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BRIDGING ANCIENT WISDOM AND MODERN NANOSCIENCE: A REVIEW OF SCANNING ELECTRON MICROSCOPY (SEM) CHARACTERIZATION OF AYURVEDIC FORMULATIONS

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ABSTRACT
Scanning electron microscopy (SEM) is a useful and modern analytical tool for the evaluation and standardization of Ayurvedic herbo-mineral formulations. In the present review, the importance of SEM for determining the particle size, surface morphology, microstructural features, and elemental composition of different Ayurvedic preparations, such as *Bhasmas*, *Kajjali*, and *Rasaushdhies*, has been highlighted. Published literature was collected from peer-reviewed articles on Ayurveda and pharmaceutical sciences that are relevant to the study. This review covers the principles, types, working mechanisms, advantages, and disadvantages of SEM and its emerging use in Ayurvedic research. The results of various studies have shown the presence of Micro and nanosized particles in various formulations, which reinforces classical Ayurvedic concepts such as *Sukshmata* and *Rekhapurnatva*. In addition, SEM-EDAX/EDS has been found to be useful for elemental analysis, purity checking, and quality control. Overall, SEM provides a valuable link between traditional Ayurvedic practices and their scientific validation, ensuring the safety, standardization, and acceptance of Ayurvedic formulations in the global market.

KEYWORDS: Nano science, Ayurveda, *Bhasma*, SEM, Rasa Shastra, Global.

INTRODUCTION
There are two types of microscopy: optical microscopy (OM) and scanning electron microscopy (SEM). The latter is more recent and has been used in the last 200 years as a simple instrument for a few easy tasks. This technique is also known as light microscopy. There are differences in operating principle and characteristics between OM and SEM:
(a) The basic operating principle behind the OM is light while the SEM is based on electrons emitted. (b) A simple OM comprises only one lens, whereas a compound OM consists of two lenses. The lenses refract light to make the images appear larger. The magnification of a typical modern optical microscope is 400-1000, compared with the ability of a scanning electron microscope (SEM) to reach magnifications of up to 300,000. Optical microscopy is suitable for studying living cells and solid materials but is primarily limited to small, thin, and transparent samples, of which very small organic structures and solid particles can be observed. SEM, on the other hand, provides extremely detailed grayscale images, providing improved surface

visualization and resolution. SEM offers better image resolution but is more expensive and maintenance-intensive than OM.

Scanning Electron Microscopy (SEM) is a common technique and analysis method used in many scientific disciplines worldwide. It is very useful for analyzing both organic and inorganic materials from nanometer to micrometer (µm) dimensions. SEM operates at very high magnifications, typically up to 300,000×, and in some high-end systems, as high as 1,000,000×, producing very high-resolution and accurate images of various materials.

SEM can be used to analyze all types of solid samples and provides several important signals in the analysis.

Secondary electrons are primarily employed for surface morphology and topography, whereas backscattered electrons aid in understanding chemical contrasts through brightness variations caused by different elements. Each element scatters electrons differently; therefore, the differences in the image are due to contrast.[1]

This method allows scientists to view and measure sub-cellular particles, which are minuscule by human standards, and near the molecular level. It has been found that many traditional Bhasmas (Ayurvedic preparations of metals/minerals) contain very small particles or what is known as Nanoparticles. (Smaller than 100 nm).[2]

METHODOLOGY

The present work is a narrative review of previous research articles related to the use of Scanning Electron Microscopy (SEM) in Ayurvedic formulation analysis.

The literature was collected from peer-reviewed journals such as AYU, Ayushdhara, Journal of Pharmacognosy and Phytochemistry, International Journal of Ayurvedic Medicine, Journal of Ayurveda and Integrative Medicine, Indian Journal of Pharmaceutical Sciences, National Journal of Research in Ayurved Science, and World Journal of Pharmaceutical Research. This review provides a general introduction, types of SEM analysis, principles of SEM, and working mechanisms of SEM.

