a 10× objective, and have one ocular equipped with a micrometer able to measure accurately particles of 10 and 25 μm linear dimension.

Particles are counted under 100× magnification with the incident light at an angle of 10° to 20°. Obviously, this is a slow and tedious process requiring patience and dedication on the part of the microscopist. Use of higher magnification, up to 400×, may be necessary occasionally to discern discrete particles from agglomerates or amorphous masses. Sometimes, particles not visible with dark field reflected light are very easily observed by means of bright field illumination at 45° polarization.

Two sizes of particles are counted, those having effective linear dimensions ≥10 and ≥25 μm. The counts obtained from the sample membranes are compared with counts obtained from a membrane treated exactly like the sample membrane minus the filtration of the product sample. Blank membrane counts rarely are zero. However, if 5 or more particles ≥25 μm and/or more than 20 particles 10 μm are counted on the blank membrane, the test is invalidated and it signifies a serious problem in one or more of the following areas: poor technique, filter breakdown in the solvent dispenser, poorly cleaned membranes, poorly cleaned filter assemblies, and/or HEPA filter leaks. The problem must be resolved before particle testing can resume.

If the USP limit of not more than 12 particles per mL ≥10 μm and not more than 2 particles per mL ≥25 μm is exceeded, the large-volume injection product fails the USP test for particulate matter. For small-volume parenterals, the test fails if more than 3000 particles/container 10 μm and/or 300 particles per container 25 μm is exceed. For ophthalmic solutions, the limit for particles measured by microscopy is 50 per mL ≥10 μm, 5 per mL ≥25 μm, and 2 per mL ≥50 μm. These limits are also stated in the ICH Q4B document.

Analysis by microscopic techniques suffers from several disadvantages—it is very time-consuming, requires technical expertise, and, because of the manpower requirements, can be very expensive. The major method for determining subvisible particulate matter in parenteral solutions, including reconstituted sterile powders, is the light obscuration technique. However, if any dispute arises regarding fulfillment of USP particulate matter specifications, such disputes must be settled by applying the official USP microscopic method.

LVI particulate matter standards in other countries governed by other compendia will be reviewed in a section at the end of this chapter.

COMPARISON OF ELECTRONIC AND MICROSCOPIC PARTICLE COUNTING METHODS

Difficulties in comparing particle-counting methods result from differences in the way in which different methods determine particle size and distribution. For example, the microscopic method measures size as the longest linear dimension of the particle. The principle of light blockage, utilized by the HIAC particle counter, expresses size as the diameter of a circle of equivalent area as the actual area consumed by the particle. Particle counting by electrical resistance (Coulter Counter) treats the particles as a three-dimensional object and measures the volume consumed by the particle. Thus, the microscope, HIAC, and the Coulter Counter methods size particles in one, two, and three dimensions, respectively.

It is virtually impossible to correlate instrumental and microscopic particle counts directly for irregularly shaped particles (15). As long as the particle is a sphere, all methods will size the sphere equally (Table 29-4). However, as the particle shape deviates from sphericity, the

Table 29-4 Summary of Sphericity Correction Factors Based on Longest Linear Dimension (15)

Shape	D_O Longest dimension	D_H Horizontal projection	D_A Light blockage	D_V Electrolyte displacement
Sphere	1.00	1.00	1.00	1.00
Cube (1:1:1)	1.00	0.90	0.95	0.88
Equant (3:2:1)	1.00	0.88	0.81	0.62
Prolate ellipsoid (2:7:1)	1.00	0.87	0.61	0.52
Flake (4:4:1)	1.00	0.90	0.81	0.55
Rod (3:1 diameter)	1.00	0.81	0.62	0.52
Fiber (rigid, 10:1)	1.00	0.64	0.36	0.25