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Pharmaceutical Standardization

Analytical study of raw *Swarna Makshika* (Chalcopyrite) and its *Bhasma* through TEM and EDAX

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Abstract

Ayurveda, the Indian System of Medicine deals with a number of metals and minerals. *Swarna Makshika* (SM), a chalcopyrite one such minerals has iron (Fe), copper (Cu), and sulphur (S) as major ingredients along with other trace elements of therapeutic importance. Studies related with characterization of SM are very few. In the current study SM and SM *Bhasma* were analysed through Transmission Electron Microscope (TEM) and Energy Dispersive X-Ray Analysis (EDAX). Analysis reveals the presence of iron, copper, and sulphur in SM. In addition to these elements, SM *Bhasma* found to contain Potassium, Magnesium, Aluminum, and Silicon in trace amount. TEM study reveals that, grain size of the SM (5-10 microns) is significantly reduced in SM *Bhasma* to 50-200 nm.

Key words: *Bhasma*, chalcopyrite, energy dispersive X-ray analysis, standardization, *Swarna Makshika*, transmission electron microscope

Introduction

Analytical study is one of the imperative parts for drug standardization in Ayurveda, Siddha, and Unani (ASU) systems of medicine. In today's era of science and information technology, it is essential that the details about the medicine consumed by the consumer must be explored to the scientific community and regulatory authority for its validation, authentication, and future development when necessary, for the greater benefit of the particular system and society as well. Though the *Bhasmas* are in clinical practice since hundreds of years, the need of the hour is to scientifically validate these by using modern tools and techniques.

Quality control methods of *Bhasmas* were well developed by seers based on the parameters prevailing in those days. Though the parameters have their own significance, they have certain limitations and are not sufficient to characterize the *Bhasma* with respect to their physicochemical nature. Therefore use of sensitive modern tools and techniques are necessary to characterize the *Bhasma*. Considering this, an effort has been made to analyze the raw material (SM), and finished product (SM *Bhasma*) through TEM and EDAX. Prior to subjecting the material to TEM or EDAX, attempts were made to examine the *Bhasma* through classical parameters of analyses.

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Pharmaceutical Process

The pharmaceutical process included *Shodhana* (purification) and *Marana* (incineration) of *Swarna Makshika* (SM) to prepare genuine *Bhasma*. For *Shodhana*, the initial material was roasted at a temperatureof about 850-900°C with frequent addition of lemon juice in a charcoal furnace till it appeared red in color, and it took three days for achieving the desired features of *Shodhana*.[1,2] Then the material was subjected to *Marana* process. For the *Marana*, lemon juice was used as *Bhavanadravya* (material for wet trituration). In conventional *Puta* (heat source) system of heating, 11 *Putas* were given to achieve desired quality of *Bhasma* and for each *Puta*, 4 kg cow dung cakes were used.[3]

After completion of the pharmaceutical process, the *Bhasma* was subjected to classical quality control parameters followed by analyses through Transmission Electron Microscope (TEM) and Energy Dispersive X-Ray Analysis (EDAX) analysis.

Materials and Methods

Materials

The raw material (SM) was collected from Ayurvedic Pharmacy, Banaras Hindu University and authenticated by studying its characters as explained in classical texts.[4,5] *Shodhita* SM and SM *Bhasma* are prepared in the Department of Rasa Shastra, Institute of Medical Sciences, Banaras Hindu University.

For TEM and EDAX: Properly calibrated EDAX attached TEM (Model-Technai-20G2, Voltage-200 KV, FEI, USA), mortar

and pestle, mounting stub, copper grid, sample holder were taken and maintained as required.

Method

Ancient analytical methods

Rekhapurnatvam: A pinch of prepared SM *Bhasma* should be taken in between the thumb and index finger and rubbed. Properly prepared *Bhasma* should enter into the creases of the fingers and should not easily cleansed out.[6] This test confirms the fineness of *Bhasma*.

Varitaratavam: Small amount of the prepared *Bhasma* is to be sprinkled over the stagnant water ina beaker. An ideal *Bhasma* should float over the surface of the water.[6]

Nisvadutvam: Genuine *Bhasma* should be tasteless when a small amount was kept over tongue.

AmlaPariksha: When a pinch *Bhasma* is mixed with little amount of *Dadhi* (curd) in a clean petridish, kept for 24 h, no color change should occur. Similar observations should occur when lemon juice is added.

Dantagrekachkachabhava: No grittiness should be observed, when a small amount of *Bhasma* is placed between the teeth.

Avami: Properly manufactured *Bhasma* should not produce any nausea or vomiting.

