Sub-Visible Particles

in Sterile Medicinal Products

WITH DALTON

Peter Pekos

Company Vision

"To make the impossible possible, Dalton Pharma Services uses its scientific and pharmaceutical expertise to bring customer ideas to life. We develop their new drug products, optimize the synthesis of therapeutic candidates, and manufacture them at the highest level of quality."

Disclaimer

This technical report is intended to provide an overview to quality and regulatory professionals on sub-visible and visible particles in sterile pharmaceuticals. This technical report should be read in conjunction with the relevant laws, regulations, and guidance's that apply to your situation.

FDA inspected, HC approved, & MRA with EMA

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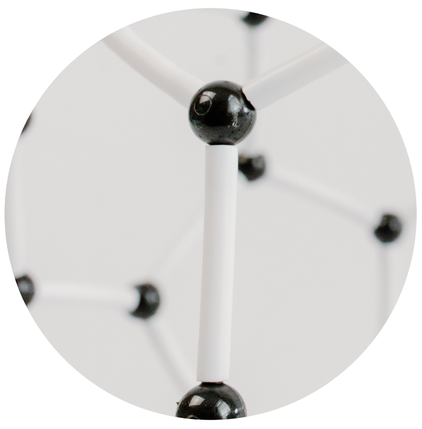
INTRODUCTION



Visible and sub-visible

particles

Visible and sub-visible particles can form in prefilled drug-delivery systems such as parenteral products, and predominantly in biologics, during the manufacture, storage, or transportation process. These particles can have an impact on the drug's effectiveness and may result unwanted immunogenic reactions.

> **FYI:** the United States Pharmacopeia (USP) recognizes drug injection, a drug for injection, drug injectable emulsion, drug injectable suspension, and a drug for suspension as parenteral products.

As a result, global authorities have increasingly tightened regulatory requirements for sub-visible and visible particles in recent years. Today, authorities expect bio/pharma industries to characterize particles and perform root-cause analysis alongside developing, validating, and implementing manufacturing processes, storage conditions, and transport operations to eliminate and/or keep these particulates and other contaminants within permissible levels.

INTERESTING FACT

Particles are one of the main reasons for recalls of sterile drugs, as well as for the issuance of Warning Letters by the US FDA.

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DO LIQUID, STERILE MEDICINAL PRODUCTS HAVE TO BE PARTICLE-FREE?

According to the pharmacopoeia, liquid, sterile medicinal products must be subject to a 100% particle freedom of visible particles. However, sub-visible particles may be present in accordance with prescribed limits set out in pharmacopoeia specifications. For instance, the Light Obscuration counting method may detect up to 25 particles (≤ 10 µm) per mL in containers up to 100 mL according to the European Pharmacopoeia.

WHAT ARE VISIBLE AND SUB-VISIBLE PARTICLES?

PARTICULATE MATTER

Particulate matter is undissolved particles (*excluding gas bubbles*) that are unintentionally present in solutions of parenteral products or biologics.

Visible particles: (approximately 100 µm –150 µm and larger) are particles that can be detected under controlled conditions with the naked eye, meaning without any optical instruments such as a magnifying glass or microscope.

Sub-visible particles: (approximately 0.1 µm –100 µm) are particles that are too large for analysis by size exclusion chromatography but too small to be visible to the unaided eye.

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THE ORIGIN OF PARTICLES

EXTRINSIC PARTICLES

Extrinsic particles originate from the outside environment of the drug product formulation or primary packaging material. Examples of these foreign particles include cellulose fibers originating from disinfectant cloths, a human user, rubber, plastic, or metals.

INTRINSIC PARTICLES

In contrast, **intrinsic particles** originate directly from the formulation of the drug product or during the manufacture of the drug product. Examples include glass flakes that delaminate from the vial wall, interactions of the drug product formulation components, or their contact with primary packing materials or processing aids.

INHERENT PARTICLES

Inherent particles originate during the formulation process, for instance, when an active ingredient or excipient forms a haze, aggregates, or crystallizes. This particle formation might be caused by increased shearing forces, among other things. Inherent particles do not influence the drug's effectiveness, and therefore, do not have adverse impacts on the patient.

Note: Although air bubbles and silicone oil droplets do not fit into the above classifications, they often appear as particles in the majority of analysis methods due to their high reflectivity.

