trum of the substance expected to be found or the spectrum of the Reference Standard exhibit similar intensities of absorption at the same wave numbers, the specimen can be identified as being the substance expected to be found. Furthermore, when several specific absorption wave numbers are specified in the monograph, the identification of a specimen with the substance expected to be found can be confirmed by the appearance of absorption bands at the specified wave numbers.

(1) *Identification by the use of a Reference Standard*

When the spectra of a specimen and the Reference Standard exhibit similar intensities of absorption at the same wave numbers, the specimen can be identified as being the same substance as the Reference Standard. When a sample treatment method for a solid specimen is indicated in the monograph in the case of nonconformity of the spectrum with that of the Reference Standard, treat the specimen being examined and the Reference Standard in the same manner as directed in the monograph, then repeat the measurement.

(2) *Identification by the use of a Reference Spectrum*

When the spectra of a specimen and the Reference Spectrum exhibit similar intensities of absorption at the same wave numbers, the specimen can be identified as being the same substance associated with the Reference Spectrum. When a sample treatment method for a solid specimen is indicated in the monograph in the case of nonconformity of the spectrum with the Reference Spectrum, treat the specimen being examined as directed in the monograph, then repeat the measurement.

(3) *Identification by the use of absorption wave number*

When several specific absorption wave numbers of the substance being examined are specified in the monograph, a specimen can be identified as being the same substance as the expected substance by confirmation of clear appearance of the absorption bands at all the specified wave numbers.

Reference spectra

Infrared Reference Spectra, in the range between 4000 cm^{-1} and 400 cm^{-1}, are shown at the end of this book for the monographs requiring the identification test by *Infrared Spectrophotometry*, except for monographs in which "Identification by absorption wave number" is specified.

24. Insoluble Particulate Matter Test for Injections

The test is to examine the size and the number of insoluble particulate matters in injections. When Method 1 is not applicable (as in the case where the preparation of a 25 mL sample solution is impossible or in another case of a protein formulation where observed data by Method 1 has exceeded the limit specified in Method I), the test should be carried out according to Method 2. The test specified herein is not applied to emulsion type or suspension type injections.

Method 1. Light Obscuration Particle Count Test

Instrument Standardization

Apparatus

The apparatus is an electronic particle counting system that uses a light-obscuration sensor with a suitable sample feeding device. Commercially available sensors employ a tungsten lamp, LED, or laser as light source, showing a difference in light wavelength for detection and consequently in sensitivity. Moreover, the sensor concentration limits (the maximum rated particle concentration) is difference of the mechanism of particulate-detecting unit.

The sample feeding device is different by its types such as the compression type or suction type, then, the standardization of the automatic light obscuration particle counter should be the basis for evaluating the performance of apparatus to be used. It is necessary to perform calibration, as well as to demonstrate the sample volume accuracy, sample flow rate, particle size response curve, sensor resolution, and counting accuracy, at least once a year.

Calibration

Particles to be used for calibration should be subject to particle-size sensitivity measurement, using spherical polystyrene particles having at least 5, 10 and 25 μm in diameter (PSL particles) in mono-dispersed suspension. The PSL particles should have either a domestic or international traceability in terms of length, with a level of uncertainly at not greater than 3%.

Manual method

The particle size response of the system to be applied should be determined using at least 3 channels for threshold-voltage setting, according to the half counting method of window moving type. The threshold-voltage window should be $\pm$20% of the measuring particle size. After measuring the sensitivity of response for the designated particle size, the size response curve is prepared by the method indicated by the manufacturer from particle-response measuring point, and threshold-voltage of 5, 10 and 25 μm of the apparatus is obtained.

Electronic method

In the use of multichannel peak height analyzer, the particle size response is measured by half-count method of moving window system same as the manual method, and the particle size response curve is prepared by the method designated by the instrument manufacturer, then, the threshold voltage of 5, 10 and 25 μm of the apparatus is obtained. In this case, the instrument manufacturer or the user should validate the obtainability of the same result as that of the manual method.

Automated method

The particle size response curve of the apparatus may be obtained by using the software developed by the user or supplied by the instrument manufacturer, whereas, the manufacturer or the user should validate the obtainability of the same result as that of the manual method.

Sample volume accuracy

Sample volume accuracy should fall within 5% of the measuring value in case measuring the decrease of test solution by the mass method after measuring the test solution of 10 mL.

Sample flow rate

The flow rate of the sample indicated into the sensor should be calculated from the observed sample volume and time, and should be conformed within the range of the manufacturer's specification for sensor used.

Sensor

There is a possibility of changes of particle size resolution

and counting rate of particle-detecting sensor in each sensor by assembling accuracy and parts accuracy even in the same-type sensor. The threshold accuracy also needs to be confirmed. Testing should be accordingly be performed for each of particle size resolution, accuracy in counting and in threshold setting, using Particle Count Reference Standard Suspension (PSL spheres having mean diameter of approximately 10 μm, of a concentration at 1000 particles/mL ±10%, not more than 5% of CV-value).