Analysis using modern parameters

The raw SM, intermediary product (*Shodhita* SM) and final product (SM *Bhasma*) were subjected to TEM and EDAX at Department of Physics, BHU.

Principle of imaging technique in TEM

In TEM, a crystalline material interacts with the electron beam mostly by diffraction rather than absorption. The intensity of the diffraction depends on the orientation of the planes of atoms in a crystal relative to the electron beam. A high contrast image is formed by blocking electrons deflected away from the optical axis of the microscope by placing the aperture to allow only unscattered electrons to pass through. This produces a variation in the electron intensity that reveals information on the crystal structures, and can be viewed on a fluorescent screen, or recorded on photographic film or captured electronically.[7]

Energy dispersive X-ray analysis

It is an analytical technique used for the elemental analysis or chemical characterization of a sample. Being a type of

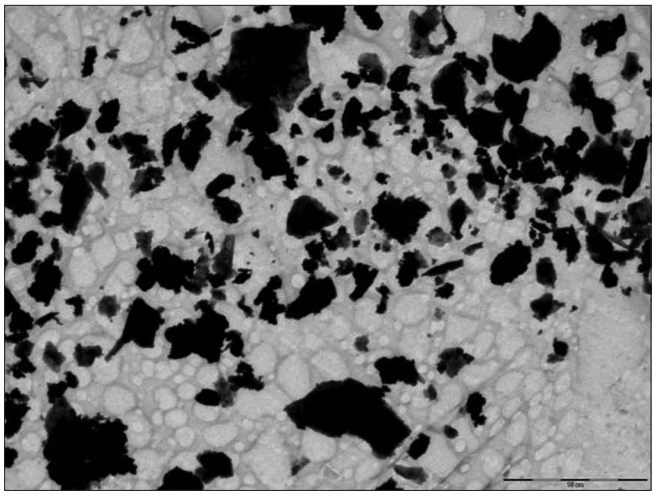


Figure 1: Raw *Swarna Makshika* Under TEM

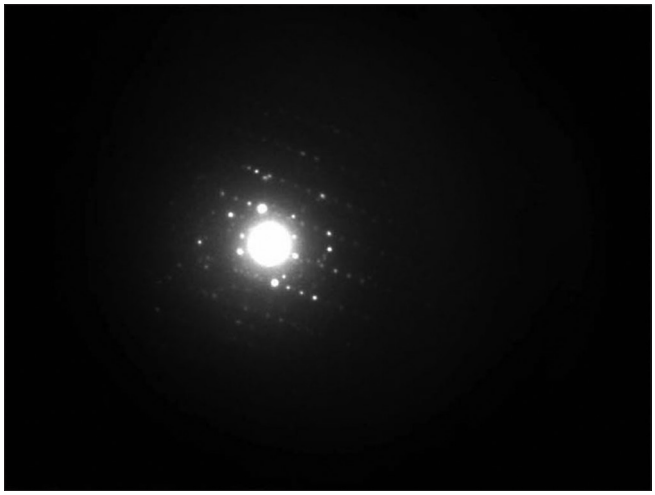


Figure 2: Diffraction pattern of raw *Swarna Makshika* under TEM

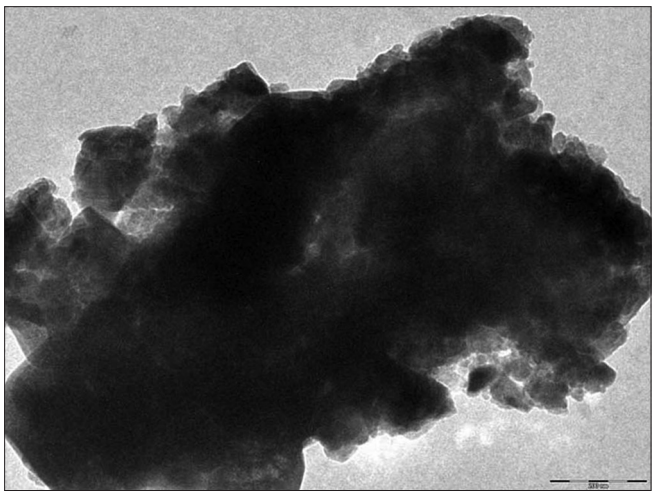


Figure 3: *Shodhita Swarna Makshika* under TEM

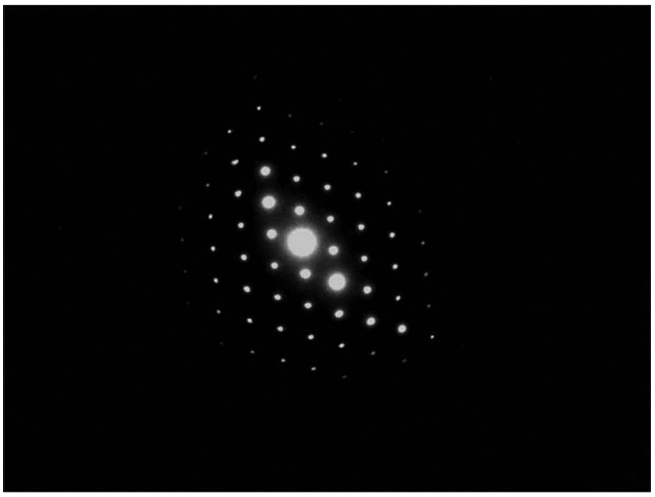


Figure 4: Diffraction pattern of *Shodhita Swarna Makshika* under TEM

spectroscopy, it relies on the investigation of a sample through interactions between electromagnetic radiation and matter and then analyzing the X-rays emitted by the mater in response to being hit with charged particles. Its characterization is due to the fundamental principle, that each element has a unique atomic structure.[8,9]

EDAX systems are found attached on TEM equipped with a cathode and magnetic lenses to create and focus a beam of electrons. A detector is used to convert X-ray energy into voltage signals; this information is sent to a pulse processor, which measures the signals and passes them onto an analyzer for data display and analysis.[10]

Preparation of the sample