RESULTS

Types of SEM

Scanning Electron Microscopy (SEM) can be classified into three main types: Conventional SEM (CSEM), Environmental SEM (ESEM), and low-vacuum SEM (LVSEM).

a. Conventional SEM (CSEM): Conventional SEM is used to perform the interaction between the electron beam and the specimen under high vacuum (approximately 10-6 torr, where torr is a unit of pressure measurement; 1 torr = 133.32 Pascal). In such a vacuum, the sample emits few secondary electrons with low energies, which have a low probability of colliding with gas molecules in the chamber. However, it is important to note that a high-vacuum environment can cause dehydration and cracking of certain materials, such as concrete, which makes the direct observation of crack propagation difficult. Although this is a limitation, some features are unaffected by the vacuum and can be effectively used, such as the identification of alkali-silica reactivity (ASR) or the analysis of the constituents of aggregates used in concrete.

b. Environmental SEM (ESEM): ESEM typically works at relatively high chamber pressures, typically 0.2 to 20 torr, with the interaction between the electron beam and specimen taking place in a gated gas environment. This operating condition has both advantages and disadvantages, depending on the sample and the analysis to be performed.[1]

Principle of SEM

It performs scanning in the place of light using a focused electron beam to scan the sample. The wavelength of an electron is much smaller than that of visible light, so SEM can focus much closer and provide much finer details.[3,4]

When the electron beam hits the surface of the specimen, it interacts with the atoms and produces several different signals.

SEM produces three important signals

Secondary electrons: These electrons help us observe the surface details, such as texture, roughness, and shape of the sample.[1,5]

Backscattered electrons: These help us observe differences between elements in the sample. Heavier elements appear brighter, whereas lighter elements appear darker.[1,6]

Characteristic X-rays: These are used to determine which elements are present in the sample. For this, SEM is connected to EDS or EDAX, which analyzes the elements.[5,7]

To avoid electron scattering by air molecules, the SEM apparatus is a complex system composed of many essential components placed in a vacuum chamber.[1,4]

Electron Source: Generally, a field emission source or tungsten filament that creates a stable stream of electrons.[5,6]

Anode: Focuses the electrons on the sample when they travel through the chamber.

Electromagnetic Lenses

An objective lens focuses the beam within a specific area.

Condenser lenses, which control the size of the beam.[1,6]

Scanning coils: Used to scan the beam in a systematic line pattern from one row of the sample surface to another.[1]

Sample Stage: The sample is placed on this stage, which can often be moved in all X, Y, and Z axes.[5]

Detectors: Specific sensors for backscattered and secondary electrons.[5,6]

