500 mm in height, which contains the developing solvent in its bottom section beforehand and the inside of which is already saturated by the vapor of the solvent, taking care to avoid contact with the walls. Immerse the paper in the solvent so that the lower edge of paper is covered with the solvent to about 10 mm from the bottom. Seal the container and allow the solvent to ascend on the paper at ordinary temperature.

When the solvent front has ascended from the starting line to the distance directed in the monograph, remove the paper from the container, make the solvent front immediately, and air-dry again. Observe the location, color, etc. of the stpots by the method specified in the monograph. Calculate the *Rf* value by using the following equation:

$$Rf = \frac{\text{distance from the starting line to the center of the spot}}{\text{distance from the starting line to the solvent front}}$$

44. Particle Size Distribution Test for Preparations

Particle Size Distribution Test for Preparations is a method to determine the particle size distribution of the granules and powders described in General Rules for Preparations.

Procedure

(1) Granules

The test is performed employing No. 10 (1700 μm), No. 12 (1400 μm), and No. 42 (355 μm) sieves with the inside diameter of 75 mm.

Weigh accurately 20.0 g of granules to be tested, and place on the uppermost sieve which is placed on the other sieves described above and a close-fitting receiving pan and is covered with a lid. Shake the sieves in a horizontal direction for 3 minutes, and tap slightly at intervals. Weigh the amount remaining on each sieve and in the receiving pan.

(2) Powders

The test is performed employing No. 18 (850 μm), No. 30 (500 μm), and No. 200 (75 μm) sieves with the inside diameter of 75 mm.

Weigh accurately 10.0 g of powders to be tested, and place on the uppermost sieve which is placed on the other sieves described above and a close-fitting receiving pan and is covered with a lid. Shake the sieves in a horizontal direction for 3 minutes, and tap slightly at intervals. Weigh the amount remaining on each sieve and in the receiving pan.

45. pH Determination

pH is defined as the reciprocal of the common logarithm of hydrogen ion activity, which is the product of hydrogen ion concentration and the activity coefficient. Conventionally it is used as a scale of hydrogen ion concentration of a sample solution.

pH of a sample solution is expressed by the following equation in relation to the pH of a standard solution (pHs), and can be measured by a pH meter using a glass electrode.

$$\text{pH} = \text{pHs} + \frac{E - E_s}{2.3026\, RT/F}$$

pHs: pH value of a pH standard solution.

E: Electromotive force (volt) induced on the following galvanic cell composed of a glass electrode and suitable reference electrode in a sample solution:

Glass electrode | sample solution | reference electrode

E_s: Electromotive force (volt) induced on the following galvanic cell composed of a glass electrode and suitable reference electrode in a pH standard solution:

Glass electrode | standard pH solution | reference electrode

R: Gas constant
T: Absolute temperature
F: Faraday's constant

The value of $2.3026\,RT/F$ (V) in the above equation means the degree of electromotive force (V) per one pH unit and it is dependent on temperature as seen in the *Table* below:

Temperature dependency of the electromotive force (V)

Temperature of solution (°C)	$2.3026\,RT/F$ (V)	Temperature of solution (°C)	$2.3026\,RT/F$ (V)
5	0.05519	35	0.06114
10	0.05618	40	0.06213
15	0.05717	45	0.06313
20	0.05817	50	0.06412
25	0.05916	55	0.06511
30	0.06015	60	0.06610

pH Standard solution

The pH standard solutions are used as a standard of pH, for standardization of a pH meter. To prepare water for preparation of the pH standard solutions, distill purified water, boil the distillate for more than 15 minutes, and cool in a container fitted with a carbon dioxide-absorbing tube (soda lime). Next, prepare individually 6 kinds of pH standard solutions as specified below.

Store the pH standard solutions in hard glass or polyethylene bottles. For storage of alkaline pH standard solutions, it is preferable to use a bottle fitted with a carbon dioxide-absorbing tube. Since the pH may change gradually during storage over a long period, it is necessary to acertain whether the expected pH value is held or not by comparison with newly prepared standard, when the solution is used after long storage.