During measurement, stirring should be made for assuring the uniformity in sample concentration.

Sensor resolution (Particle size resolution of apparatus)

Measurement should be made by either one of the following methods.

1. Manual method to be obtain the spread of histogram prepared from the counting value of the apparatus.
2. Electronic method to obtain the spread of histogram of the classification of system-responding signal by using the multichannel peak height analyzer.
3. Automated method to obtain the spread of histogram of responsive signal of the test-particle by using the software prepared by the manufacturer or the user.

The difference between the threshold of particle size counting 16 and 84% of the total counts and the test-particle size should be within 10%, whereas, electronic method and automated method should be both validated for obtaining the same result as that of the manual method.

Particle counting accuracy

Data obtained by counting particles of 5 μm and greater should be 763 to 1155 particles per 1 mL.

Threshold accuracy

Particle size calculated from a threshold corresponding to 50% counts for particles of 5 μm and greater should fall within ±5% of the mean diameter of the test particles.

Reagents

Purified water for particulate matter test: The purified water containing not more than 5 particles of 10 μm or greater size, and not more than 2 particles of 25 μm or greater size in 10 mL of the insoluble particle number measured by the light obscuration particle counter.

Procedure

The test should be performed in a clean and preferably dust-free equipment of device, with care to avoid particulate contamination as much as possible.

Aqueous injections (1 unit of less than 25 mL in volume)

Open a proper number of units of injections, and collect the content in clean container to prepare sample solution of not less than 25 mL. If the unit volume is too small to prepare 25 mL of sample solution, use a proper number of units and an appropriate diluting solution to prepare sample solution. Deaerate the test solution by sonication for 30 seconds, or by allowing to stand for a proper time.

Gently stir the test solution by hand-swirling or mechanical means to homogenized the particles in the solution while avoiding the generation of air bubbles and contamination with foreign matter. Withdraw a minimum of three sample aliquots into the light obscuration counter sensor, each not less than 5 mL in volume. Obtain average particle number by discarding the initial measured value, then calculate the number of particles in 1 mL of the test solution.

Aqueous injections (1 unit of more than 25 mL in volume)

Repeat inverting the sample unit 20 times to mix the content well, and deaerate by the sonication or by allowing to stand for a while. Open the container, mix the content by hand-swirling or mechanical means, and set directly to the tube for inducing the test solution, or transfer the test solution to a clean container, and use as the test solution. Repeat the measurement at least three times, each with a volume of not less than 5 mL of the test solution. Obtain average particle number by discarding initial measured value, then calculate the number of particles in 1 mL of the test solution.

Injectable sterile solids or lyophilized injections

While avoiding any contamination with foreign matter, remove the closure to open the container. Add a proper amount (a claimed volume for constitution) of purified water for particulate matter test or appropriate diluent without contamination of particles when the purified water is not suitable for constitution. Mix the reconstituted solution by hand-swirling or mechanical means, and then test as directed in the method for aqueous injections.

Powder injections with constituted solution

Dissolve the content by the method described on the label and test the solution on the method for aqueous injections.

Method 2. Microscopic Particle Count Test

Apparatus

Use a microscope, filter assembly for retaining insoluble particulate matter and membrane filter for observation.

Microscope: The microscope is equipped with an ocular micrometer calibrated with an objective micrometer, a moving stage and an illuminator, and is adjusted to 100 magnifications.

Filter assembly for retaining insoluble particulate matter: The filter assembly for retaining insoluble particulate matter consists of a filter holder made of glass or a proper material uncapable of causing any trouble in testing, and a clip. The unit is capable of fitting with a membrane filter 25 mm or 13 mm in diameter and can be used under reduced pressure.

Membrane filter for testing: The membrane filter is white in color, 25 mm or 13 mm in diameter, 0.45 or 0.5 μm in nominal pore size and is imprinted with about 3 mm grid marks. Upon preliminary testing, the insoluble particulate matter on the filter is not more than 5 particles that are equal to or greater than 10 μm, and particles that are equal to or greater than 25 μm should not be found. When necessary, wash the filter with purified water for particulate matter test.

Reagents

Purified water for particulate matter test: Purified water which contains not more than 10 particles of 10 μm or greater size in 100 mL. Prepare before use by filtering through a membrane filter with a nominal pore size of 0.5 μm or less.

Procedure

Large-volume injections

Carry out all operations carefully in clean equipment and facilities which are low in dust. Fit the membrane filter onto the membrane filter holder, and fix them with the clip. Thoroughly rinse the holder inside with the purified water for particulate matter test, and filter under reduced pressure with 200 mL of the purified water for particulate matter test at a rate of 20 to 30 mL per minute. Apply the vacuum until the surface of the membrane filter is free from water, and remove the membrane filter. Place the filter in a flat-bottomed

petri dish with the cover slightly ajar, and dry the filter fully at a temperature not exceeding 50°C. After the filter has been dried, place the petri dish on the stage of the microscope. Under a down-light from an illuminating device, adjust the grid of the membrane filter to the coordinate axes of the microscope, adjust the microscope so as to get the best view of the insoluble particulate matter, then count the number of particles that are equal to or greater than 10 μm within the effective filtering area of the filter, moving the mobile stage, and ascertain that the number is not more than 20. In this case the particle is sized on the longest axis.