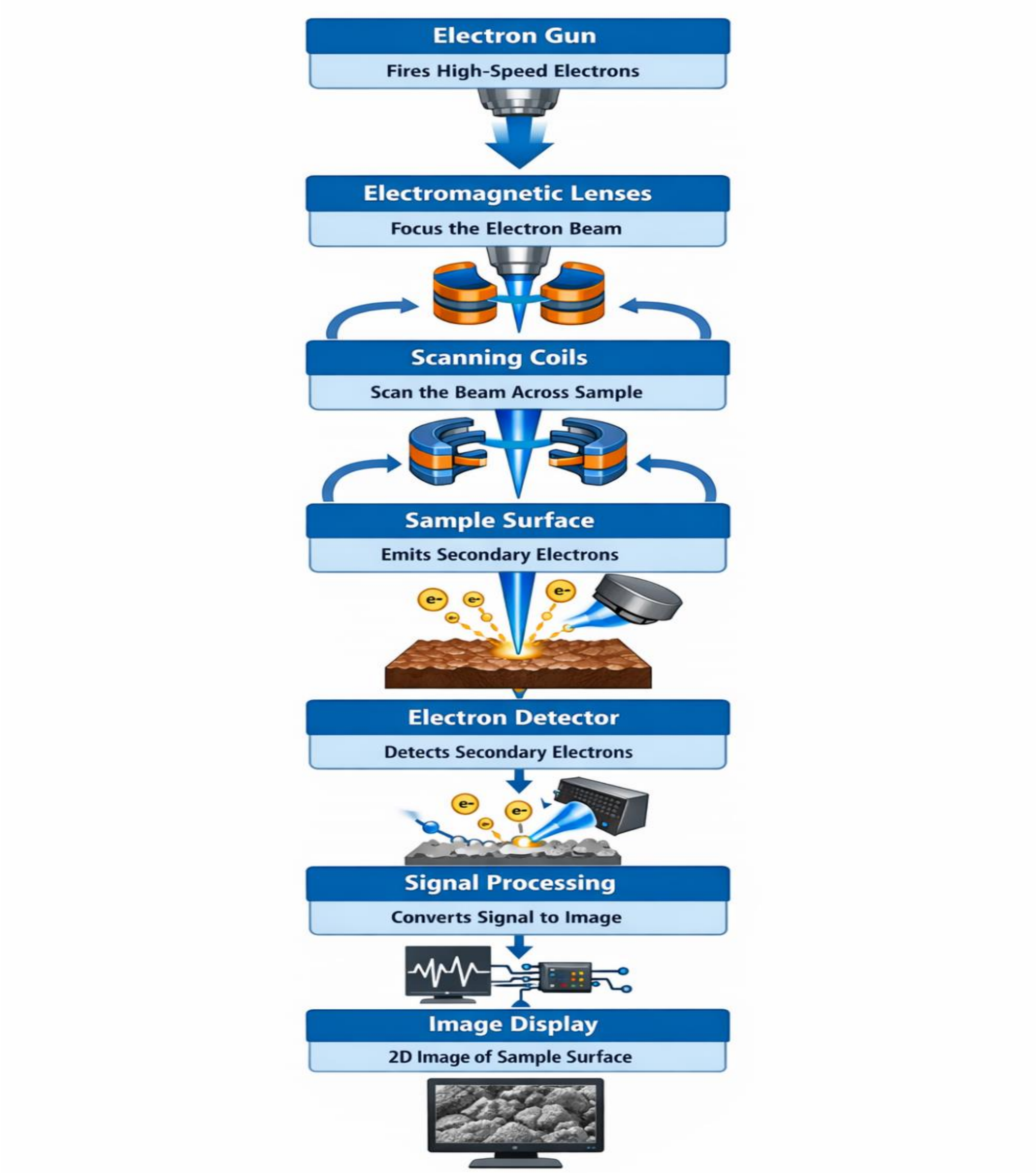

Figure 1: Working Mechanism of SEM.

Working Mechanism: Here is how the entire process works, step by step:

First, an electron gun spits out electrons (high voltage accelerates the electrons). Subsequently, the beam is focused on a small, concentrated area using electromagnetic lenses. Then, this spot is scanned by the scanning coils, which hit the sample one by one. The beam hits the surface of the sample, and secondary electrons are ejected from the surface atoms of the sample. These electrons are collected by a detector, which transforms them into an electrical signal. Eventually, the signal is converted to an image on a monitor in the form of pixels, building up a two-dimensional image that reveals the surface texture, topography, roughness, and morphology of the sample.[1,5]

Advantages

High Magnification: 20X, 30,000X, or greater.[3,4]

Depth of Field: Makes surface structures appear in 2D.[1]

Concurrent chemical identification with the integration of EDAX was performed in the Elemental Analysis.[5,7]

Limitations

Imaging wet or live samples is challenging because a high vacuum is necessary.

Specimen Preparation: Samples were dried and solid.

Conductivity Problems: To stop charging, non-conductive samples such as some herbs need to be coated with gold or graphite.

SEM's suitability for Ayurvedic samples
SEM has proven to be a very useful technique for the study and demonstration of some substances in Ayurveda research, such as *Bhasmas* (incinerated mineral/metallic powder) and *Kajjali* (mercurial preparations).

Nanoparticle confirmation: According to Ayurveda, *'Bhasma-Pariksha'* or *Bhasmas* can penetrate the fine lines of the facial skin (*Rekhapurnatwa*). SEM is the scientific evidence of this, as it can detect the sizing of particles at the micrometer and nanometer levels.[8,9]

Morphological fingerprinting: Uniform surface patterns are obtained from various preparations (Puta count, heat cycle application). For quality control purposes, SEM analyses were used to recognize these "fingerprints" as the shape and surface texture of the formulation are indicative of the formulation's morphological fingerprint, which is unique to each formulation or drug.[10,11]

Harmonization: Harmonization is necessary for regulatory clearance in a global sense, as it helps move towards quantitative results (size distribution, shape) from sensory evaluation (*Grahya Lakshanas*).[9,12]

Safety Evaluation: SEM and EDAX were used to verify that the particles were less than 1 micron, confirming a very fine particle size. The surface morphology of the sample was uniform. Signs of proper *Bhasmikarana* (incineration process). Sizable metal particles were not found, which indicates that there is no free mercury.[7]