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METHOD OF ANALYSIS

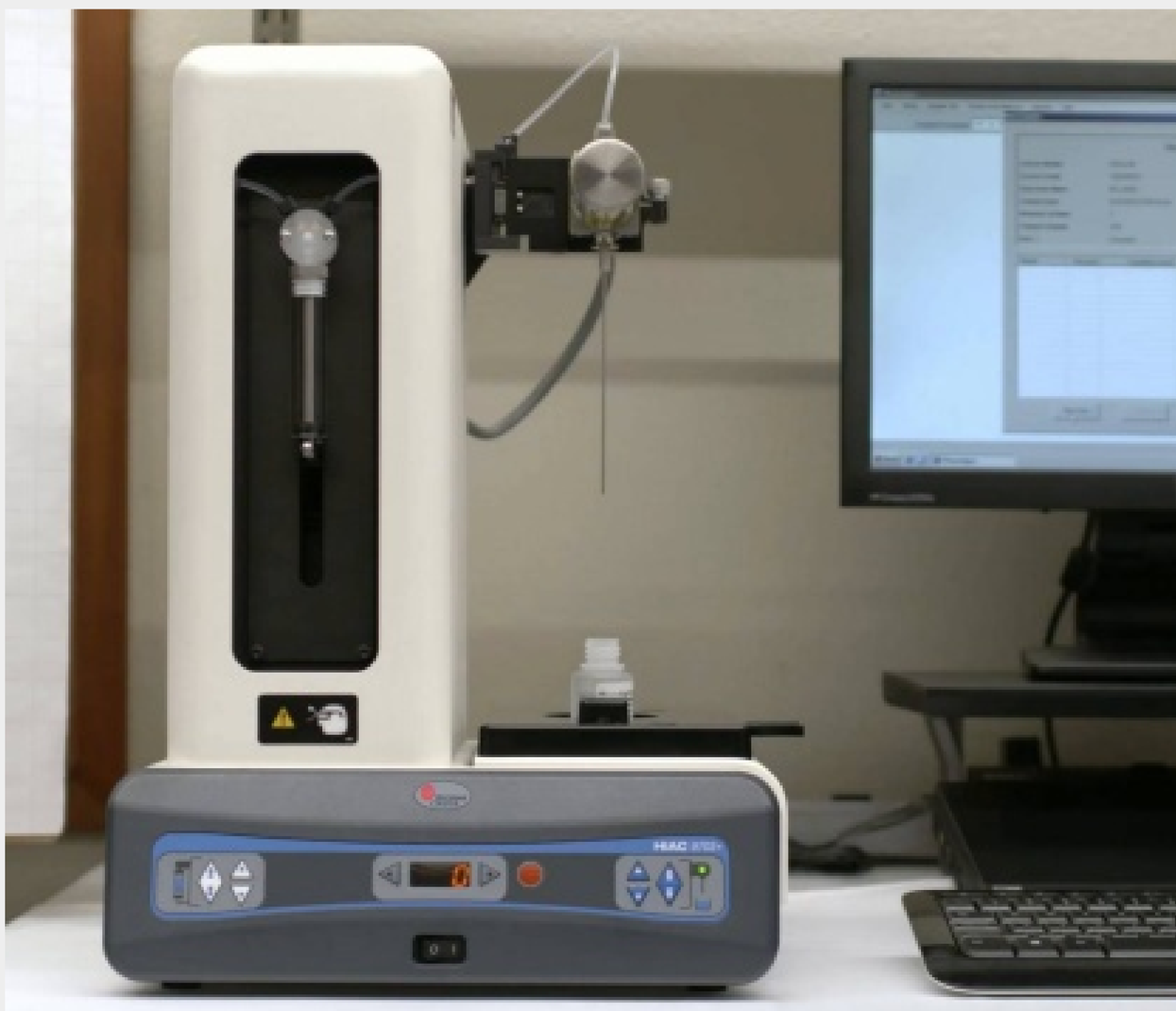
Visible particles

The standard procedure to identify the presence of visible particles is a visual check of the filled units combined with a subjective description of any visible contaminants.

Sub-visible particles

For sub-visible particles, the standard procedure for quantification is a Light Obscuration Particle Count Test and a Microscopic Particle Count Test.

- For both traditional parenteral products and biologics, Light Obscuration Particle Count Testing is preferred. This technique identifies the quantity and size of sub-visible particles in the 1–100 µm range but it cannot describe the particle's morphology and chemical properties.
- However, for traditional parenteral products with reduced clarity or increased viscosity (i.e., emulsions, colloids, and liposomal formulations) Microscopic Particle Count Testing is preferred. Note that microscopic particle count testing cannot provide reliable measurements for protein biologics.



