Scanning Electron Microscope (SEM) Coupled with Energy Dispersive X-ray Spectroscopy (EDS)- A Potential Analytical Tool for Physico-chemical Characterization of API in Complex Drug Formulations

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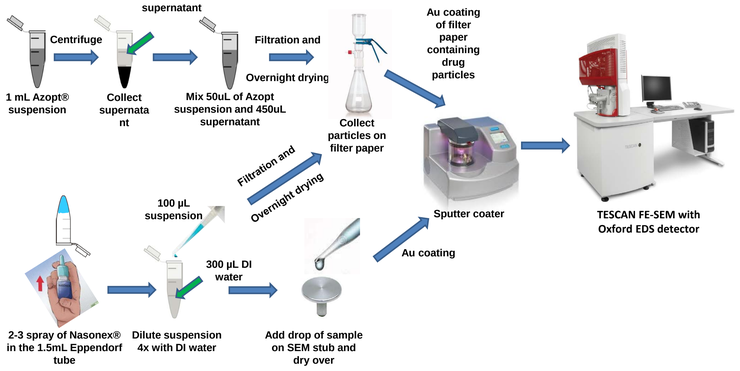
BACKGROUND

Comprehensive physicochemical characterization (e.g., morphology and composition) of the active pharmaceutical ingredient (API) in complex drug formulations provides critical information to support bioequivalence of complex generic drug formulations to the reference listed drug (RLD) product. However, such characterization is rather challenging due to many factors, such as complex nature of formulation (e.g. solution vs. suspension), unique physicochemical properties of API (e.g., solubility, stability) as well as interference from the other excipients (e.g., similar chemical composition/properties and morphology as API). In this work, through two case studies, Nasonex® (mometasone furoate monohydrate nasal spray suspension) and Azopt® (brinzolamide ophthalmic suspension), we demonstrated that SEM–EDS can be a valuable tool for morphological and compositional characterization of the API in complex drug formulations.

EXPERIMENTAL

- For Nasonex®, samples were shaken for 5 seconds and sprayed twice into 1.5 mL Eppendorf tube and then 100 µL of suspension was diluted 4 times with 300 µl of deionized water as shown in below schematics. After that, 100 µL of sample was deposited on an aluminum stub or vacuum filtered on 0.2 µm polycarbonate (PC) filter paper and then air-dried overnight in fume hood.
- For Azopt®, 1mL of suspension was first centrifuged at 15,000 g for 15 minutes and supernatant was collected. A fresh aliquot (50 µL) of Azopt® suspension was diluted 10 times using the 450 µL of supernatant and deposited on 0.05 µm PC filter paper via vacuum filtration and air-dried overnight in fume hood.
- After drying, both samples were sputter coated with gold (~ 5 nm thick) prior to SEM-EDS analysis using TESCAN Mira 3 (Tescan USA Inc., Warrendale, PA, USA) equipped with an Oxford X-Max 80 EDS detector (Oxford Instruments, Abingdon, UK). Particle size distribution of APIs in suspension was performed via analyzing SEM images using ImageJ software.



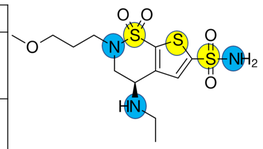
SEM-EDS Sample Preparation Procedure

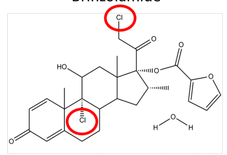
RESULTS

- Both EDS mapping and point-shoot analysis of Nasonex® and Azopt® products shows that the API's in Nasonex® and Azopt® can be distinguished from other excipients based on the detection of specific characteristic element, such as Chlorine (Cl) in mometasone furoate (Figure 1) and Sulfur (S) in brinzolamide.

RESULTS

Nasonex	AZOPT 1%
Active ingredient: Mometasone furoate (50 µg/spray)	Active ingredient: Brinzolamide (10 mg/mL)
Inactive ingredients • Glycerin • sodium citrate • citric acid • polysorbate 80 • carboxymethylcellulose sodium • microcrystalline cellulose	Inactive ingredients: • Mannitol • carbomer 974P • Tyloxapol • Edetate disodium • sodium chloride • purified water with hydrochloric acid and/or sodium hydroxide to adjust pH.
Preservative: benzalkonium chloride	Preservative: benzalkonium chloride