(1) Oxalate pH standard solution—Reduce potassium trihydrogen dioxalate dihydrate for pH determination to a fine powder, and dry in a desiccator (silica gel). Weigh 12.71 g (0.05 mole) of it accurately, and dissolve in water to make exactly 1000 mL.

(2) Phthalate pH standard solution—Reduce potassium hydrogen phthalate for pH determination to a fine powder, and dry at 110°C to constant mass. Weigh 10.21 g (0.05 mole) of it accurately, and dissolve in water to make exactly 1000 mL.

(3) Phosphate pH standard solution—Reduce potassium dihydrogenphosphate for pH determination and disodium hydrogenphosphate for pH determination to fine powders, and dry at 110°C to constant mass. Weigh 3.40 g (0.025 mole) of potassium dihydrogenphosphate and 3.55 g (0.025 mole) of disodium hydrogenphosphate 12-water accurately, and dissolve in water to make exactly 1000 mL.

(4) Borate pH standard solution—Allow sodium tetraborate for pH determination to stand in a desiccator (saturated sodium bromide aqueous solution) until it reaches constant mass. Weigh 3.81 g (0.01 mole) of it accurately, and dissolve in water to make exactly 1000 mL.

(5) Carbonate pH standard solution—Dry sodium hydrogen carbonate for pH determination in a desiccator (silica gel) to constant mass, and weigh 2.10 g (0.025 mole) of it accurately. Dry sodium carbonate for pH determination between 300°C and 500°C to constant mass, and weigh 2.65 g (0.025 mole) of it accurately. Dissolve both reagents in water to make exactly 1000 mL.

(6) Calcium hydroxide pH standard solution—Reduce calcium hydroxide for pH determination to a fine powder, transfer 5 g to a flask, add 1000 mL of water, shake well, and allow the solution to become saturated at a temperature between 23°C and 27°C. Then filter the supernatant at the same temperature and use the clear filtrate (about 0.02 mol/L).

The pH values of these pH standard solutions at various temperatures are shown in the *Table* below. pH values at an arbitrary temperature not indicated in this *Table* can be calculated by the interpolation method.

The pH values of the pH standard solutions

Temperature (°C)	Oxalate pH standard solution	Phthalate pH standard solution	Phosphate pH standard solution	Borate pH standard solution	Carbonate pH standard solution	Calcium hydroxide pH standard solution
0	1.67	4.01	6.98	9.46	10.32	13.43
5	1.67	4.01	6.95	9.39	10.25	13.21
10	1.67	4.00	6.92	9.33	10.18	13.00
15	1.67	4.00	6.90	9.27	10.12	12.81
20	1.68	4.00	6.88	9.22	10.07	12.63
25	1.68	4.01	6.86	9.18	10.02	12.45
30	1.69	4.01	6.85	9.14	9.97	12.30
35	1.69	4.02	6.84	9.10	9.93	12.14
40	1.70	4.03	6.84	9.07		11.99
50	1.71	4.06	6.83	9.01		11.70
60	1.73	4.10	6.84	8.96		11.45

Apparatus

A pH meter generally consists of an electrode system of a glass electrode and a reference electrode, an amplifier and an indicating unit for controlling the apparatus and for displaying the measured value of electromotive force. The indicating unit is usually fitted with dials for zero and span (sensitivity) adjustment. Sometimes a temperature compensation dial is included.

The reproducibility of a pH meter should be within 0.05 pH unit, when measurements for an arbitrary pH standard solution are repeated five times, following the procedure described below. After each measurement it is necessary to wash the detecting unit well with water.

Procedure

Immerse the glass electrode previously in water for more than several hours. Start the measurement after confirming stable running of the apparatus. Rinse well the detecting unit with water, and remove the remaining water gently with a piece of filter paper.

To standardize the pH meter, two pH standard solutions are usually used as follows. Immerse the detection unit in the phosphate pH standard solution and adjust the indicat-

ed pH to the pH value shown in the *Table*. Next, immerse the detection system in the second pH standard solution, which should be selected so that the expected pH of the sample solution to be determined is between the pH values of the two pH standard solutions, and measure the pH under the same conditions as used for the first pH standard solution. Adjust the indicated pH to the defined pH value using the span adjustment dial, when the observed pH is not identical with that tabulated. Repeat the above standardization procedure until both pH standard solutions give observed pH values within 0.02 pH unit of the tabulated value without further adjustments. When a pH meter is fitted with a temperature compensation dial, the standardization procedure is done after the setting of the temperature to that of the pH standard solution to be measured.

