

# Let's Get SMALL - Really SMALL

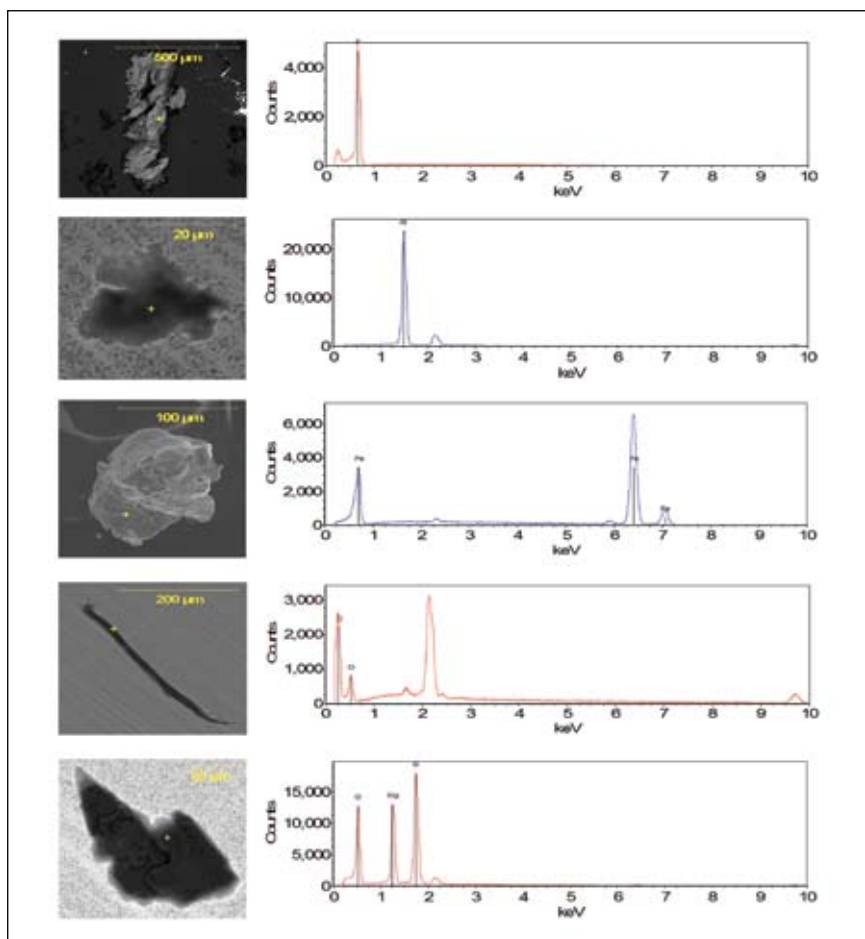
Foreign particle size characterization and distribution with a high-throughput electron beam analyzer

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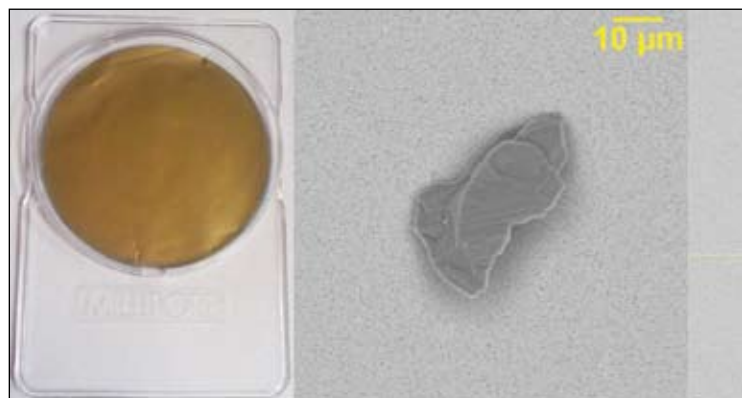
In an effort to meet FDA recommendations, pharmaceutical manufacturers and contract research facilities are adopting the Quality by Design (QbD) approach to assess the quality and safety of solid and parenteral drug products with respect to foreign particles. The QbD approach, which can be applied during developmental studies, or through routine quality control, is based on the idea that the manufacturers of drug products, devices, and formulation components are expected to understand their processes thoroughly. This concept can be incorporated in pharmaceutical companies by using analytical testing