USP LIMITS FOR SUB-VISIBLE PARTICLES

Light Obscuration Particle Count Limits for Therapeutic Protein Injection and Other Parenteral Products.

- For sample preparations with a volume of less than or equal to 100 mL, the average number of particles present in the units tested should not exceed 6000 particles per container equal to or greater than 10 µm, and not exceed 600 particles per container equal to or greater than 25 µm.
- For sample preparations with a volume greater than 100 mL, the average number of particles in the units tested should not exceed 25 particles per mL equal to or greater than 10 µm and should not exceed 3 particles per mL equal to or greater than 25 µm. Additionally, for product volumes greater than 100 mL, the total particle load should meet the same limits as the particle load for products less than or equal to 100mL. This means the total particle load should not exceed 6000 particles per container equal to or greater than 10 µm and should not exceed 600 particles per container equal to or greater than 25 µm.
- Note that for products first reconstituted in less than 100 mL and then diluted for infusion in a volume greater than 100 mL, particle content should be assessed both before and after dilution and evaluated based on their final volume.
- The limit values for ophthalmic drugs are even more strict: particles greater than 10 µm in size are limited to at or below 50 particles/container and particles greater than 25 µm in size are limited to at or below 1 particle/container

FYI: Ophthalmic are anti-infectives and include eyedrops, gels, or ointment.

Microscopic Particle Count Method Limits for Parenteral Products That Are Not Protein Therapeutics.

For sample preparations with a volume of more than 100 mL, the average number of particles present cannot exceed 12 particles per mL equal to or greater than 10 µm and cannot exceed 2 particles per mL equal to or greater than 25 µm. For samples with a volume of fewer than 100 mL, the average number of particles present in the units tested cannot exceed 3000 per container equal to or greater than 10 µm and cannot exceed 300 per container equal to or greater than 25 µm.

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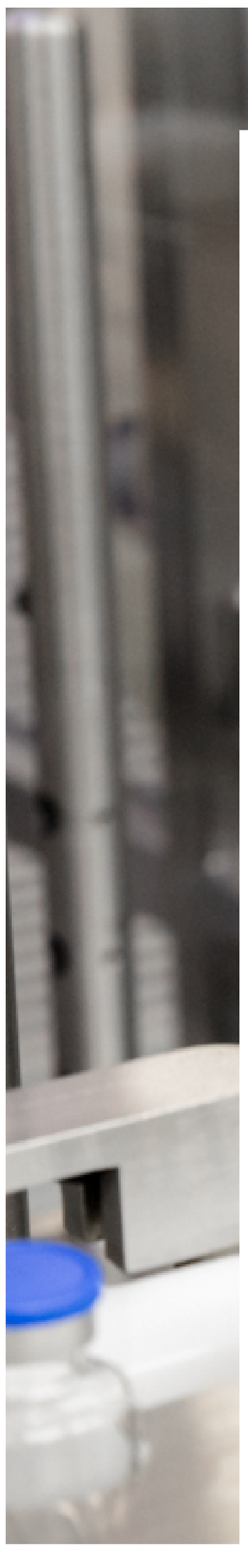
KEY INNOVATIVE TECHNOLOGY TO DETECT AND CHARACTERIZE SUB-VISIBLE PARTICLES

A range of novel analytical techniques are on the rise to provide comprehensive particle characterization and identification, particularly for sub-visible particles. The detection and characterization methods available are dependent on several factors: particle size, number, shape, optical properties, and chemical identification.



Archimedes' Resonant Mass Measurement (RMM): This method is used to detect sub-visible particles between 50 nm – 5 µm. Method: Measures changes in resonant frequency as individual particles pass through a mechanically resonating microfluidic channel. This data generates buoyant mass, dry mass, and morphology information. This technique is, therefore, able to distinguish between silicone oil, which is less dense than water, and proteinaceous particles, in the size range where flow microscopy loses resolution. Furthermore, RMM has the advantage of being able to *directly* differentiate silicone oil droplets from protein particles without relying on post-measurement data manipulations.