Sample was grinded in mortar and pestle and was made sufficiently fine. A stub was taken; the sample to be analyzed taking in a grid, was mounted to the stub, and was subjected to scanning under TEM.

Scanning the sample under TEM

The mounted sample was placed inside the vacuum chamber of microscope through an air-tight door, and then the air was pumped out. After the air was pumped out of the chamber, a beam of high electron beam was emitted by an electron gun from the top. This beam travels downward through a series of magnetic lenses designed to focus the electrons to a very fine spot. Near the bottom, a set of scanning coils made the focused beam to move back and forth across the mounted sample, row by row. As the electron beam hits each spot on the sample, secondary electrons are back scattered from its surface. A detector counts these electrons and sends the signals to an amplifier. The final image was built up from the number of electrons emitted from each spot on the sample.[8]

Observations

It was found during the classical analytical parameters that, the *Bhasma* passed all the tests of analysis. It implies that, the finished product is genuine.

TEM reveal that, the grains in the raw material and the *Shodhita* sample are not uniformly arranged [Figures 1-4], while they were distributed uniformly in *Bhasma* [Figure 5]. More clustering of grain was observed in the final product [Figure 6], whereas in raw material [Figure 1] and in the intermediary product [Figure 3] the grains were found scattered. The size of the grain was significantly reduced in the final product. The grain in the raw material was in between 5 to 10 microns [Figure 1] and in the *Bhasma* it was 50 to 200 nm [Figure 6].

EDAX study reveals that raw material contains Cu-33.08%, Fe-28.60%, S-32.85%, and O-5.47% w/w [Figure 7]. *Shodhita* material found to contain Cu-21.45%, Fe-49.25%, S-08.38%, and O-20.92% w/w [Figure 8], while SM *Bhasma* contains Cu-29.40%, Fe-32.26%, S-02.45%, O-24.05%, Mg-03.05%, Al-02.23%, Si-05.28%, and K-01.23% [Figure 9]. It was observed from the analysis that the quantity and quality of trace elements are increasing with succeeding the number of *Putas* with decreasing of the sulfur percentage and increasing of the oxygen percentage.