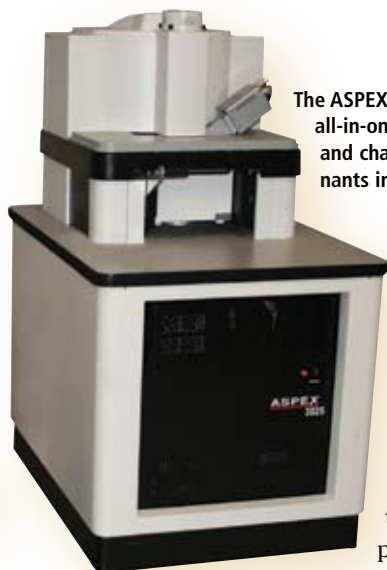
techniques that will allow the monitoring and understanding of every step of the manufacturing process. By successfully implementing the QbD approach, manufacturers can identify and improve areas that may constitute a risk of contributing foreign particulate material to the drug product delivered dose.

In general, pharmaceutical products are required to be essentially free of visible foreign particles. For parenterals, the USP General Chapter <788> specifies light obscuration and microscopy as the methods for the counting and sizing of particles in the  $\geq 10\mu\text{m}$  and  $\geq 25\mu\text{m}$  size range. In addition, there is a large variety of techniques for particle analysis, including infrared spectroscopy, raman microprobe, light obscuration, laser diffraction, optical microscopy, and scanning electron microscopy. All of these techniques have advantages and limitations, and the final decision for the most appropriate testing technique lies on each facility. In this case, the use of automated Scanning Electron Microscopy-Energy Dispersive Spectrometry (SEM-EDS) is illustrated as part of the QbD approach to monitor the particle content in the components of an asthma kit.



Above: Secondary Electron and Backscatterer Electron Images of particles identified in the different components of the sample. Top to bottom: aluminum, iron, synthetic fiber, talc, and stainless steel. Right: Representative sample preparation for organic particulate analysis; typical foreign particulate image; illustration of particle detection methodology.





The ASPEX Rx micro-analysis system is an all-in-one system designed for detecting and characterizing microscopic contaminants in pharmaceuticals.

**PARTICLE CHARACTERIZATION TECHNIQUES**

The International Pharmaceutical Aerosol Consortium on Regulation and Science (IPAC-RS) has published several reviews that summarize various particle analysis technologies commonly used for the particle analysis of orally inhaled and nasal drug products (OINDP).

- **Fourier transform infrared spectroscopy (FTIR)** is a technique mostly used to identify organic, polymeric, and some inorganic materials. When using FTIR, particles must be harvested individually, preventing the technique to be used for particle enumeration.

- **Raman microprobe** is typically used as a complimentary method to FTIR. Even though Raman microprobe systems can be automated, they have no utility for the study of metals and fluorescent materials. Both vibrational techniques are used to physically characterize solids.

- **Light obscuration** is used for fast particle counting. This technique is dependent on particle shape and is prone to sizing errors when testing non-spherical or high aspect ratio particles.

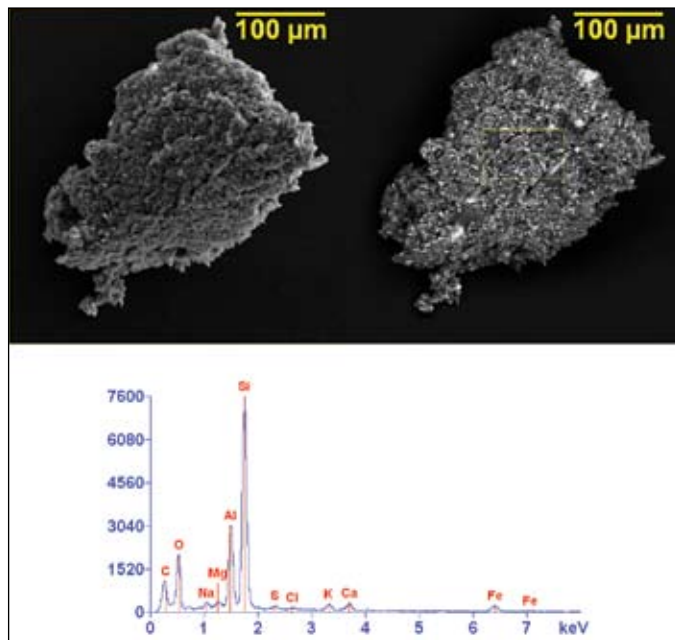
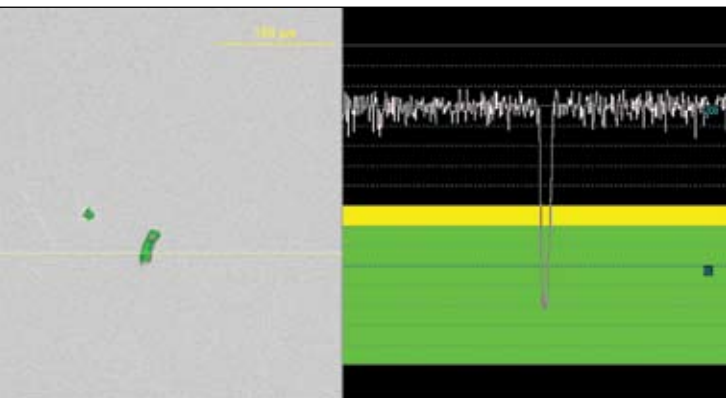
- **Optical Microscopy** is a reliable technique that allows the direct visualization of particles. Low depth field makes this technique impractical for particles smaller than 5 microns.