Micro-flow imaging (MFI): This technique is used to detect sub-visible particles between 1 µm – 70 µm. Method: Merges digital microscopy with modern microfluidics, by running a continuous sample stream through a flow-cell positioned in the field of view of a microscopic system while bright-field images are taken in successive frames. An image morphology analysis software enables the determination of particle number, size, intensity, coincidence, and particle type (i.e., air bubble, silicone oil droplet, protein aggregate, or fiber).

Automated Particle Analysis and Raman Spectroscopy (APA & RS): This technique is used to detect sub-visible particles between 1 µm and 1000 µm. Method: This approach combines the automated static imaging capabilities of a high-resolution modern digital microscope with Raman spectroscopy to obtain the chemical identification, size, and shape of individual particles.

FYI: Raman spectroscopy is insensitive to the presence of water, enabling the characterization of aqueous solutions as well.

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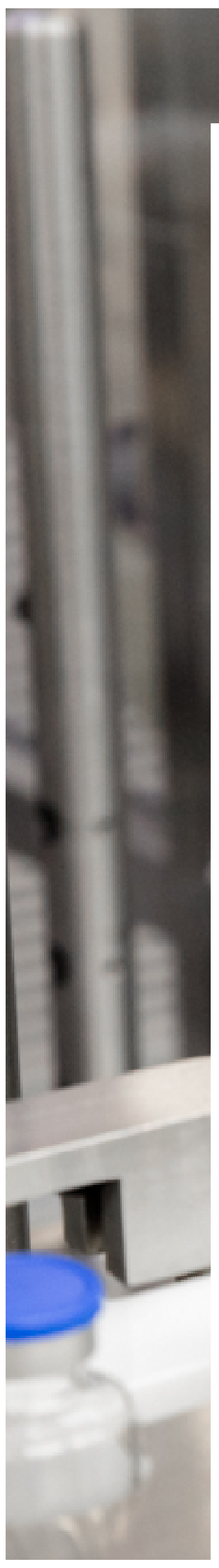
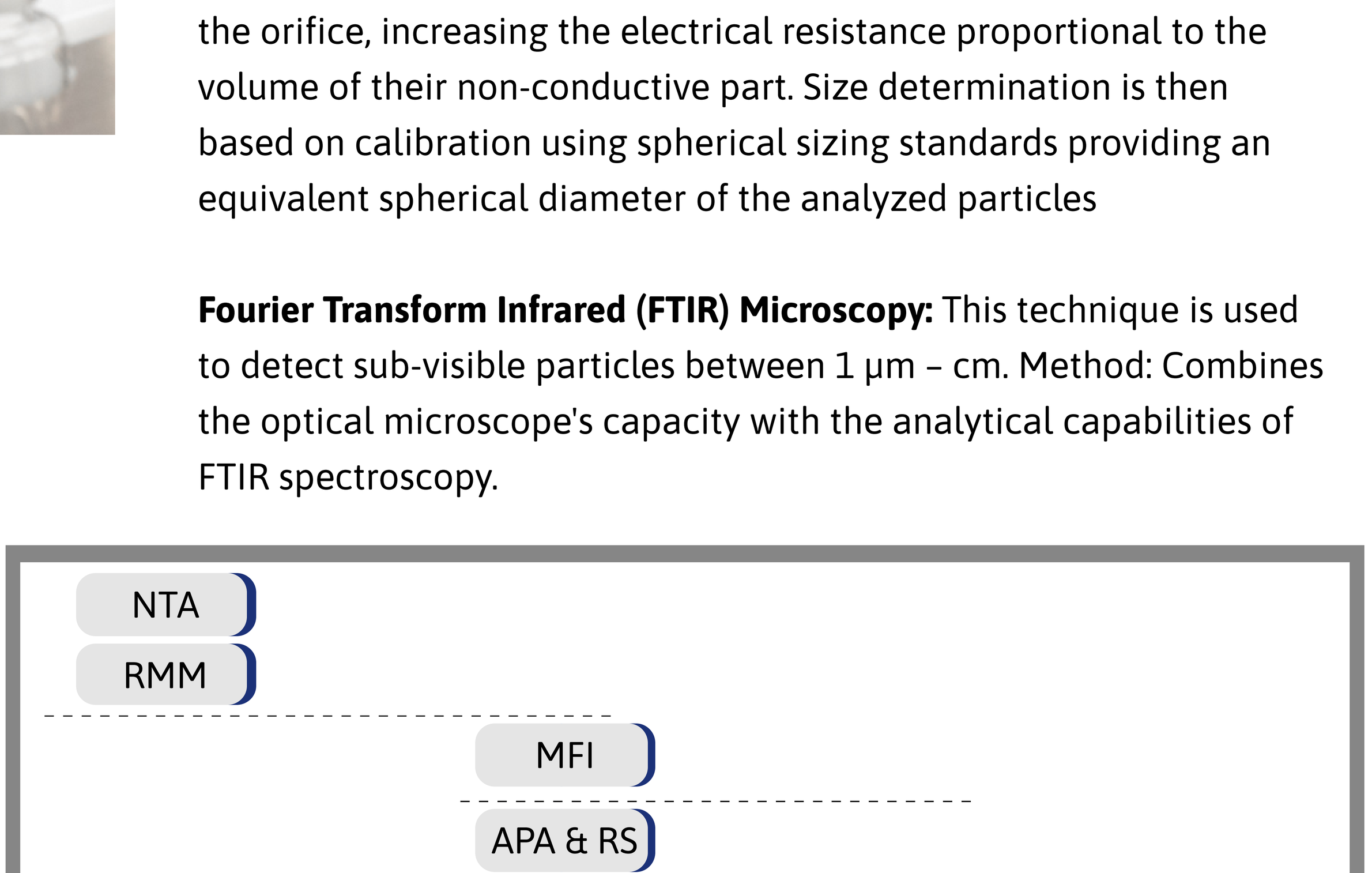