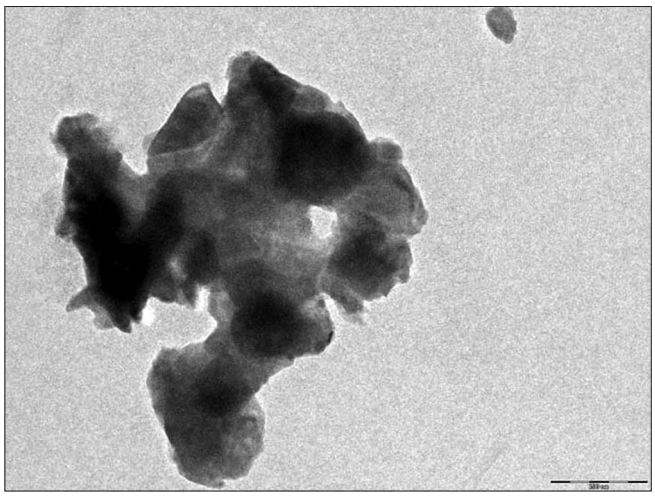


Figure 5: *Swarna Makshika Bhasma* under TEM

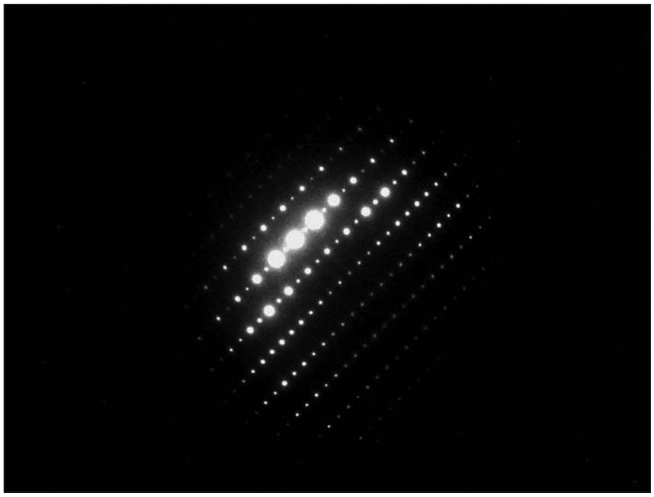
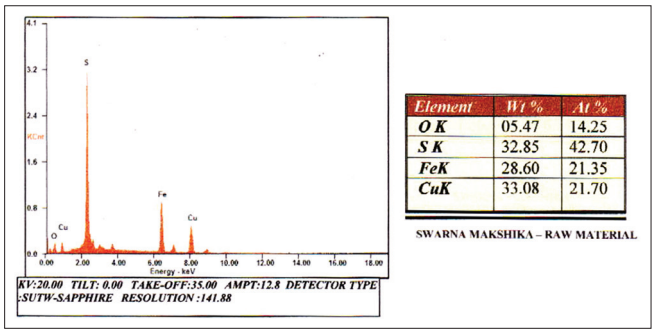


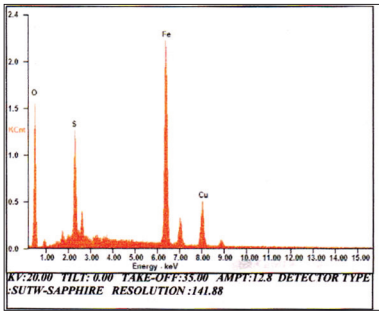
Figure 6: Diffraction pattern of *Swarna Makshika Bhasma* under TEM



Element	Wt %	At %
O K	05.47	14.25
S K	32.85	42.70
FeK	28.60	21.35
CuK	33.08	21.70

SWARNA MAKSHIKA – RAW MATERIAL

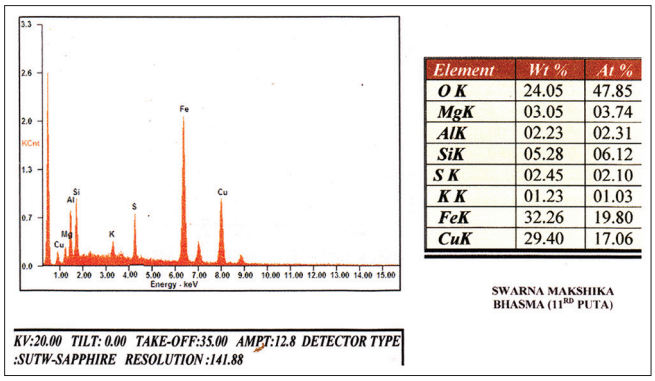
Figure 7: EDAX report of raw *Swarna Makshika*



Element	Wt %	At %
O K	20.92	46.89
S K	08.38	09.37
FeK	49.25	31.63
CuK	21.45	12.11

Swarna Makshika- Shodhita

Figure 8: EDAX report of *Shodhita Swarna Makshika*



Element	Wt %	At %
O K	24.05	47.85
MgK	03.05	03.74
AlK	02.23	02.31
SiK	05.28	06.12
S K	02.45	02.10
K K	01.23	01.03
FeK	32.26	19.80
CuK	29.40	17.06

Figure 9: EDAX report of raw *Swarna Makshika* after 11th *Puta* (*Swarna Makshika Bhasma*)

Discussion

Ancient pharmaceutical processes are known to convert metals and minerals into therapeutically potential drugs that are vogue since centuries. But need of the time is to explain the physico-chemical nature of raw material and the finished products as well. Considering importance of this aspect, the raw SM and its *Bhasma* were analyzed by TEM and EDAX todevelop analytical profile of both the samples.

