# Use of Scanning Electron Microscopy in the Authentication of Botanicals

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**ABSTRACT** This article provides an overview of scanning electron microscopy and its applications in the identification and authentication of botanicals. The USP Dietary Supplements—Botanicals Expert Committee plans to include scanning electron microscopy as an additional tool in addition to those currently included in *USP* General Chapter *Identification of Articles of Botanical Origin* ⟨563⟩ (*1*). The objectives of this *Stimuli* article are to initiate discussion, to solicit public comments, and to invite the participation of interested parties in the efforts of the Expert Committee.

## INTRODUCTION

Microscopy plays a vital role in the authentication and quality control of botanical articles. By the turn of the 20th century, light microscopy became the primary means of identification of pharmacopeial articles of botanical origin, contributing to one of the greatest improvements in the quality control of botanicals (*2*). The analyst identifies plant material by comparing the salient microscopic features of a test sample to voucher or authenticated specimens and to descriptions and drawings found in various compendia.

The first commercially available scanning electron microscope (SEM) was introduced in 1965 (*3*). Unlike the conventional optical light microscope, which uses a series of glass lenses to bend the light and create a magnified image, SEM creates a magnified image using electrons instead of light and yields both topographic images and elemental information when used in conjunction with energy-dispersive X-ray analysis or wavelength-dispersive X-ray spectrometry. SEM is useful for characterizing the size and morphology of microscopic specimens. Typically, SEM analysis requires a small amount ($10^{-10}$ to $10^{-12}$ g) of a solid specimen that is coated with a conductive substance such as a metal if it is not itself conductive. The sample is placed in an evacuated chamber and is scanned in a controlled raster pattern by an electron beam. Interaction of the electron beam with the specimen produces a variety of physical phenomena that, when detected, are used to form images and provide elemental information about the specimen. Additional details covering SEM as applied to pharmacopeial articles in general can be found in the *USP* General Information Chapter *Scanning Electron Microscopy* ⟨1181⟩ (*4*).

SEM produces a higher resolution compared to that possible using a light microscope, and the images obtained are three-dimensional. The maximum resolution for SEM (minimum distance by which the two objects can be separated and observed as distinct objects) is 10–20 nanometers compared to 200–300 nanometers for light microscopy (*5*). SEM has the advantage of providing images with large depth of field, which allows a substantial portion of the sample to be in focus at one time (*Figure 1*). SEM allows the analysis of specimens as large as 50 mm, making it possible to produce SEM electron micrographs of an object that is clearly visible to the naked eye (*6*). Modern compound light microscopes, under optimal conditions, can magnify an object by as much as ×1000 to ×2000. Typical SEM magnification ranges from ×10 to ×300,000. Commercial SEM instruments also are available with magnifications as low as ×5 and as high as ×2,000,000. At low magnification SEM often is more useful than light microscopy. In general, SEM not only produces images that are analogous to those from an optical microscope, but it also can produce images whose contrast is based on compositional variations of specimens. However, SEM is much more expensive when compared to the light microscope. Although SEM typically yields black and white images, researchers have attempted to use low-vacuum SEM to insulate specimens without a metal coating in order to preserve the color information on their surfaces (*7*).

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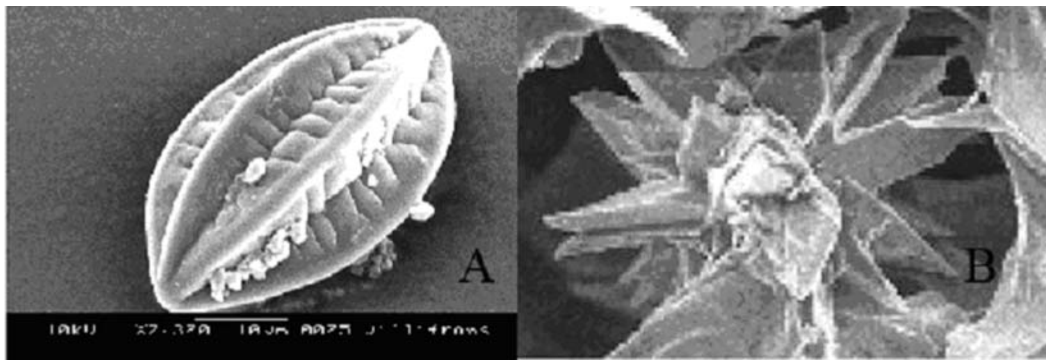


