

Applications of automated scanning electron microscopy—energy dispersive spectroscopy (SEM-EDS) for inhalable drug products

Case studies for an MDI, a DPI and a nebulizer suspension

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This article provides an introduction to automated scanning electron microscopy—energy dispersive spectroscopy (SEM-EDS) and the capabilities it can offer in development of inhalable drug products. Case studies are presented for a foreign particulate matter investigation of a metered dose inhaler (MDI); an active pharmaceutical ingredient (API)-specific particle size distribution of a dry powder inhaler (DPI); and a quantitative sameness of shape analysis for a nebulizer liquid suspension.

Scanning electron microscopy

In comparison to light microscopy, the magnification and resolution capabilities that can be obtained from scanning electron microscopy are unmatched and magnifications of 25,000x can be achieved.

Characteristics of SEM and EDS are described by Frederick H. Schamber in “An Introduction to the Personal SEM” and are provided here regarding SEM as well as in the following section on EDS.¹

A scanning electron microscope (SEM) uses a highly focused beam of electrons to form a magnified image of objects placed within it. The electron beam interacts with the sample to emit multiple types of signals by the emission of secondary electrons, backscatter electrons and x-rays. The secondary electrons emitted by a sample after interaction with the electron beam allow for the detection of minute topographical changes on the surface of a sample and can result in extremely high-resolution images of a sample. The image generated by the secondary electrons is based on the height variance across the sample, with areas closer to the

detector appearing brighter than those further from the detector or lower on the sample height. Samples often require gold-coating, using a sputter coater during sample preparation, in order to be imaged with SED (Secondary Electron Detector) SEM, depending on the conductivity of the sample surface.¹

In addition to imaging, elemental or chemical information via generation of backscatter electrons and x-rays can also be obtained. The backscatter electron signal is sensitive to changes in the average chemical makeup of a sample, while the x-ray signal permits the chemical makeup of the specimen to be measured. The images generated by backscatter electrons are based on the atomic number of the elements present; higher atomic number elements appear brighter than darker elements in the field of view. This allows for materials that are optically consistent with one another to be differentiated by their chemical or elemental signal. In conjunction with the backscatter electron image formation comes the ability to obtain unique elemental spectra, via energy dispersive x-ray spectroscopy, to allow for the chemical identification of the elements present.¹

Energy dispersive x-ray spectroscopy

Energy dispersive x-ray spectroscopy (EDS or EDX) is based on the principal that all elements possess unique valence electrons that, when excited by the electron beam of the SEM, give off specific x-ray signals that can be detected in the form of a spectrum. EDS has allowed the SEM to evolve into a powerful tool, combining collection of high-magnification and

high-resolution images plus characterization of materials based on their elemental makeup.¹

Manual versus automated SEM-EDS

Using a manual SEM can provide two principal types of information: images and elemental spectra. An SEM can provide high-magnification images in order to assess features, such as texture of the particles, in minute detail. The manual high-resolution imaging capabilities of the SEM allows analysis of individual particles for in-depth morphological features, which are often critical in the aerodynamics and adsorption of the active pharmaceutical ingredient particles of inhalable products.

A representative backscatter image of particles from a dry powder inhaler is shown in Figure 1. The bright regions illustrate the API particles attached to the lactose surface, which is represented by the dark regions. A representative high-resolution SED image of a particle from a dry powder inhaler is shown in Figure 2. The detailed texture of the particles is apparent in this image.

Manual imaging allows better understanding of both API and excipient morphologies and their relationship to one another. An elemental spectrum and image can also be collected manually; an example is shown in Figure 3. The manual EDS spectrum allows identification and characterization of materials based on the elements present. However, manual analysis poses limitations such as the inability to analyze a large particle population of a sample, the inherent subjectivity of manual analysis and extended analysis times.

Automation of scanning electron microscopy-energy dispersive spectroscopy (SEM-EDS) uses the addition of an automated stage and software, allowing for the accumulation of compositional information about the sample while gathering images of every particle analyzed. In addition, size and morphological data from the sample is collected and stored based on the elemental signal of the sample that is detected via the backscatter electron detector.

