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RESEARCH ARTICLE

Physico-chemical characterization of Ayurvedic *Swarna Bhasma* by using SEM, EDX, XRD and PSA

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ABSTRACT

Introduction: *Swarna Bhasma* (SB) is a charismatic Ayurvedic drug known for its miraculous therapeutic functions for protecting human health. Consistency in the efficacy and safety of SB has largely been attributed to its manufacturing process. The quality assurance of SB has been based on the parameters listed in ancient texts of Ayurveda, however, there is a need for machine-based, reliable and repeatable characterization methods for estimation of gold content, particle shape and size, etc.

Methods: The present study was undertaken to evaluate the physico-chemical characteristics of SB manufactured by Shree Baidyanath Ayurved Bhawan Pvt. Ltd. Jhansi, India. SB was characterized using X-Ray Diffraction (XRD), Scanning Electron Microscopy (SEM), Energy Dispersive-ray analysis (EDX) and Particle Size Analyzer (PSA).

Results: In XRD analysis, the diffraction pattern showed the presence of four major peaks at 2θ value of 37.17^0, 44.35^0, 64.53^0 and 77.50^0, which corresponds to gold as per the International Centre for Diffraction Database (ICDD) reference. The EDX results showed high purity of gold (97.40% w/w) in the sample along with Na, Fe, Al, K, Ag and Ca in trace amounts. The mean particle size of gold is found to be 2.633 µm by PSA. The particle size by SEM is in the range of 3-4 µm. The morphological characteristics of the gold particles were spherical and mostly irregular in shape and seen to be agglomerated.

Conclusion: The physico-chemical analysis of each batch of samples of SB should be carried out by the manufacturing companies, to rule out variations during manufacturing practices for ensuring its therapeutic efficacy and safety.

KEYWORDS:
Baidyanath, Gold, *Swarna Bhasma*, SEM, XRD EDX, PSA

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INTRODUCTION

Ayurveda uses several metals and minerals as therapeutic agents. *Rasa Shastra* is a branch of Ayurveda, where metals/minerals are converted into therapeutic forms known as *Bhasma* by various chemical and physical processes. These metals and minerals are triturated with several herbs with intermittent heating and cooling as per a complex manufacturing process described in *Rasa Shastra*, the texts of the Ayurvedic system of medicine. *Charaka Samhita*, one of Ayurveda's scheduled texts, contains a large number of references regarding the preparation of various *Bhasma*.[1] Metals like gold, copper, iron, mercury, lead and silver are very frequently used in the preparation of the *Bhasmas* and are known to play important roles in the therapy and management of human diseases.[2]

Gold is a valuable metal known to Indians since antiquity. Several types of gold preparations for therapeutic uses have been mentioned in Indian, Arabic and Chinese medicines.[3] The charismatic health benefits of Gold Ash or *Swarna Bhasma* or *Suvarna Bhasma* (SB) has been documented in Ayurvedic medicine since ancient times.[4] Several pharmacological activities like antioxidant and free radical scavenging,[5] analgesic, anti-cataleptic, anti-depressant,[6] anti-cancer,[7] and reproductive system enhancement[8] have been reported from SB. It has also been utilized for the treatment nervous system disorders and rheumatoid arthritis.[9] Studies also have claimed that it has been used as a rejuvenating and revitalizing agent by aging human beings.[10]

In SB preparations, Gold is subjected to various traditional pharmaceutical processes; *Shodhana*, *Bhavana* and *Marana*

with certain additives including medicinal plant extracts. The gold metal work as an adsorbent/carrier for these phytochemicals and this complex is responsible for the therapeutic potential of SB.[11,12] Recent researches have indicated that the size, surface charge and chemical phase of the gold in SB play a crucial role for its biological activity as well as safety. The emerging field of nanomedicine has indicated that the size of the therapeutic molecule is important for its characteristics pharmacokinetics and pharmacodynamics.[10] Gold nanoparticles have been projected as efficient drug delivery systems for enhancing the bioavailability and efficacy of a large number of therapeutic agents.[13] Although SB has a long history of human use, some of the recent reports have raised concerns about its safety. These safety issues have been largely because of the improper manufacturing practices and usage of poor quality ingredients during the preparation of SB. The quality assurance and reproducibility in *Bhasma* preparation is difficult at present due to lack of understanding of conventional preparation methods.[14,12] Here, the characterization of gold in SB in terms of size, shape, phase, etc. may be of great significance in nanomedicine-based drug delivery as well therapy and safety. In this paper, we have carried out the physio-chemical characterization of *Swarna Bhasma* manufactured by Shree Baidyanath Ayurved Bhawan Pvt. Ltd., Jhansi, India.