Figure 1. Surface view of *Ephedra* spp. pollen grain (A); oxalate crystal in *Opuntia ficus-indica* (L.) P. Mill. (B). Procedure: *Ephedra* pollens and *Opuntia ficus-indica* (L.) P. Mill. samples were freeze dried for 48 h. The freeze-dried samples were sputter coated with gold using a Hummer 6.2 Sputter Coater, examined and photographed using a JEOL JSM-5600 SEM at an accelerated voltage of 10–12 kV.

The combination of higher magnification, larger depth of focus, greater resolution, and ease of sample observation makes SEM a useful instrument for contemporary research. All operations can be performed using a personal computer, and most adjustments are automated.

## USE OF SCANNING ELECTRON MICROSCOPY IN BOTANICAL AUTHENTICATION

Botanicals require both authentication and identification. Authentication is arriving at a final answer regarding the correct identity of the source plant for a botanical product. Authentication requires positive identification of the plant and its derived products, along with detection of contaminants and adulterants. Botanicals in commerce often are presented as powders or pieces, making authentication by cross-sectioning of the article difficult if not impossible. Structures that are resistant to these processes are useful in identification. Structures such as the xylem vessels and tracheids may be broken into smaller portions, making detection of pitting and lignification on the walls difficult if not impossible using the light microscope. SEM has been used extensively to investigate the surface topology of a wide variety of plant materials. SEM can play a vital role in authentication of entire botanicals and those in powder form; it can distinguish between closely related species; and it finds applications in the examination of powder mixtures.

### A. Identification of Botanicals

SEM has been used extensively for morphological analysis of botanicals for several years. Although its usage in authentication has been limited, recent publications show a rise in its use for botanical authentication. Ragusa et al. used SEM to authenticate *Harpagophytum procumebens* DC (*8*). They identified morphological elements along with superficial granular material containing harpagoside and harpagide that assist in identification. Rao et al., using SEM, determined the structure of glandular and nonglandular trichomes for rose-scented Geranium cultivars (*Pelargonium* spp.) (*9*). Liu et al. determined the botanical origin of the Chinese crude drug Jiaomu using SEM (*10*). Kwanhwa et al. used this technology to obtain information about the relation of glandular trichomes and essential oils of *Elsholtzia ciliata* (Thunb.) Hylander, a native Korean aromatic medicinal plant (*11*). Chauhan et al. used SEM to identify *Centella asiatica* Urban, a botanical used either whole or in powder form (*12*), and identified unusual structures in the epidermis as a useful characteristic for identification of this plant. Ragusa et al., using SEM, carried out a micro-morphological investigation on *Entada africana* Guill. & Perr. roots and leaves (*13*). Rapisarda et al. used SEM to examine *Nepeta sibthorpii* Benth., an aromatic plant from southern Greece, and characterized the trichomes that are abundantly present on the calyx and leaves (*14*). The glandular hairs distributed on the adaxial leaf surface and on the outer surfaces of the calyx and corolla showed unusual features of the epidermal cells, which are useful diagnostic features for the identification of this medicinal plant. Researchers have used SEM to examine the pulp component of *Aloe vera* L. leaf. Ni observed that the pulp consists of large clear mesophyll cells (*15*). Sibert used SEM for localization of salvinorin A and related compounds in the glandular trichomes of the psychoactive sage, *Salvia divinorum* Eppling & Játiva (*16*). Srivastva et al. used SEM along with light microscopy and other analytical tools for a pharmacognostic evaluation of *Cassia angustifolia* Vahl (*17*).