Particles of interest can be detected and plotted, based on their elemental signal compared to the background on which they are prepared. Elemental differences due to the sample interaction with the electron beam are displayed as brightness differences across the field of view. Particles that fall above the brightness thresholds set by the analyst are detected and collected automatically by the system. Size and elemental (EDS) information are automatically collected for every detected particle in a field of view. Various morphological parameters are simultaneously collected, which can be used to place a quantitative value on the shape and morphology of particles. These can then be used for comparison and images of each particle identified can be automatically collected by the system for manual review following the analysis, if necessary. The automated software offers a range of options, including random fields of view collected or every field of view in a given sample area defined by the analyst. Every particle detected in a given sample can be collected or a defined number of particles collected (either all particle classifications or a defined classification such as an API). Automation also allows for the accumulation of reliable data in the sub-

Figure 1

A representative backscatter image of particles from a dry powder inhaler; lactose and API. (Magnification: 2,500x)

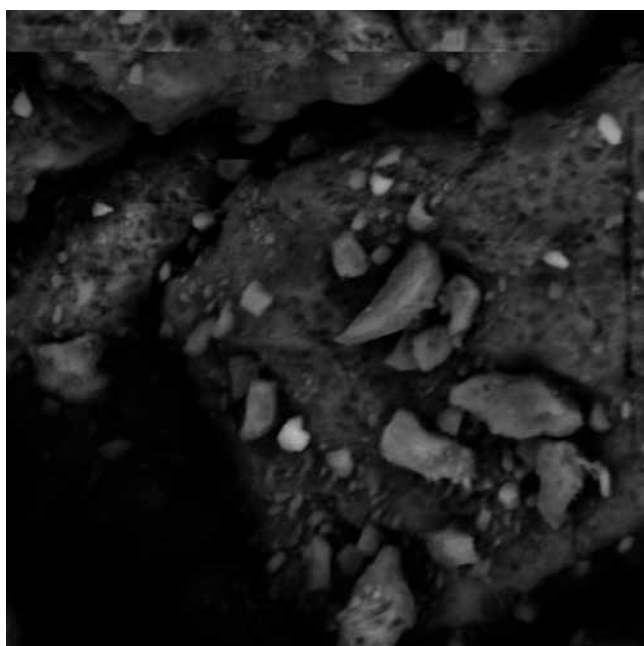
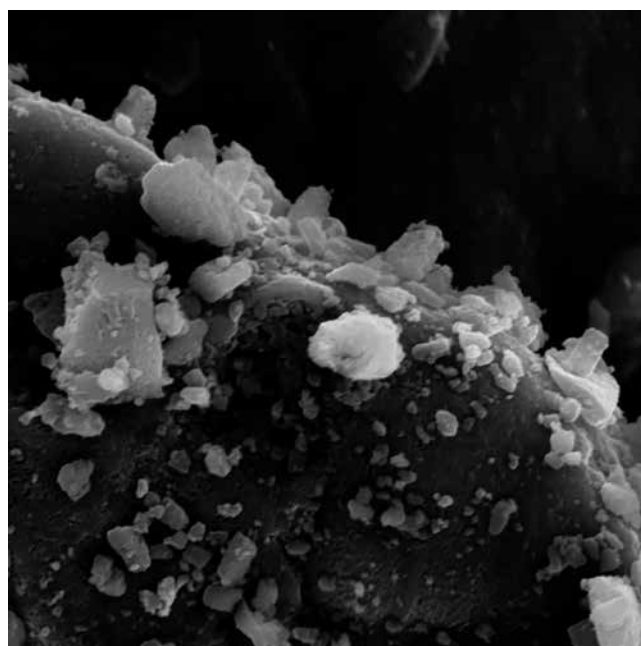


Figure 2

A representative high-resolution secondary electron image of particles from a dry powder inhaler; lactose and API. (Magnification: 5,020x)



micron range, which is of increasing importance in inhalable product analyses with respect to deposition in the lungs and nasal passages.

Automated SEM-EDS also enables the efficient collection of large sets of data—several hundred particles in a matter of hours—with no destruction of samples. Minimal manual input is required and results have no scientist bias regarding particle selection.