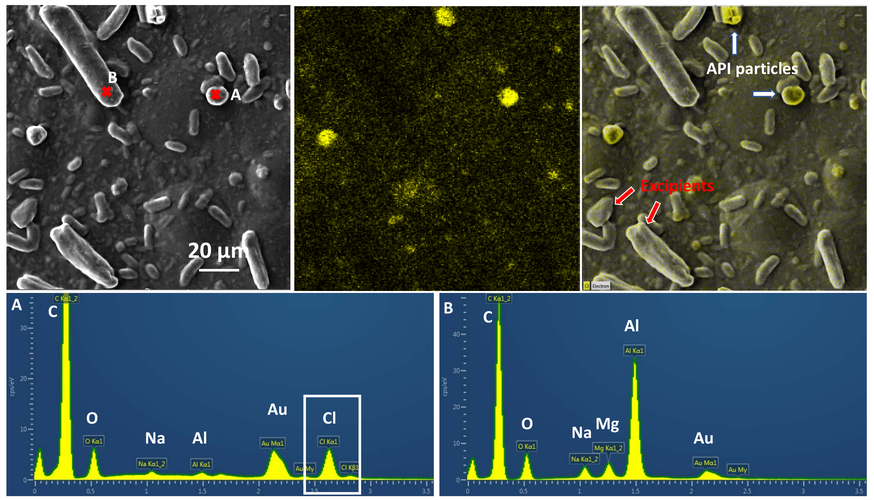


Figure 1. SEM and EDS analysis of Nasonex® nasal spray (suspension of mometasone furoate monohydrate). Top left is a SEM image, in middle is "Cl" element EDS mapping image and right is overlay image of SEM and "Cl" element mapping of Nasonex® nasal sprays. Mometasone furoate monohydrate particles and excipient particles were shown in white and red arrow respectively. Bottom left is point-shoot EDS spectrum of Momentasone furoate monohydrate API particles and bottom right is point-shoot EDS spectrum of other inactive excipients.

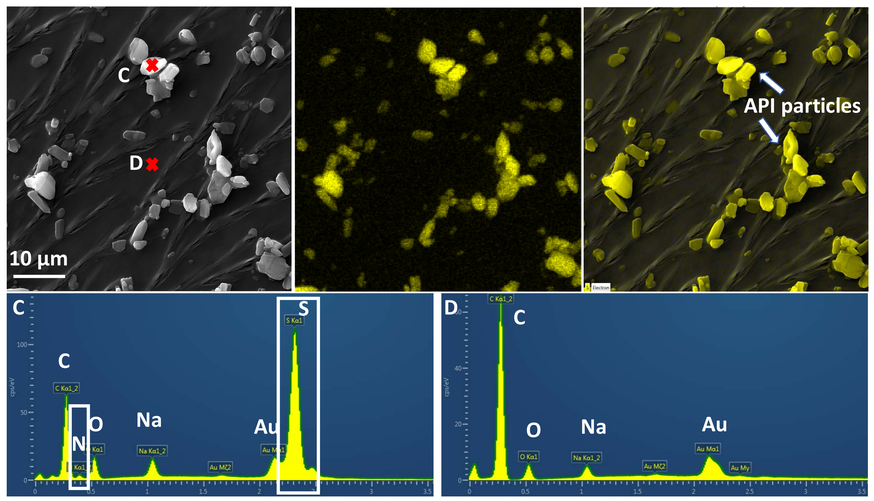
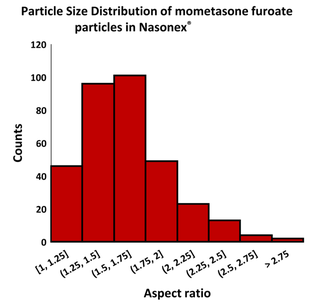


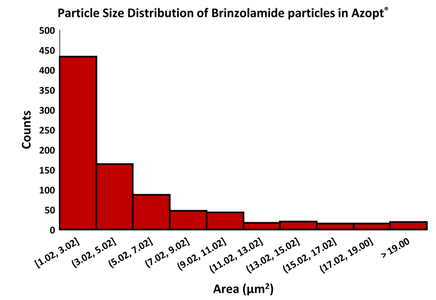
Figure 2.SEM and EDS analysis of brinzolamide API in Azopt® formulation. Top left is a SEM image, in middle is "S" element EDS mapping image and right is overlay image of SEM and "S" element mapping of Azopt® formulation. Bottom left is a point-shoot EDS spectrum of brinzolamide particles and bottom right is point-shoot EDS spectrum of background.

RESULTS

- For Nasonex® product, SEM image analysis (Figure 1) showed the mometasone furoate particles have an aspect ratio approximately 2:1 which is consistent with the result from Morphologically-Directed Raman Spectroscopy (MDRS) analysis.[1] While for Azopt® product, the brinzolamide particles have a linear dimensions ranging from 1 to 5 µm, which is complementing to the value we obtained from laser diffraction analysis (D50 = 2.5 µm).



Total particle count	Length ~~(µm)~~	Width (µm)	Aspect ratio
334	8.46 ± 2.88	5.38 ± 1.88	1.61 ± 0.36



Total particle count	Area (µm²)	Feret diameter (µm)	Equivalent circle diameter (µm)*
860	4.95 ± 5.66	3.40 ± 1.65	2.26 ± 1.09

* Equivalent circle diameter (µm) was calculated using equation Diameter = 2 * (area/3.14)$^{1/2}$ assuming particle shape is spherical.

CONCLUSIONS

- Both EDS mapping and point-shoot analysis of Nasonex® and Azopt® products shows that the API's in Nasonex® and Azopt® can be distinguished from other excipients based on the detection of specific characteristic element, such as chlorine (Cl) in mometasone furoate (Figure 1) and sulfur (S) in brinzolamide.
- Based on EDS spectral detection and subsequent SEM image analysis of two complex drug formulations (Nasonex® nasal spray suspension and Azopt® ophthalmic suspension), SEM-EDS appear to be an analytical tool capable of simultaneous morphological characterization (quantitative) and chemical identification (qualitative) of API in these complex drug formulations. This work reveals SEM-EDS as an additional option for API characterization which may provide relevant information to support bioequivalence and drug product quality.

REFERENCE

[1] Liu, Q., et al., *Scientific Considerations for the Review and Approval of First Generic Mometasone Furoate Nasal Suspension Spray in the United States from the Bioequivalence Perspective.* The AAPS journal, 21(2), p.14.

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