Analysis of TEM reports of raw material, and *Bhasma* elucidates gradual reduction of the grain size, increase in regularity, and uniformity of the grain with increasing the number of *Putas*. The significant reduction of the particle size of the final product justifies the *Rekhapurnata* and *Varitaratwa* character of *Bhasma* claimed in the ancient texts. It highlights the ancient technology of size reduction and their applicability in biomedical system in view of the current nanotechnology.

Size of the grains of SM was 5-10 microns, whereas size of the grains in SM *Bhasma* was 50-200 nm. It was observed that the grain size of the *Bhasma* was not having a particular fixed size rather; it was in a range and the particle size was significantly less in comparison with that of the raw material. This indicates that the process generates uniformity, regularity, and gradual reduction in size. In addition, the study revealed the crystalline diffraction pattern and three dimensional observation of surface phenomenon of the sample under high resolution.

From the EDAX study, it was observed that the amount of sulphur gradually decreases and amount of oxygen increases from raw material to *Bhasma* and the *Bhasma* contains least amount of sulphur and maximum amount of oxygen. This happens due to sublimation of sulphur from raw material while heating and substitution of oxygen during the process. Thus, the *Bhasma* may be more suitable to the body as it contains more oxygen and least sulphur.

It was also observed that trace elements like Mg, K, Si, and Al were found in the prepared *Bhasma*, which might enter into finished product through the process of *Bhavana* (wet trituration) with lemon juice. Lemon juice is reported to contain many trace elements.[9] The presence of silicon may be due to using of earthen casseroles in the *Puta* system of heating in *Marana* process. The relative percentage of iron is increased in prepared *Bhasma* because of the loss of sulphur, and some iron may be present in the *Bhavana* drugs used and some may be entered from the utensils used in roasting of raw material during *Shodhana*. Analyzing critically, it was observed that the trace elements increase both quantitatively and qualitatively with increasing of the number of *Puta*.

Conclusion

This study concludes that the ancient pharmaceutical processes are very effective in converting the macro elements into therapeutically effective medicines of micro form. The size of the raw material (5-10 microns) was reduced to 50-200 nm in the *Bhasma*. It implies that, the classical procedures reduce the particle size to a considerable level. Increase of Fe and Cu along with other trace elements and decrease of sulphur was observed in the *Bhasma* in EDAX study. Findings of elemental analysis of EDAX showing quantitative analysis of the samples can be considered in future studies.

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हिन्दी सारांश

खनिज स्वर्णमाक्षिक एवं भस्म का TEM व EDAX परीक्षणों के माध्यम से विश्लेषणात्मक अध्ययन

सुधलदेव मोहपत्रा, चन्द्रभूषण झाँ

भारतीय चिकित्सा पद्धति में औषधि निर्माण हेतु अनेक खनिज पदार्थों का व्यवहार होता आ रहा है । इनमें से स्वर्णमाक्षिक एक महत्वपूर्ण खनिज द्रव्य है, जिसमें लोह, ताम्र, गन्धक पर्याप्त मात्रा में पाया जाता है । अन्य चिकित्सीय तथा कुछ भस्म प्रक्रिया कालीन नमूनों का अत्याधुनिक यन्त्रोपकरण के माध्यम से विश्लेषणात्मक अध्ययन किया गया तथा औषधि द्रव्य का मानकीकरण करने हेतु प्रयास किया गया है । इस सन्दर्भ में विभिन्न नमूनों का TEM एवं EDAX उपकरणों के माध्यम से विश्लेषण किया गया । अध्ययन के निष्कर्षानुसारत खनिज स्वर्णमाक्षिक द्रव्य व अन्तिम भस्म दोनों का परीक्षण करने पर यह पाया गया कि खनिज स्वर्णमाक्षिक में लोह, ताम्र, गन्धक, उपस्थिति और भस्म में इन उपरोक्त तत्वों के उपरांत Potassium, Magnesium, Aluminum, and Silicon कुछ अंश में उपस्थिति पाई गई।परीक्षण में यह दर्शाया गया कि प्राचीन औषधीय निर्माण प्रक्रियाओं के प्रभाव से खनिज स्वर्णमाक्षिक के कणों का आकार ५-१० से अत्याधिक ह्रास होकर भस्म में ५०-२०० पा तक हो गया । इस शोधपत्रमें इन तथ्यों को वैज्ञानिक पद्धति से स्थापित करने का प्रयास किया गया है । यह लेख विद्वान जनादृत होगा ऐसी अभिलाषा है ।