Limitations of automated SEM-EDS

While automated SEM-EDS has proven effective in many scenarios, the method has limitations. The particle being evaluated must contain a significant contribution from an inorganic element; or should be the only solid component in the formulation, such as in a suspension; or contain a distinct morphological feature different than the excipient population. The sample must also be able to undergo preparation for placement inside the SEM chamber, under vacuum. Therefore,

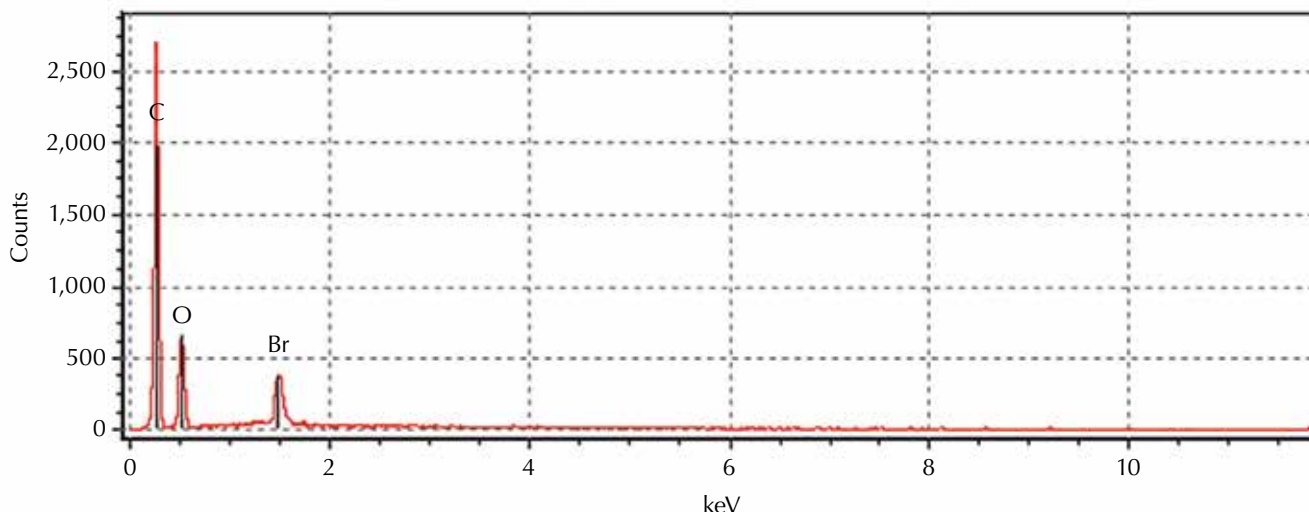
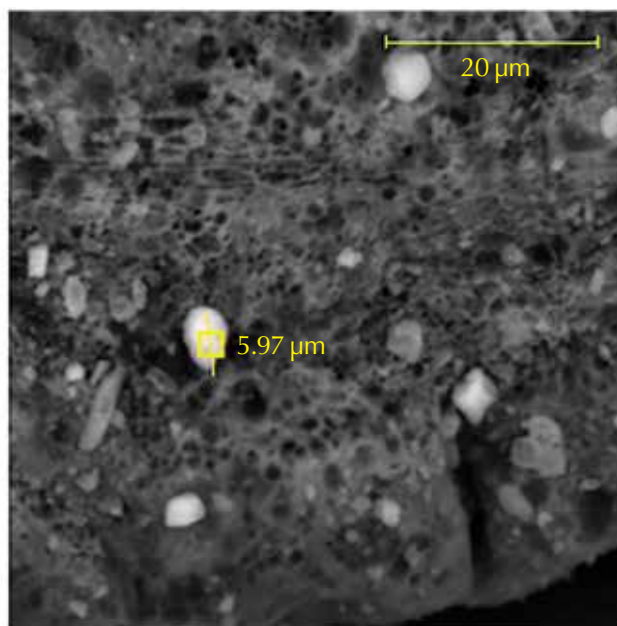
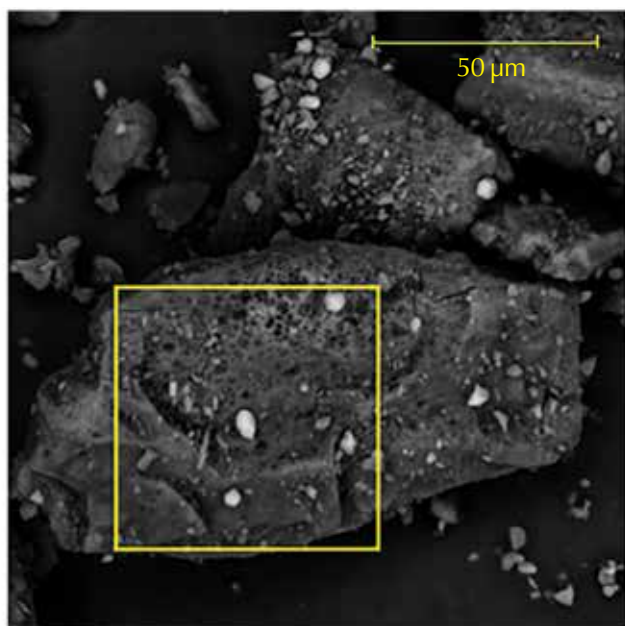
liquid samples cannot be analyzed by SEM without additional preparation. Also, the size of the sample is limited to the size of the SEM chamber, which varies among instruments. Limitations compared to light microscopy include the inability to use transparency of a sample for viewing and analysis. SEM is a surface analysis, as the electron beam penetration varies depending on the type of material being investigated. This means the viewing and elemental analysis is often restricted to surface features of the sample. Also, the SEM technique is based on electron interaction rather than light, making it impossible to discern color. Therefore, color differentiation of a sample cannot be used during imaging or x-ray analysis.