- **SEM-EDS** is used to perform size, shape, and elemental composition analysis of particles at low micron and submicron range. It has limited applicability to the detection of submicron organic materials.

For this study, the ASPEX Personal Scanning Electron Microscope (PSEM) with Automated Feature Analysis (AFA)

**HOW DOES AN AUTOMATED SEM-EDS WORK?**

The ASPEX Personal Scanning Electron Microscope (PSEM) system dynamically scans the sample. Rather than capturing a high-resolution image of the field, which is time consuming and inefficient, the PSEM instead moves the beam across this field in an array of fairly coarse steps. At each point, the brightness of the backscatter signal is noted. If the signal is bright enough to indicate that a particle is present at this position, then the software initiates a particle-sizing sequence. There are several algorithms that can be used for this purpose, but for simple particle shapes, the "rotating chord" algorithm is both accurate and exceptionally fast. Once the "coarse" scanning identifies a particle, the center is identified and chords are drawn on the particle to define the particles size and shape. The major reason for this improvement in speed is because it only spends time collecting detailed data where particles are known to be present, rather than capturing and transferring vast numbers of "empty" pixels. Since there is almost always much more empty space on the specimen than space occupied by particles, the result is a big speed advantage. For particles analysis, the Automated Feature Analysis software (AFATM) provides enumeration, location for easy post analysis relocation, size, aspect ratio, individual image, and energy dispersiv spectrum of major and minor elements of every particle.



Personal Scanning Electron Microscope Response Signals. Secondary Electron Image, Backscatter Electron Image and X-Ray Spectrum of a particle in a pharmaceutical product.

Particle Type	Total
Dolomite	15
Talc	41
Al-Si-Ca	8
Mixed Clay	1397
Cl Rich	13
Al Rich	42
Misc. Salts	51
High Oxygen_C	2196
Low Oxygen_C	1388
Fe Rich	8
Ti Rich	4
Unclassified	5
All particles analyzed	5436

DMAX> 10 microns	
Particle Type	Total
Dolomite	1
Talc	25
Al-Si-Ca	6
Mixed Clay	545
Cl Rich	114
Al Rich	21
Si Rich	87
Misc. Salts	30
High Oxygen_C	1585
Low Oxygen_C	626
Fe Rich	5
Ti Rich	1
Unclassified	2

DMAX> 25 microns	
Particle Type	Total
Dolomite	1
Talc	3
Al-Si-Ca	4
Mixed Clay	84
Cl Rich	4
Al Rich	5
Si Rich	12
Misc. Salts	6
High Oxygen_C	447
Low Oxygen_C	228
Fe Rich	1
Ti Rich	0
Unclassified	0

Figure 4: Data reporting following USP <788> guidelines. The amount of particles equal to or greater than 10 microns and 25 microns were tabulated for every sample analyzed.

software was used to evaluate the components of a pharmaceutical asthma product for particulate content and composition. As a guideline, the limits for the subvisible particles allowed specified in the USP <788> were used. Samples collected from different lots were carefully prepared in a particle limiting environment. Enumeration and characterization of particulate material from the packaging, encapsulated powder, and the exterior of the inhalable device was performed. Particle size distribution data combined with compositional information was utilized to identify potential sources of contamination and improve process quality.