SEM Analysis of Some *Bhasmas* and Formulations

1. *Swarna Bhasma* (Gold *Bhasma*): In the case of *Swarna Bhasma* (Gold *Bhasma*) the SEM analysis reveals the presence of globular particles in general. The exact amounts used vary from study to study, but the transformation of gold into an extremely fine, biocompatible material has been reported.[8]
2. *Rajata Bhasma:* The mean particle size of *Rajata Bhasma* was approximately 1.04 µm. In the SEM-EDX analysis mode, silver is first altered to the sulfide state (Acanthite: Ag_2S), which reveals a significant reduction from the state of raw metal.[13]
3. *Tamra Bhasma*: According to SEM analysis, the particle size of *Tamra Bhasma* was < 2 µm. Furthermore, commercial manufacturers may produce products with different particle sizes and chemical compositions; however, they all aim to produce a fine, absorbable powder.[14]
4. *Lauha Bhasma: Teekshna Lauha Bhasma* is prepared by burning 20 cycles (15 *puta*), and the particle size is 100-500 nm (15). For General *Lauha Bhasma* revealed fine and coarse structures and a uniform size distribution.[16]
5. *Yashada Bhasma*: Based on SEM images, it is observed that *Yashada Bhasma* consists of small particles, generally of the order of ~324 nm which is smaller than the size of raw Zn metal (1-2µm).[17] The smaller particles were mainly composed of zinc oxide nanoparticles.[18]
6. *Mandura Bhasma*: It is an Iron-based Mineral, which is first extracted from raw form of *Mandura* (undispersed form with big grains of 10-20 microns). During the initial processing procedure to prepare *Mandura Bhasma*, the structure of *Mandura* is changed, where smaller aggregates of approximately 200–300 nm are formed with a more coherent structure.[19]
7. *Abhraka Bhasma*: Scanning electron microscopy (SEM) revealed the existence of square-shaped nanoparticles of size 92.3 nm (fine) and smooth, easily visualized in *Krishna Vajra Abhraka Bhasma* (KVB).[20]

When analyzed using energy X-ray spectroscopy (EDAX/EDS), the primary elements identified were Fe (22 %), Si (13 %), Ca (11 %), and K (8 %) (28). Moreover, another study established the presence of other elements in the nanoparticles of Krishna Vajra Abhraka Bhasma, such as Ti (1 %), Al (2 %), Na, Cl, and P (20 %).[21]

8. *Kajjali* is mixture of *Parad* and *Gandhak*: SEM photomicrographs of *Samagandhak Kajjali* shows that majority of particles are very fine with the size generally being 10µm and many are of the size less than 5 µm and some are as small as 0.23 µm. The size distribution of the particles appeared to be mostly in the micrometer range based on the images. The particles predominantly have a cubic shape, with some having rhombus features. The overall sample exhibited a combination of small particles and geometric shapes.[22]
9. *Rasa Sindura*: Particle Morphology and Size: The SEM images revealed isolated particles. At 80.00 KX, the particle sizes were found to be in the 56.02-60 nm range, and at 20.00 KX amplification, the ranges were 118-138.2 nm.

In terms of the elemental composition of EDX/EDAX.

a) The analysis showed the presence of mercury (Hg) and sulfur (S).
b) Elemental weights for Hg (84.08%) and S (15.92%) yielded atomic ratios (or Atomic %) of Hg (45.77%) and S (54.23%),
c) No other heavy metals were detected in this product.[23]

10. *Naga Bhasma* (lead-based formulation)
Characterization: *Naga Bhasma* yields nanocrystalline Lead Sulfide as its end product.

- Processing Relevance: Tamarind and peepal bark herbal powders were used to maintain Pb in the +2-oxidation state.[24]

The purification (*Sodhana*) process removes heavy elements other than Pb and prepares the material before trituration.[25]

11. *Tarakeshwara Rasa* (TR): A scanning electron microscope (SEM) analysis of TR found that the chemicals were aggregated together as particles with a size ranging from 0.5 to 2 μm. These data are helpful in developing a standardized fingerprint for this unique set of substances for treating the condition called *Prameha* (diabetes mellitus).[26]

12. *Shatpala Gandhaka Churna*: The SEM micrographs of *Shatpala Gandhaka Churna* reveal the particles are evenly distributed and arranged in a cluster in both elliptical and spherical patterns. The particle size distribution (PSD) and quantitative analysis of SEM showed much smaller particle sizes of 10.9 nm. This nanoscale size provides insight into the stability and structural properties of the formulation, with a zeta potential of 40.9 mV.[27]

13. *Shankhapani Rasa*: The Scanning Electron Microscopy of *Shankhapani Rasa* at different magnifications, the grain size was commonly found to range between 109.2 nm at 5K magnification to 77.97 nm at 7K. The smallest grain size was 77.97 nm at a magnification of 7 K in the *Shankhapani Rasa* sample.[28]

Interpretation of SEM analysis

All 14 formulations were characterized using SEM, which illustrates the importance of this characterization technique in the validation of Ayurvedic medicine based on the *Suksma* (fineness) aspect.