Applications of automated SEM-EDS to inhalable drug products

With respect to inhalable drug products, automated SEM-EDS has two main applications of interest: for-

Figure 3

A representative SEM-EDS and spectrum of a dry powder inhaler and API on lactose.



eight particulate matter testing and API-specific particle sizing. While these two avenues of investigation differ greatly, automated SEM-EDS can be applied to both with the same intention: to utilize the elemental signal of a material for its detection and sizing. Each of these applications will be discussed briefly, along with a case study. In addition, a case study of API morphology testing is presented.

Foreign particulate matter testing

The presence of foreign particulate matter in drug products is a common problem that can have major implications for pharmaceutical companies, from both financial and safety perspectives.² Characterization of a foreign particulate in a sample may enable source determination of the contaminant(s) and thereby lead to the eventual improvement of manufacturing and packaging processes as well as assessment of the safety of a product prior to release.

Manual particulate characterization analysis is useful when a limited number of unknown particles must be identified. However, automated SEM-EDS analysis provides elemental composition of a sample, which is particularly useful for metals and inorganic materials. Automation of the method allows for the identification of an entire population of particles versus a small number of particulates that can be analyzed manually. SEM-EDS also allows characterization of particulates to submicron sizes. For unique particle types, classification rule files can be written to further mine the data and provide discrimination between potential sources. This technique can efficiently provide a more

comprehensive understanding of a large population of foreign particles.

Case study: Foreign particulate matter investigation of an MDI

Methods: A metered dose inhaler (MDI) was characterized for the foreign particulate present. Twenty actuations of the MDI were collected and the drug product was dissolved using methanol. The solution containing the foreign particulate matter was filtered onto a carbon-rich filter to better increase the contrast and detection of the inorganic and metallic particulate. Automated SEM-EDS analysis was performed using an Aspx Explorer SEM-EDS System (Thermo Fisher Scientific, Waltham, MA, US), characterizing particles 2 μm and larger. SEM-EDS can detect carbon-rich particulate only if prepared onto an appropriate, elementally contrasting background. However, it cannot distinguish different carbon-rich particle types such as various polymers, cellulose, etc. Therefore, a complementary technique such as Raman spectroscopy or Fourier-transform infrared spectroscopy (FTIR) is often used in conjunction with SEM-EDS to distinguish these carbon-rich particles.

Results: Particle size and elemental classification results of the foreign particulate present in the sample are summarized in Table 1. In this case, the size of the particles is based on the D_{max} morphology parameter, which is equivalent to a length parameter on other instruments. The instrument draws sixteen chords (or straight lines) through the center of the particle; the D_{max} is the longest of those chords. The most frequently identified materials in the sample were silicon-magnesium mineral, gen-

Table 1

Foreign Particulate Matter Investigation of an MDI. Elemental Classification and Particle Size Results.