In the case of using an apparatus having an auto-calibration function, it is necessary to confirm periodically that the pH values of two pH standard solutions are identical with the tabulated values within 0.05 pH unit.

After finishing the standardization procedure described above, rinse well the electrodes with water, remove the attached water using a filter paper, immerse the electrode system in the sample solution, and read the indicated pH value after confirming the value is stable. If necessary, a sample solution can be agitated gently.

In the pH determination, the temperature of a sample solution must be controlled to be the same as that of the pH standard solutions with which the pH meter was standardized (within 2°C). When a sample solution is alkaline, the measurement should be done in a vessel with a cover and if necessary, in a stream of inert gas such as nitrogen. Furthermore for a strongly alkaline solution above pH 11 containing alkali metal ions, an alkali error may be induced in the pH measurement. Thus, in such a case, an electrode with less alkali error should be used and an appropriate correction should be applied to the measured value.

Note: Construction and treatment in detail are different for different pH meters.

46. Powder Particle Size Determination

Powder Particle Size Determination is a method to determine directly or indirectly morphological appearance, shape, size and its distribution of powdered pharmaceutical drugs and excipients to examine their micromeritic properties. Optical microscopy and analytical sieving method may be used depending on the measuring purpose and the properties of test specimen. "Powder" here means a gathering of numerous solid particles.

Method 1. Optical Microscopy

The optical microscopy is used to observe the morphological appearance and shape of individual particle either directly with the naked eye or by using a microscopic photograph, in order to measure the particle size. The particle size distribution can also be determined by this method. When examining the crystallinity, a polarizing device is attached to the microscope, or a polarizing microscope is used.

This method can generally be applied to particles in the size range between 0.5 and 100 μm. It is also possible with this method to measure the size of the individual particle even when different kinds of particles mingle if they are optically distinguishable.

Apparatus

An optical microscope consists of a lens barrel that houses the optical system consisting of the objective and ocular, a mirror stand and column to support the illumination system, a stage for holding the test specimen, and microscope base to support all these sections. The lens barrel is moved up and down in the column with handles for coarse and fine adjustments, so that the focus can be adjusted. In addition, there is usually a built-in optical system (light source, reflecting mirror, diaphragm, and condenser) making the path for the enlarged image of the sample through the objective and ocular.

The microscopic magnification (product of the objective magnification and ocular magnification) must be sufficient to allow adequate characterization of the smallest particles in the test specimen.

Data processing techniques, such as image analysis, can be useful for determining the particle size distribution. The polarizing devices and color filters of relatively narrow spectral transmission are also useful to adjust the contrast with the background.

Preparation of Test Specimen

In order to ensure the uniformity of test specimen, preprocessing of the particulate matter is performed using an appropriate reduction method. The test specimen should be in a state where it accurately represents the particle size distribution of the original particulate matter. After the preprocessing, the test specimen is prepared with the following methods: It is necessary to make it possible to adequately distinguish the individual particle within the field of view.

(1) Dry method: The sample material is sprinkled onto the slide glass, little by little, and this is used as the test specimen.

(2) Wet method: The sample material is suspended in an appropriate liquid which does not dissolve the sample. One drop of the suspension is placed on a slide glass and used as the test specimen directly, or used after drying.

Procedure

When the particle size is measured, an ocular micrometer is inserted at the position of the ocular diaphragm, and a calibrated stage micrometer is placed at the center of the microscope stage and fixed in place. The ocular is attached to the lens barrel and adjusted to the focus point of the stage micrometer scale. Then, the distance between the scales of the two micrometers is determined, and the sample size equivalent to 1 division of the ocular scale is calculated using the following formula:

The particle size equivalent to 1 division on the ocular scale (μm)

$$= \frac{\text{Length on the stage micrometer } (\mu m)}{\text{Number of scale divisions on the ocular micrometer}}$$

The stage micrometer is removed and the test specimen is placed on the microscope stage. After adjusting the focus, the particle sizes are determined from the number of scale divisions read through the ocular.