Verification at the nano level: In SEM, the traditional incineration process (*Bhasmikarana*) has been shown to enable the dissolution and reduction of bulk metals such as gold and mercury to biocompatible nanoparticles, for example, *Swarna Bhasma*[8] or micron-sized *Rasa Sindura* (56-60 nm).[23]

Surface morphology as quality grade: The appearance of the particles, such as square-shaped nanosized particles in *Krishna Vajra Abhraka Bhasma*[21] and spherical-shaped nanosized particles in *Swarna Bhasma,*[8] provides a standard for researchers to ensure that the study has been conducted correctly with these medicines.

Stability analysis: High-resolution imaging along with the Zeta Potentials of the particles enables manufacturers to estimate the stability of the herbo-mineral cluster and predict its shelf life. For example, the zeta potential of the particle of *Shatapala Gandhaka Churna* is 40.9 mV, and hence its stability is known.[27]

Efficiency of processing: Dramatic size reduction from the raw material size (10-20 μm) to the final formed product size (200-300 nm) is a good example of the use of SEM in the efficiency of processing.

SEM is an extremely powerful tool for the chemical fingerprinting of materials and can be used to identify the standard material and determine its purity using SEM and energy-dispersive X-ray analysis (EDAX/EDX). In addition, SEMEDX will not only inform you if a metal has turned into a therapeutic compound, but it will also alert you to the possibility that the metal could have turned into a poisonous compound.

In the case of *Rajata Bhasma,*[13] silver would become Acanthite (Ag2S), whereas in *Yashada Bhasma,*[18] zinc would change to Zinc Oxide. Elemental fingerprinting uses the percentage of weight of specific elements in complex formulations to standardize them (e.g., in *Shankhapani Rasa,*[28] 4.52% of mercury and 12.79% of calcium).

Therefore, the standardization of this type is necessary for a safe formulation, as it ensures that the formulation does not contain unsafe levels of heavy metal contaminants. (e.g., *Rasa Sindura*[23] and *Naga Bhasma.*[24]

Finally, SEM and EDAX/EDX can be used to obtain the purity, chemical fingerprinting, chemical transformation, elemental fingerprinting standardization, and verification of contaminants.

DISCUSSION

In the latest report from Schenstad et al., between discussing the success of its research on the evaluation of Ayurvedic drugs using SEM technology, it has mentioned a significant number of research gaps that persist in the progression towards standardization of Ayurvedic medicine. In the latest report by Schenstad et al., while talking about the success in evaluating the Ayurvedic drugs using SEM technology, the report mentions a considerable number of research gaps that still hinder the full standardization of Ayurvedic medicine. For example:

1. Lack of Validated Markers: Researchers have identified the morphology of some Ayurvedic preparations, such as *Shatpala Gandhaka Churna*, which consists of elliptical clusters. However, an Official Pharmacopoeial Standard that defines the morphology that best provides therapeutic benefits is not yet available for all drugs.[27]
2. Inconsistent Sample Preparation: Particles may be prepared with inadequate sample preparation, resulting in an incorrect size estimation of the particles, which may not be the true size, with 2. Inconsistent Sample Preparation (*Mandura* Bhasma[19]) was considered. The SEM results may be affected considerably by poor sample preparation, particularly by sample dispersion in the SEM.

3. Commercial Variability: For instance, a scholarly article reviewed by *Tamra Bhasma*[14] showed significant variations in the chemical phase and particle size attributes manufactured by various drug makers in the Ayurvedic industry; therefore, the industry has no standardized "Puta" cycles at present.
4. Biologic Correlation: Many researchers report only on physical parameters of SEM findings such as the size measurement of *Shatpala Gandhaka Churna* (10.9nm or 27 units) and no correlation with the human bioavailability and the related toxicity.

CONCLUSION

Scanning Electron Microscopy (SEM) has become an important tool for bringing scientific clarity to Ayurvedic herbo-mineral medicines. It is useful for the close examination of the size, shape, and elemental composition of particles, as well as for investigating the processes by which raw metals and minerals are converted to very fine, body-friendly particles. This enables SEM to link traditional Ayurvedic testing methods with modern scientific testing, thereby ensuring the safety, purity, and uniformity of *Bhasmas*. However, there are some gaps in this understanding, such as the absence of fixed standards, variations in the methods of preparation, and inadequate knowledge of the relationship between the results and real biological effects. These challenges suggest the need for further research to make Ayurvedic preparations more widely recognized and properly standardized on a global scale.

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