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:

$$\text{The particle size equivalent to 1 division on the ocular scale } (\mu\text{m}) = \frac{\text{Length on the stage micrometer } (\mu\text{m})}{\text{Number of scale divisions on the ocular micrometer}}$$

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.

Method 2. Analytical Sieving Method

The analytical sieving method is a method to estimate the particle size distribution of powdered pharmaceutical drugs by sieving, which is usually applicable to powdered materials having a particle size of more than about 75 μm. Essentially, this method is to evaluate the two-dimensional size of the samples. The particle size determined by this method is shown as the size of a minimum sieve opening through which the particle passes.

Apparatus and instruments

(1) Sieves

Use the sieves specified in Measuring Instruments, Appliances under General Tests, Processes and Apparatus. Unless otherwise specified, principally use 200 mm-sieves made of stainless steel. To avoid loosing sample and the change in sieve opening caused by distortion of the frame, it is necessary to handle the sieves carefully. Remove the particles from the aperture of sieve, without beating the frame of the sieve or strongly brushing the mesh-wires of the sieve, by using a brush for sieve, an air jet, cleaning agents or organic solvents carefully not to injure the mesh-wires. After cleaning, dry the sieves in a suitable drying chamber at temperatures below 100°C. The sieves treated as described above should be used after checking that they are not disordered.

(2) Balances

Use balances readable to the nearest 0.1 g.

(3) Apparatus

Use either a ro-tap-type or electromagnet-type sieve shaker.

Sampling

(1) Sample should be taken so as to be representative of the test specimen. When the amount of the sample taken is larger, divide it into a suitable amount by an adequate way.

(2) Usually, the amount of sample is between 25 and 100 g, depending on the bulk density of the sample.

(3) The most appropriate sample mass is determined as the following example: Take 25, 50, 75 and 100 g of the sample, and proceed test sieving with them as directed in Procedure tentatively. If the results obtained with 25-, 50- and 75-g samples are similar, but the percentage through the finest sieve for the 100-g sample is lower compared with the cases of 25-, 50- and 75-g samples, 100 g is too large as the sampling size of the specimen.

(4) In the case that the amount of sample is less than 25 g, 75-mm diameter sieves may be used, though the amount of sample should be not less than 10 g.

Pretreatment of sample

The following treatments may be performed, depending on the properties of the sample:

(1) Drying agglomerated samples owing to their hygroscopicity under a condition which does not change the essential qualities of the sample.

(2) Sieving the agglomerated sample through a coarse mesh sieve previously to deagglomerate it.

(3) Addition of adequate additives to adhesive or agglomerated samples due to their electrostatic charge in an amount which does not affect the results to avoid the generation of electrostatic charge.

Procedure

Usually, this method is proceeded under the controlled temperature and humidity conditions, taking into consideration of the physicochemical characteristics such as hygroscopicity or static electricity.

Unless otherwise specified, select sieves which cover the entire particle size range of the sample to be tested. Place the sieves one upon another on a collecting pan in order from small to large opening, place the sample on the top sieve, replace the lid, and fix the nest of sieves on a mechanical shaker. Agitate the nest of sieves for the time period previously obtained by the endpoint determination and then remove each sieve from the nest. If there is some fine powder on the down surface of each sieve, take it off by the brush gently, and combine it with the sieve fraction retained on each next down sieve, then weigh each sieve and the collecting pan. Determine the mass of material on each sieve and in the collecting pan by the following equation to obtain the particle size distribution. The difference between the mass of the sample taken and the total mass of sample on each sieve and in the collecting pan, the total loss, must not exceed 2% of the mass of the original test specimen.

$$\text{Amount of the material on each sieve (\%)} = \frac{W_i}{W_T} \times 100$$

W_i: Mass of the material on each sieve (g)
W_T: Total mass of the material on each sieve and in the collecting pan (g)

Particle size distribution: Cumulate the amount of the material on each sieve in order from smaller to larger opening, and obtain cumulative amount undersize (%) corresponding to the sieve opening (μm).

Endpoint Determination

Unless otherwise specified, it is decided as the endpoint when the change in mass of material on any sieve is not more than either 5% or 0.1 g after repeating the continuous agitating for every 5 minutes.

47. Pyrogen Test

The Pyrogen Test is a method to test the existence of pyrogens by using rabbits.

Test animals

Use healthy mature rabbits each weighing not less than 1.5 kg which have not lost body mass when kept on a constant diet for not less than one week. Do not use the rabbits repeatedly in the same test unless as long a resting period as possible is taken. Animals should be excluded which have been used for a previous test that was decided as pyrogen-positive.

Record the rectal temperature four times at 2-hour intervals during 1 to 3 days prior to the test. House the animals individually during this period in an area free from disturbances likely to excite them, and exercise particular care to avoid disturbances on the day of the test.

Keep the temperature in an area of performing test uniform between 20°C and 27°C and preferably maintain constant humidity for at least 48 hours before the test.

Apparatus

(1) Thermometer—Use a rectal thermometer or any other temperature-recording devices of equal sensitivity for which the time necessary for reading the rectal temperature