Nanoparticle tracking analysis (NTA): This technique is used to detect sub-visible particles between 50 nm – 1000 nm (or 1 µm). Method: NTA employs a high-resolution digital camera, a laser, and specially designed software to track the Brownian motion of individual particles under a microscope. Because each particle is sized separately and within a defined sample volume, the particle concentration, or particle count, may also be calculated. These capabilities offer a solution to the rising need to understand the contribution of all sub-visible particle size fractions.

Electrical Sensing Zone (ESZ): This technique is used to detect sub-visible particles between 0.5 µm – mm. Method: An electrical field is applied between opposing sides of an orifice, whereby particles suspended in a conductive electrolyte solution are aspirated through the orifice, increasing the electrical resistance proportional to the volume of their non-conductive part. Size determination is then based on calibration using spherical sizing standards providing an equivalent spherical diameter of the analyzed particles

Fourier Transform Infrared (FTIR) Microscopy: This technique is used to detect sub-visible particles between 1 µm – cm. Method: Combines the optical microscope's capacity with the analytical capabilities of FTIR spectroscopy.



REGULATORY OVERVIEW

The increasing global regulatory requirements on sub-visible particles demonstrate its evolving importance in the quality, safety, and effectiveness of parenteral products and biologics.

USP: The current recommendation reflects rising concern about particles in the 0.1 µm – 10 µm size range and suggests determining particle concentrations for the 2 µm and >5 µm fractions, as well as enumerating particulates in the >10m and >25m size ranges. It also asks for the ongoing examination of particles in the 2 µm – 10 µm range of a product's shelf life. Particle concentration limitations are given for the larger size ranges, but the guidance also allows for product-specific restrictions to be established. The USP issued the amended chapter on testing for sub-visible particles <1788> In May 2020, along with new draft monographs on the following testing methods: Light Obscuration, Membrane Microscopy, and Flow Imaging.

FDA: The new FDA guidance emphasises the need to differentiate silicone oil from proteinaceous, inherent, and intrinsic particles, and strongly recommended to characterize particles in smaller (0.1 – 2 µm) size ranges as new technologies become available.

EMA: Similarly, the EMA guidance, specifies that the formation of aggregates, sub-visible, and visible particulates in the drug product should be extensively researched and monitored on batch release and throughout stability studies. The guidance also indicates that other orthogonal analytical procedures, in addition to the pharmacopoeial test for particulate matter, may be required to assess the amount and kind of particles.

Health Canada: Health Canada adopted the ICH guidance Q4B Annex 3(R1): Evaluation and Recommendation of Pharmacopoeial Texts for Use in the ICH Regions on Test for Particulate Contamination: Sub-Visible Particles General Chapter, in 2010.

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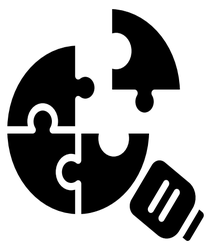
WHAT DOES THIS MEAN FOR MANUFACTURERS?