Fit another membrane filter to the filtration device, and fix them with the clip, then wet the inside of the filter holder with several mL of purified water for particulate matter test. Clean the exterior of the container, and mix the sample solution gently by inverting the container several times. Remove the closures carefully and pour out approximately 50 mL of the solution with gentle mixing in such manner as to wash the opening of the container. Immediately after that, measure 40 mL of the solution using a measuring cylinder, which has been rinsed well with purified water for particulate matter test, and pour it into the filter holder along the inner walls. Apply the vacuum and filter mildly so as to keep the solution always on the filter. As for viscous sample solutions, dilute previously the solution with an appropriate diluent or purified water for particulate matter test, and then filter as described above. When the amount of the solution on the filter becomes small, add 30 mL of purified water for particulate matter test in such manner as to wash the inner walls of the filter holder. Repeat the process 3 times with 30 mL of the water. Apply the vacuum gently until the surface of the membrane filter is free from water. Place the filter in a petri dish, and dry the filter fully at a temperature below 50°C with the cover slightly ajar. After the filter has been dried, place the petri dish on the stage of the microscope, and count the number of particles which are equal to or greater than 10 μm and equal to or greater than 25 μm within the effective filtering area of the filter according to the same procedure of the microscope as described above. In this case the particle is sized on the longest axis.

Small-volume injections

Proceed the test in the manner for large-volume injections, except for using a membrane filter of 13 mm diameter and a filter holder of 4 mm in diameter of particle-retaining mouth.

Aqueous injections

Clean the exterior of container of aqueous injections, mix the content by inverting slowly, then, open carefully, and withdraw the whole content using such a plastics syringe or the like that has not been contaminated with foreign matter. In getting along the inside wall of the filter, pour the sample slowly, and suction slowly for keeping the sample on the filter at any time. For viscous sample, dilute previously with an appropriate diluent or purified water for particulate matter test, and filter similarly. When the sample amount on the membrane filter is reduced, add 30 mL of purified water for particulate matter test or diluent in the manner to wash the inside wall of the filter holder. Moreover, repeat this washing 3 times with each 30 mL, whereas, in the use of the diluent, finally wash with the purified water for particulate matter test, then, suction slowly from the top of membrane filter until water runs out, and proceed in conformity with the operation for the above mentioned large-volume injections.

Injectable sterile solid or lyophilized injections, or injectable sterile solids injections with constituted solution

Prepare sample solution according to the sample preparation procedure for the light obscuration particle count test, and perform the test as directed in the method for aqueous injections.

25. Insoluble Particulate Matter Test for Ophthalmic Solutions

The Test is to examine for the size and the number of insoluble particulate matter in Ophthalmic Solutions.

Apparatus

Use a microscope, filter assembly for retaining insoluble particulate matter and membrane filter for determination.

Microscope: The microscope is equipped with a micrometer system, a mobile stage and an illuminator, and is adjusted to 100 magnifications.

Filter assembly for retaining insoluble particulate matter: The filter assembly for retaining insoluble particulate matter consists of a filter holder made of glass or a proper material uncapable of causing any trouble in testing, and a clip. The unit is capable of fitting with a membrane filter 25 mm or 13 mm in diameter and can be used under reduced pressure.

Membrane filter for testing: The membrane filter is white in color, 25 mm or 13 mm in diameter, not more than 10 μm in nominal pore size and is imprinted with about 3 mm grid marks. Upon preliminary testing, the insoluble particulate matter equal to or greater than 25 μm in size should not be found on the filter. When necessary, wash the filter with purified water for particulate matter test.

Reagents

Purified water for particulate matter test: Purified water which contains not more than 10 particles of 10 μm or greater size in 100 mL. Prepare before use by filtering through a membrane filter with a nominal pore size of 0.5 μm or less.

Procedure

Aqueous ophthalmic solutions

Carry out all operations carefully in clean equipment and facilities which are low in dust. Fit the membrane filter onto the membrane filter holder, and fix them with the clip. Thoroughly rinse the holder inside with the purified water for particulate matter test, and filter under reduced pressure with 200 mL of the purified water for particulate matter test at a rate of 20 to 30 mL per minute. Apply the vacuum until the surface of the membrane filter is free from water, and remove the membrane filter. Place the filter in a flat-bottomed petri dish with the cover slightly ajar, and dry the filter fully at a temperature not exceeding 50°C. After the filter has been dried, place the petri dish on the stage of the microscope. Under a down-light from an illuminating device, adjust the grid of the membrane filter to the coordinate axes of the microscope, adjust the microscope so as to get the best view of the insoluble particulate matter, then count the number of particles that are equal to or greater than 150 μm within the effective filtering area of the filter, moving the mobile stage, and ascertain that the number is not more than 1. In this case the particle is sized on the longest axis.

Fit another membrane filter to the filtration device, and fix