D_{max} /Length (μm) Class	Total	≥ 2	≥ 5	≥ 10	≥ 25	≥ 50	≥ 100
SiMg Mineral	1,849	850	659	323	15	2	0
Mineral	744	553	156	35	0	0	0
Al-Rich	494	218	165	104	6	1	0
Steel	41	26	11	3	1	0	0
Fe-Rich	40	27	11	1	1	0	0
Ca-Rich	35	19	10	6	0	0	0
Si-Rich	11	6	4	1	0	0	0
SiAlMg Mineral	4	4	0	0	0	0	0
Si-Al-Fe	2	0	2	0	0	0	0
SiAl Mineral	1	0	1	0	0	0	0
SiAlCa Mineral	1	1	0	0	0	0	0
Particles of Interest	3,222	1,704	1,019	473	23	3	0

Al = aluminum, Ca = calcium, Fe = iron, Mg = magnesium, Si = silicon

eral mineral and aluminum. Common sources of the silicon-magnesium mineral category are talcum and other synthetic magnesium silicates. Mineral is a generic category that contains some or all of the following: silicon, aluminum, sodium, calcium, potassium and magnesium, when combined, in abundance of greater than 60 relative weight percent. Mineral particles are placed in this category when they do not meet the more-specific criteria of other classifications, such as silicon-rich (Si-rich), silicon-magnesium (Si-Mg), aluminum-rich (Al-rich), etc. In Table 1, the three highlighted categories accounted for 95.8% of the total particles identified. Approximately 18% of the identified particles were metallic in nature; the majority of them were consistent with aluminum and only trace levels of steel and iron were present. This information can be used to set allowable particulate levels in a given product and be used for comparison to future stability or batch release sample testing results.

API-specific particle sizing

Determining the active pharmaceutical ingredient (API) particle size distribution within the final formulation of a drug product is critical, as it relates to bioavailability and rate of absorption of the drug product.³

Inhalable products such as dry powder inhalers (DPIs) and nasal sprays can be challenging to characterize, due to the complexity of their formulations. Such formulations can include excipients that may prevent obtaining API-specific particle size data without significant interference. Furthermore, DPIs can pose challenges for differentiating API and excipient particles based on morphology, which is commonly characterized by methods such as morphology-driven Raman spectroscopy (MDRS) and optical-microscopy-based techniques.

Automated SEM-EDS can differentiate particles based solely on their elemental differences and can characterize the submicron particle size range to 0.2 μm , depending on the drug product and elements present. In a combination product, multiple APIs can also be distinguished, provided they possess different elemental compositions. Classification rules can be written and applied to differentiate the various APIs. The only requirements for using automated SEM-EDS for API-specific particle sizing is the needed presence of an inorganic component within the API that is not present among the other product components. In addition, the product must be prepared in a manner that is suitable for the SEM chamber under high vacuum, while not affecting the particle morphology of the API. Liquid samples are not suitable for analysis using the Aspex Explorer SEM-EDS System.

Case study: API-specific particle size distribution of a DPI

Methods: Automated SEM-EDS was used for the determination of the API particle size distribution of a dry powder inhaler (DPI). In this instance, automated

SEM-EDS was applied to characterize a bromine-containing API component within the carbon-rich and oxygen-rich lactose excipient matrix of a DPI. Following cascade impaction, a thin layer of drug product was deposited onto an adhesive carbon tab.

Prior to automated analysis by SEM-EDS, manual SEM-EDS spectra were collected from the API and excipient to determine the suitability of the SEM-EDS information for differentiating the various components of the product based on elemental differences. These elemental distinctions were utilized to write specific rule files for the identification and classification of the API within the sample. Automated analysis of the sample was conducted using the Aspex Explorer SEM-EDS System on a minimum of 500 API particles. For a submission to the US Food and Drug Administration (FDA), a full population study would be needed to determine the minimum number of particles that are statistically equivalent to the population for a given sample type. The work described above was performed for demonstration purposes only and is not meant to constitute a statistically significant result.

Results: Particle size distribution results for the bromine-containing API in the sample are summarized in a histogram in Figure 4 and in Table 2. For each particle analyzed, an image, the sizing data and the SEM-EDS spectrum were collected. The particle size distribution of the API was reported based on the D_{avg} morphology parameter, which is the average diameter. The instrument draws sixteen chords (or straight lines) through the center of the particle; D_{avg} is the average length of those chords. Average diameter was used, using the assumption of a spherical API particle. SEM-EDS can determine particle size data in the submicron range. Statistical calculations were performed on the API data obtained for particle size and are shown in Table 2. These results can be used to compare specific products or lots, for bioequivalence testing or for choosing an appropriate candidate during drug development.