**SEM-EDS TECHNOLOGY**

Scanning Electron Microscopy (SEM) combined with Energy Dispersive Spectrometry (EDS) is a powerful combination of analytical techniques for the evaluation of particulate matter. The SEM-EDS is a powerful instrument that combines three types of signals for a full detailed sample analysis. The first component is Secondary Electron (SE) imaging, which is used to obtain surface information for a qualitative description of the sample. Surface defects, porosity, and the tri-dimensional structure can be evaluated using high-resolution imaging. The second component is Backscatter Electron (BSE) imaging which is commonly used for the automation of the SEM-EDS analysis. BSE

imaging displays compositional contrasts based on the atomic number of the various elements present in the sample. The Backscatter Electron signal is used to find features during the automated analysis. The third component of the SEM-EDS system is the Energy Dispersive Spectrometer (EDS). Nowadays, Silicon Drift Detectors (SDD) have been incorporated in almost every SEM-EDS platform, resulting in state-of-the-art equipment capable of providing high throughput particle analysis. The SDD provides chemical composition and their relative proportions on the sample. Data is typically displayed as the number of X-rays received and processed by the detector (counts per seconds) vs. the energy levels of the counts.

**METHOD DEVELOPMENT**

*Compositional Contrast*

A sample preparation method was developed in order to analyze both organic and inorganic particles in a single analysis. All the samples were prepared by filtering a solution containing the particles washed from the different components of the asthma kit, through a polycarbonate filter membrane (0.2 micron pore size). Preliminary evaluation showed that the majority of the particles were of organic nature (carbonaceous). Since the polycarbonate filter membrane has a similar backscatter signal than these particles, a sputter coater was

used to deposit a thin layer of gold on each filter membrane prior to filtration. The gold provided a backscatter signal contrast ideal for the detection of organic particles. In addition, other materials such as silica, aluminum, and stainless steel have sufficient contrast with gold for the automated detection. As mentioned before, the automated analysis is dependent on the backscatter signal contrast between the substrate and the elements present in the sample.

*Sample Charging*

Non conductive samples, such as organic particles, can build up charge which alters the secondary electron contrast and deflects the electron beam. These artifacts results in poor imaging. A traditional solution is the application of conductive coating for high resolution images. For this case study, the Variable Pressure option of the ASPEX PSEM was utilized. Chamber pressure was set up at a 0.2 torr. This pressure set point allowed automated analysis without further sample preparation or coating insulating materials typically encountered in pharmaceutical settings, such as, plastics, paper, glass, rubber, and wipes.

*Sample Evaluation*

Manual imaging and X-ray spectrum acquisition was performed on a series of tests samples to evaluate size, shape, elements, particle deposition on the filter membrane, EDS acquisition time, magnification, and scan speed. These parameters were opti-

mized for a fast and accurate automated particle analysis. Chemical composition and size information were utilized to define particle classification rules.

Initial sample evaluation showed, but was not limited to, the presence of aluminum, glass, iron, synthetic fibers, talc, stainless steel, and carbonaceous particles.

**CLASSIFICATION RULES AND DATA FORMAT**

Samples were analyzed in triplicates using the method developed for automated particle analysis. Blank samples were collected by passing particle-free water through gold-coated polycarbonate filter membranes and analyzed to monitor the cleanliness of the equipment used to prepare samples. AFA Data Viewer™ software was used to review particle-by-particle data (images and spectra), as well as create histograms and ternary plots. Data collected from thousands of particles per analysis provided enough statistical information to define classification rules to categorize particles. By combining chemical composition, size, and morphology information, several types of particles were identified in the three components of the analyzed testing kit. Among the most abundant were talc, clays, silicates, and carbonaceous (carbon and oxygen).

Data reports were based on the guidelines for microscopic analysis specified in the USP <788>. The USP <788> is used by pharmaceutical companies to evaluate particulate matter in injections and parenteral infusions either by light obscuration or microscopic analysis. By treating these samples as parenterals, the use of the microscopic analysis was ideal for the determination of the particulate matter in each component of the testing kit. In addition to particle count, in this case, particle tables were created to highlight particles below 10 microns and size/source histograms were plotted to get a better understanding of the potential source of the identified particles.

**SUMMARY**

Monitoring particulate material in pharmaceutical products is an example of how the Quality by Design approach can be incorporated in the development or manufacturing process of pharmaceutical companies and contract research labs. In

this case, automated SEM-EDS analysis was used as a reliable quality control technique to obtain relevant information such as enumeration and characterization of particles from different components of an asthma kit. The type of particulate, morphology, and size distribution information can be used to identify the sources of potential contamination, eliminate or minimize the root cause of contamination, and develop specifications

and controls for production of drugs in FDA-regulated environments

**REFERENCE:**

J. Blanchard, et al. Pharmaceutical Research, Vol. 21, No. 12, December 2004

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