### B. Morphological Variation within Species

Closely related species often have greater similarities compared to species belonging to different genera and families, which makes differentiation within a genus a difficult task. In such cases SEM can play a vital role because it can resolve the differences due to its higher magnification, larger depth of focus, and greater resolution. Rapisarda et al. used SEM to study the genus *Cordia,* which is made up of several species widely distributed in tropical and subtropical regions of the world (*18*). They observed the histological characteristics of the leaves of *Cordia francisci* Ten., *C. martinicensis* Link, *C. serratifolia* H., B., & K., and *C. ulmifolia* Juss. spp. grown in Sicily and compared them with those of *C. myxa* L. from Africa. They revealed morphological elements that allow leaf identification and species differentiation. Xiao et al. used SEM to identify the morphology and distribution characteristics of calcium oxalate crystals in the bark of several species of *Cinnamon* (*19*). Chen examined seeds of five species of medicinal *Rhododendron* spp. and was able to distinguish the five species based on the form, wings, and ribs of the seeds (*20*).

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### C. Detection of Adulterants/Substitution

Few reports indicate the use of SEM for the detection of adulterants and substitutes. Recently Xia et al. used SEM to identify *Kochia scoparia* and its substitutes, including the fruits of *Chenopodium album, C. serotinum,* and *Kochia scoparia* (L.) Schrad. var. *trichophila* Schirz and Thell (*21*). Their work showed that *K. scoparia* displayed some differences from substitutes, notably in the trichomes, stomata, and shape of seeds. Joshi et al. used SEM to differentiate between the *Illicium verum* Linn and its toxic adulterant *I. anisatum* (*22*). The two species could not be differentiated using light microscopes, but SEM showed distinct variation in the surface morphology and in the follicles of the two species (*Figure 2*). SEM also permitted differentiation of the two species in powder form. Wierzbick et al. detected and localized lead in *Allium cepa* L. cells using an X-ray micro-analyzer attached to SEM, which illustrates the use of SEM in the detection of heavy metals in botanicals (*23*).



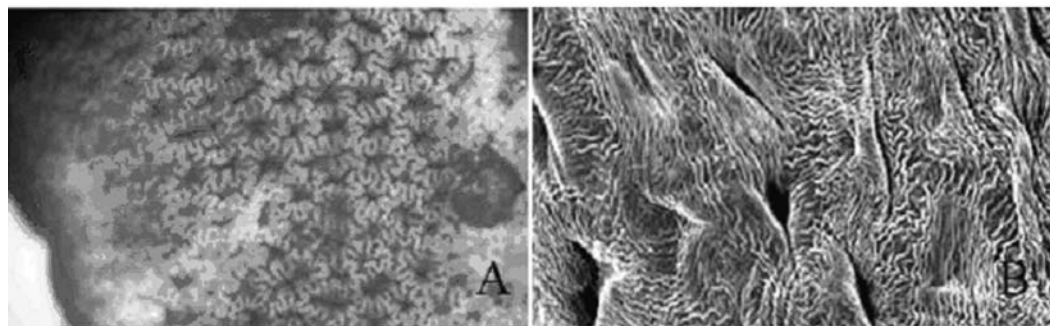


Figure 2. Surface view of *Illicium anisatum* L. follicle: light microscopy (A) vs. scanning electron microscopy (B). Procedure: *Fluorescent scope analysis:* Follicles of *I. anisatum* L. were freeze dried, examined under a Nikon Eclipse E 600 fluorescent microscope at UV Ex 330–380 wavelength, and photographed with attached Kodak Digital camera. *Scanning electron microscopic analysis:* Follicles of*I. anisatum* were freeze dried for 48 h. The freeze-dried samples were then sputter coated with gold using a Hummer 6.2 Sputter Coater, examined and photographed using a JEOL JSM-5600 SEM at an accelerated voltage of 9–11 kV.

## CONCLUSION

The USP Dietary Supplements–Botanicals Expert Committee plans to add SEM to General Chapter ⟨563⟩ and welcomes public comments and participation in the process of revising this General Chapter.

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