Figure 4

API-specific particle size distribution of a DPI.
Histogram depiction of results.

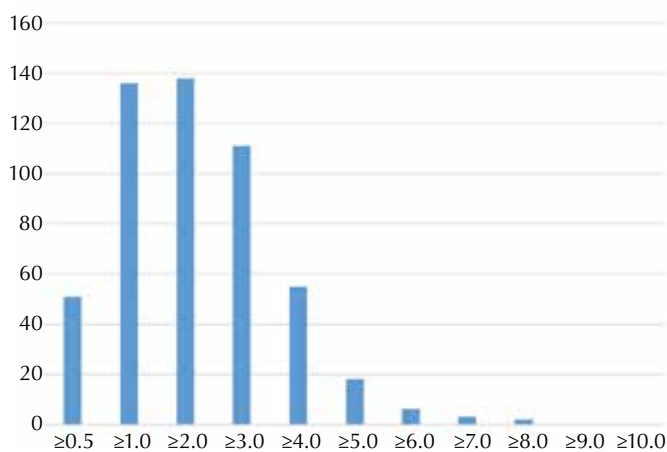


Table 2

API-Specific Particle Size Distribution of a DPI: Results.

API	Number of Particles	D10	D50	D90	Std. Dev.	Min.	Max.	Mean
D _{avg} (μm)	525	0.99	2.5	4.5	1.38	0.37	8.55	2.66

API morphology testing

In addition to particle size of the API, guidance documents may include references to particle morphology or sameness of shape (crystalline habit) of a drug substance.^{4,5} Crystal(line) habit is defined as “the characteristic external shape of an individual crystal or crystal group; a description of its general shape and its crystallographic forms.”⁶

X-ray diffraction (XRD) is commonly used to probe and detect this internal crystalline structure rather than the external structure or morphology. Other analytical methods capable of defining particle morphology, such as laser diffraction and BET (Brunauer-Emmett-Teller), may be considered bulk analysis methods, more suitable for bulk powders rather than analysis of individual drug particles in a final formulation. Automated SEM-EDS can determine detailed particle morphology parameters about a given population of particles.⁷ Such parameters can help place quantitative values on various morphological features and be used to compare two given populations.

Manual imaging of the API is a qualitative analysis, which raises the issue of setting relevant bioequivalence criteria when comparing generic and reference listed drug products. Image analysis typically cannot be used for quantitative comparison between a reference listed drug and generic drug. Even with manual measurements such as aspect ratio acquired within the imaged particles, manual bias and extensive time constraints exist. Therefore, an automated method for determining the sameness of shape or particle morphology of the drug particles may provide a better alternative.

Case study: Quantitative sameness of shape analysis for a nebulizer liquid suspension

Automated SEM-EDS was utilized for the analysis of a budesonide suspension and determination of particle size and morphology (sameness of shape) of the API particles. Various morphological parameters were explored to determine the most descriptive parameters of the particle shape in order to compare two different brands of the drug formulation. This simulates a bioequivalence investigation for sameness of shape or the crystalline habit of the drug particles.

Methods: Budesonide (the API in this case) was the only solid component of the suspension among liquid excipients. This made the API in final formulation amenable to various sample preparation and automated particle sizing analysis methods that are not fea-

sible in more complex drug products, where significant excipient interference exists. A small amount of the suspension was centrifuged to force the API out of suspension, the supernatant containing the excipients was removed and discarded, and the API re-suspended in deionized, ultra-filtered (DIUF) water. The API/water suspension was then placed on aluminium-coated slides and allowed to dry prior to testing. (As noted earlier, a liquid sample cannot be placed inside the sample chamber of the Aspex Explorer SEM-EDS System.)

Automated SEM-EDS was employed to determine particle size and morphology of the budesonide API particles.⁷ The aluminum-coated slide provided an elementally contrasting background to the carbon-rich particles of the budesonide. This allowed for detection and analysis of the API particles while ignoring residual excipients such as sodium chloride, which may have remained from the preparation process. Approximately 1,000 API particles were analyzed automatically by the system for each sample, for demonstration purposes. For an FDA submission, a full population study would be required to determine the minimum number of particles that are statistically equivalent to the population.