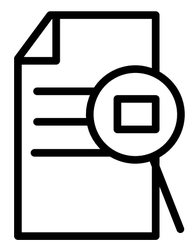


Challenges

- Detection for each product is a time-consuming process
- Advancing regulatory requirements to improve upon existing methodologies
- Limited number of methods especially for high-concentration protein solutions
- Difficult to quantify due to limitations of analytical techniques

New Ways of Working

- Required to learn the application of uprising innovative technologies
- Pharmaceutical industries and manufacturers must collaborate to help define the quantitative capabilities of current particle counting methods



Tips

- Monitor particulate matter throughout the products lifecycle
- Determine the best test methodology for your product
- Self-regulate and adopt innovative methods of analysis

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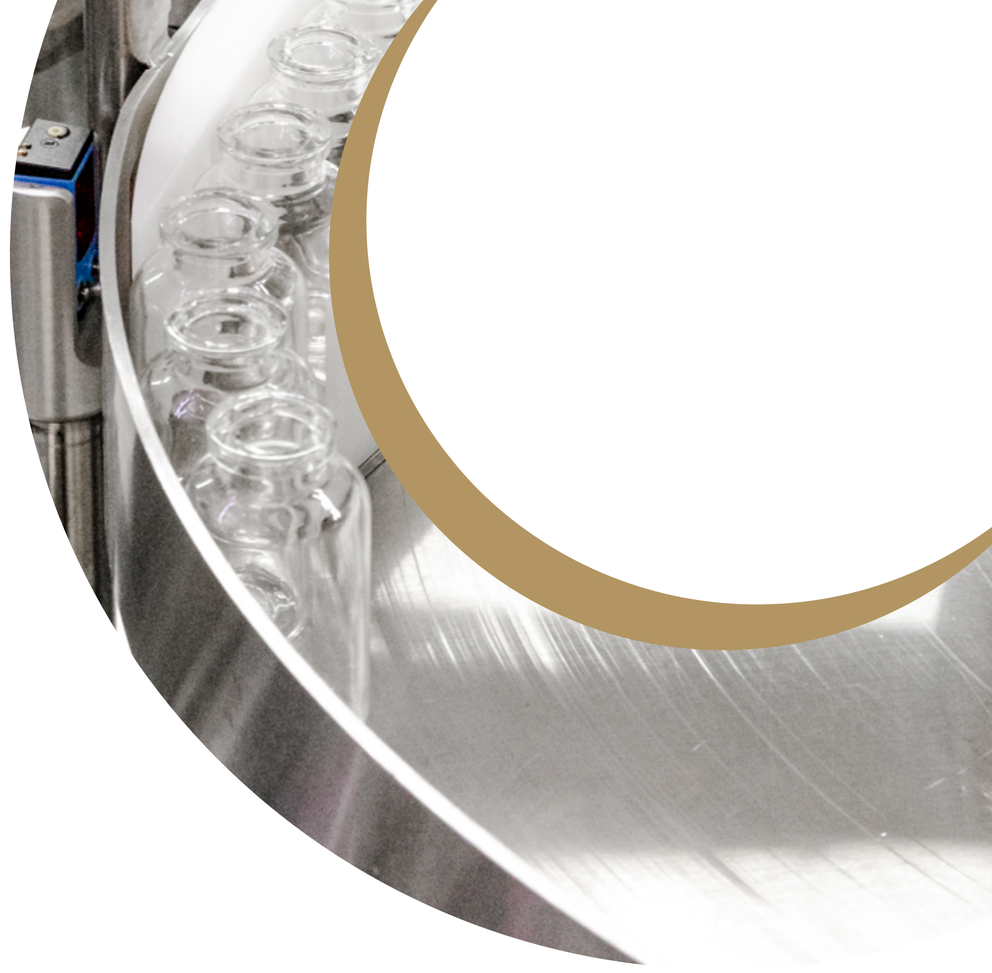

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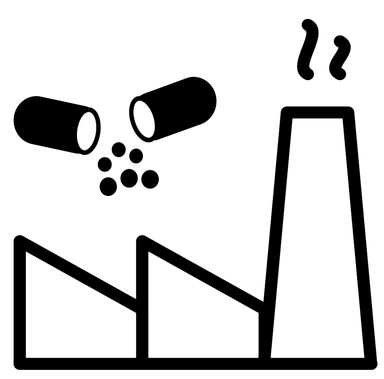

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