# **GatewayAnalytical**<sup>®</sup>

## Particulate Matter in Injections and Ophthalmic Solutions: Particle Counting

### **Purpose**

Particulate matter, in both parenteral and ophthalmic drug products, consists of extraneous mobile, undissolved particles, other than gas bubbles, unintentionally present in the solutions. These particles can originate from various sources including, but not limited to: the environment, the manufacturing process, packaging materials and undissolved formulation ingredients. This particulate matter can be harmful when introduced to the body. Therefore, the US Pharmacopeia places limits on the amount of sub-visible particles that are allowed in drug products based on these risks. The chapters recommend two test methods for particulate matter, Light Obscuration Particle Count Test and Microscopic Particle Count Test which determine the amount of particles in the final product in the sub-visible  $(10 - 100 \ \mu m)$ range. Depending on the drug product, its viscosity, potential to create air bubbles, etc., one method might be preferable over the other. Light obscuration, via instrumentation like the familiar HIAC system, provides particle counts within specified size bins, while Method 2 provides particle counts and sizes, along with the opportunity to visualize particulate. Each method, as with any analytical technique, has its advantages and disadvantages. Method 1 provides a robust, unbiased, and efficient way to assess the population of particulate present in a drug product; light obscuration, however, is uninformative regarding the identity of the particles detected. Method 2 provides not only size and count of the particles present, but also the opportunity to make note of the appearance (color and shape) of the particulate; however, manual microscopy is a tedious and subjective technique. Often times, a combined analytical approach of the two methods can provide you with the most information for a given sample.

## **Sample Preparation**

All glassware and filtration ware to be used during preparation for either method is carefully cleaned and rinsed with particlefree water per the USP method. A negative control, or blank, is performed prior to any sample preparation and must pass the specified requirements prior to the commencement of preparation of the samples for either test method.

The sample for testing is slowly inverted twenty times, being careful not to introduce air bubbles during the inversion process. For large volume parenterals, or small volume parenterals with a volume greater than 25mL, single units are tested directly

without the need for dilution with either analytical method. For small-volume parenterals less than 25mL in volume, the contents of 10 or more units are combined in a clean container to obtain a volume a minimum of 25mL. If necessary, ten or more units can be combined and the volume diluted to 25mL with particle-free water. The number of test specimens must be adequate to provide a statistically sound assessment.

For Method 1, light obscuration particle testing, the given volume is sampled directly from the container by the instrument. For Method 2, microscopic particle count test, the given volume is filtered following the specified preparation method outlined in the guidelines, utilizing black or grey, gridded filters with no larger than a 1.0um pore size.

#### USP <788>/<789> Requirements:

- Laminar Flow Environment
- Particle Free Water
- Proper Negative Control/Blank
  - <25 particles greater than 10µm</li>
- Proper Sizing Standard (Liquid Particle Counting)
  - Dispersion of spherical particles of known sizes between 10-25µm
- Proper Stage Micrometer/USP <788>/<789> Graticule/ Reticule
- Up to date USP guidelines online subscription

## **Analysis Methods and Results**



# **Featured Application**

# **GatewayAnalytical**<sup>®</sup>

**Method 1:** Light obscuration particle count test utilizes an automated instrument that performs counting and sizing based on the amount of light that is blocked by the particle as it passes a sensor. This is the preferred method for counting and sizing sub-visible particulate in drug products. Four portions, or aliquots of 5mL each, of the sample are tested by the instrument, disregarding the first aliquot. The mean number of particles is then calculated from the remaining three aliquots/analyses and reported for the sample. An example of data obtained from light obscuration is shown below as well as a corresponding histogram of the particle counts vs. acceptable particle levels.

Run No	Particle Size (µm)	Differential Counts/ Container (10mL)	Differential Counts/mL
Run 1	10.000	7720.00	772.0
	25.000	845.00	84.50
Run 2	10.000	6875.00	687.50
	25.000	754.00	75.40
Run 3	10.000	6456.00	645.60
	25.000	723.00	72.30
Run 4	10.000	6632.00	663.20
	25.000	704.00	70.40
Average	10.000	6654.33	665.43
	25.000	727.00	72.70

\*\*Run 1 is discarded.



\*\*The above data is from a small volume parenteral, with ten vials of drug product pooled together. For small volume parenterals, the passing criteria for Method 1 is no more than 6,000 particles per container equal to or greater than 10 $\mu$ m and no more than 600 particles per container equal to or greater than 25 $\mu$ m per USP <788>. The acceptable particle numbers are different for both large volume parenterals per USP <788>, as well as ophthalmic solutions per USP <40>.

However, certain drug products are not conducive for light obscuration particle counting and can result in artificially high particle counts due to issues like air bubbles, silicone droplets, etc. Drug products may be too viscous, even after dilution to adequately pass through the instrument channels, or the drug product may result in high levels of air bubbles when being injected by the instrument for analysis. **Method 2:** Microscopic Particle Counting is utilized when Method 1 is not suitable for a drug product and is often utilized following a failing result with Method 1. The particle sizing is performed using a suitable binocular microscope, with a magnification of  $100\pm10$  and with the use of a circular ocular graticule. The ocular graticule consists of a large circle divided by crosshairs into quadrants, transparent and black reference circles that are 10um and 25um in diameter at 100x magnification, as well as a linear scale in  $10\mu$ m increments, as shown below. Two different forms of illumination are required, both episcopic and oblique light.



Particle sizing is performed by a scientist by estimating the equivalent circular diameter of the particle in comparison with the 10µm and 25µm reference circles on the graticule. The inner diameter of the transparent circles is used to size white and transparent particles, while dark particles are sized by using the outer diameter of the black opaque graticule reference circles. Using the microscopic particle count test, amorphous, semiliquid, or otherwise morphologically indistinct materials are not taken into account. For this reason, some types of particles that may be counted using light obscuration would not be counted using Method 2. An example of data obtained from the Microscopic Particle Count Test is shown below as well as a corresponding histogram of the particle counts vs. acceptable particle levels.

	Particles per Container >10um	Particles per Container >25um	Particles per mL >10um	Particles per mL >25um
Sample 1	328	54	3.28	0.54

# **GatewayAnalytical**<sup>®</sup>





\*\*The above data is from a large volume parenteral (100mL vial). For large volume parenterals, the passing criteria for Method 2 is no more than 12 particles per mL equal to or greater than 10 $\mu$ m and no more than 2 particles per mL equal to or greater than 25 $\mu$ m per USP <788>. The acceptable particle numbers are different for both small volume parenterals per USP <788>, as well as ophthalmic solutions per USP <789>.

Industry considers one of the valuable aspects of performing microscopy to be the ability to start sourcing/determining root cause of particulate problems, based on what the particles looks like (shape/color). While microscopy does provide basic information that light obscuration particle counting cannot provide, caution should be used when it comes to the ability to source particulate based on microscopy alone. For true particle sourcing and root cause analysis, additional analysis methods should be utilized.

# Representative Particle Images During Method 2 Microscopic Particle Counting:







# **GatewayAnalytical**



2009 Kramer Rd. Gibsonia, Pa 15044 |Tel: +1 724-443-1900, Fax: +1 866-658-1445 info@gatewayanalytical.com |www.gatewayanalytical.com © 2019 All Rights Reserved Gateway Analytical LLC.