Statistical calculations, such as D10/D50/D90, mean and standard deviation, were performed on the budesonide sizing and shape analysis data to quantify the differences between the sample populations. Such statistical data is useful during method development and throughout the validation process, as well as during the comparison of generic and branded drug formulations for bioequivalence studies.

Results: The automated system also enables gathering images of the identified particles. Various shapes of budesonide particles were observed, as shown in Figure 5. Some particles exhibited a more rounded, circular shape while others exhibited a more elongated, crystal-like shape with sharper edges. The statistical data calculated from the two samples is shown in Table 3. This study was conducted for demonstration purposes. However, for an FDA submission, morphology parameters would be established for sameness of shape along with pass/fail criteria.

In summary

Automated SEM-EDS can provide high-magnification images and elemental information, as well as detailed morphological and shape information in a quantitative method for easier comparison of two products.

Depending on the drug type and preparation method, it can detect particles in the submicron range. It also enables efficient collection of large data sets in a matter of hours, limiting the burden of scientist time and eliminating the subjectivity of manual analysis.

In the development of inhalable drug products, automated SEM-EDS can be used for foreign particulate matter testing, API-specific particle sizing and API morphology testing. A case study of each technique has been presented in this article.

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Figure 5

Representative budesonide API particles from a suspension for nebulizer, collected during automated analysis.

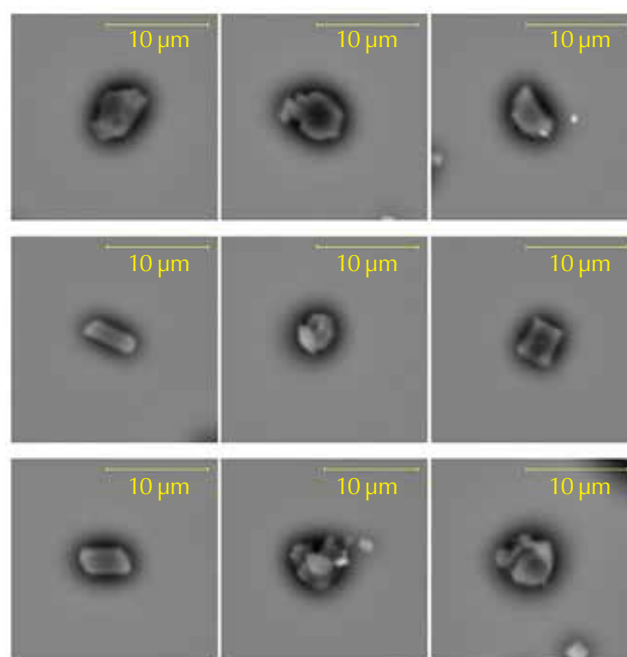


Table 3

**Quantitative Sameness of Shape Analysis for a Nebulizer Liquid Suspension.
Results for Budesonide Particle Size and Morphology.**

	Morphology Factor	D10	D50	D90	Min.	Max.	Average	Std. Dev.
Product 1	$D_{max}(\mu\text{m})$	4.83	6.64	11.03	1.66	31.40	7.45	3.03
	$D_{ave}(\mu\text{m})$	4.55	6.17	10.02	1.48	29.91	6.88	2.73
	Aspect Ratio	1.07	1.21	1.58	1.00	3.37	1.28	0.25
	Roundness	0.42	0.56	0.72	0.20	0.89	0.57	0.12
	Equivalent Circular Diameter (ECD)	3.31	5.19	8.15	1.43	20.02	5.57	2.19
Product 2	$D_{max}(\mu\text{m})$	4.74	6.60	11.53	1.61	25.92	7.51	3.35
	$D_{ave}(\mu\text{m})$	4.36	6.12	10.37	1.55	23.68	6.90	2.98
	Aspect Ratio	1.08	1.24	1.71	1.00	3.72	1.34	0.33
	Roundness	0.38	0.56	0.74	0.21	0.96	0.56	0.14
	Equivalent Circular Diameter (ECD)	3.18	5.17	8.26	1.32	17.48	5.51	2.18

Additional resources

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