MATERIALS AND METHODS

Sampling of *Swarna Bhasma*

Swarna Bhasma (Batch No.47) was obtained as a gift from Shree Baidyanath Ayurved Bhawan Pvt. Ltd., Jhansi, India is popularly known as Baidyanath, a leading Ayurvedic products manufacturing company in India.

X-ray diffraction

XRD was recorded on RIGAKU, Smart lab XRD facility available at the Innovation Centre, Bundelkhand University, Jhansi, Uttar-Pradesh, India. The powdered sample was scanned with X-rays 40KV, 40mA, scanning range from 3.0 to 80.0 deg in continuous mode, at a scanning rate of 4^0/ minutes.

SEM

SEM imaging was performed at the Sophisticated Analytical Instrument Facility, AIIMS, New Delhi, using ZEISS, EVO18 SEM. The electron beam was generated at 10kV, EHT 200V-30KV and the equipment was equipped with a BSD detector with a magnification range of 5x ~1000000x. For the SEM analysis, the SB (50 mg) was placed on a double-sided carbon tape and mounted on the analysis platform. On the mounted sample, 10 kV was applied, and the scanning was done at different magnifications from 300x to 1000x with a distance ranging from 20-22 mm.

EDX

EDX analysis was performed at the National Institute Technology (NIT), Raipur, Chhattisgarh, on Oxford-Energy Dispersive X-ray system fitted with INCA 250 EDS with X-MAS 20 mm detector.

PSA

PSA was performed on Anton Paar, Litesizer-500 instrument available in Department of Pharmacy, IGNTU, Amarkantak, Madhya Pradesh, India. SB sample (0.1-0.2 mg) was dissolved in ultra-pure grade water and mean particle size was recorded using a measurement cell.

RESULTS

XRD

The four major XRD peaks of SB were observed at 2θ value of 37.17^0, 44.35^0, 64.53^0 and 77.50^0. All the four peaks corresponded to Bragg reflections face-centered cubic lattice (111), (200), (220), and (311) for gold as per available literature (Figure 1). The element was also identified with the help of a standard ICDD (International Centre for Diffraction Database) reference values. The sharp peak in the XRD pattern suggests the crystalline nature and good purity of gold in this sample of SB.

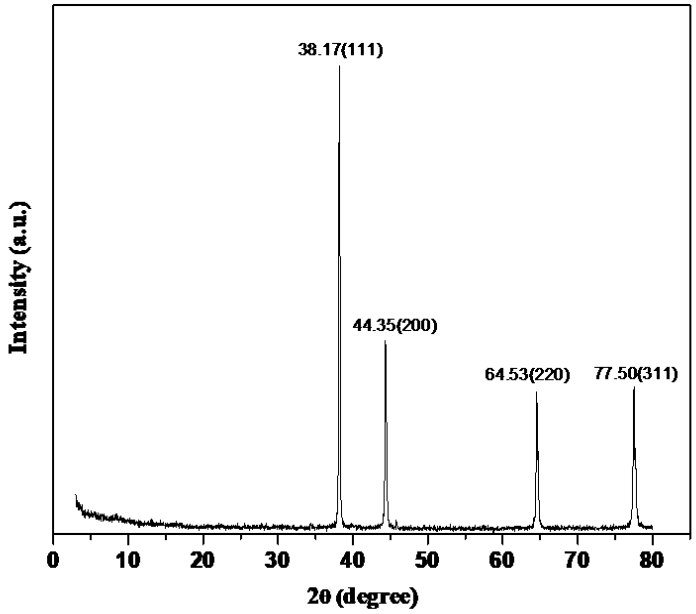

Fig.1: XRD spectra of SB with d-spacing values at corresponding $2\theta^0$

SEM

The SEM images of SB were recorded at four different magnifications viz. 1µm, 2 µm, 10 µm and 100 µm. The images showed that the particle size was in the range of 3-4 µm, which was also further confirmed by ImageJ software. The morphological characteristics of the particles were spherical and mostly irregular in shape and seen to be agglomerated. The particles were largely uniform in size and shape (Figure 2).

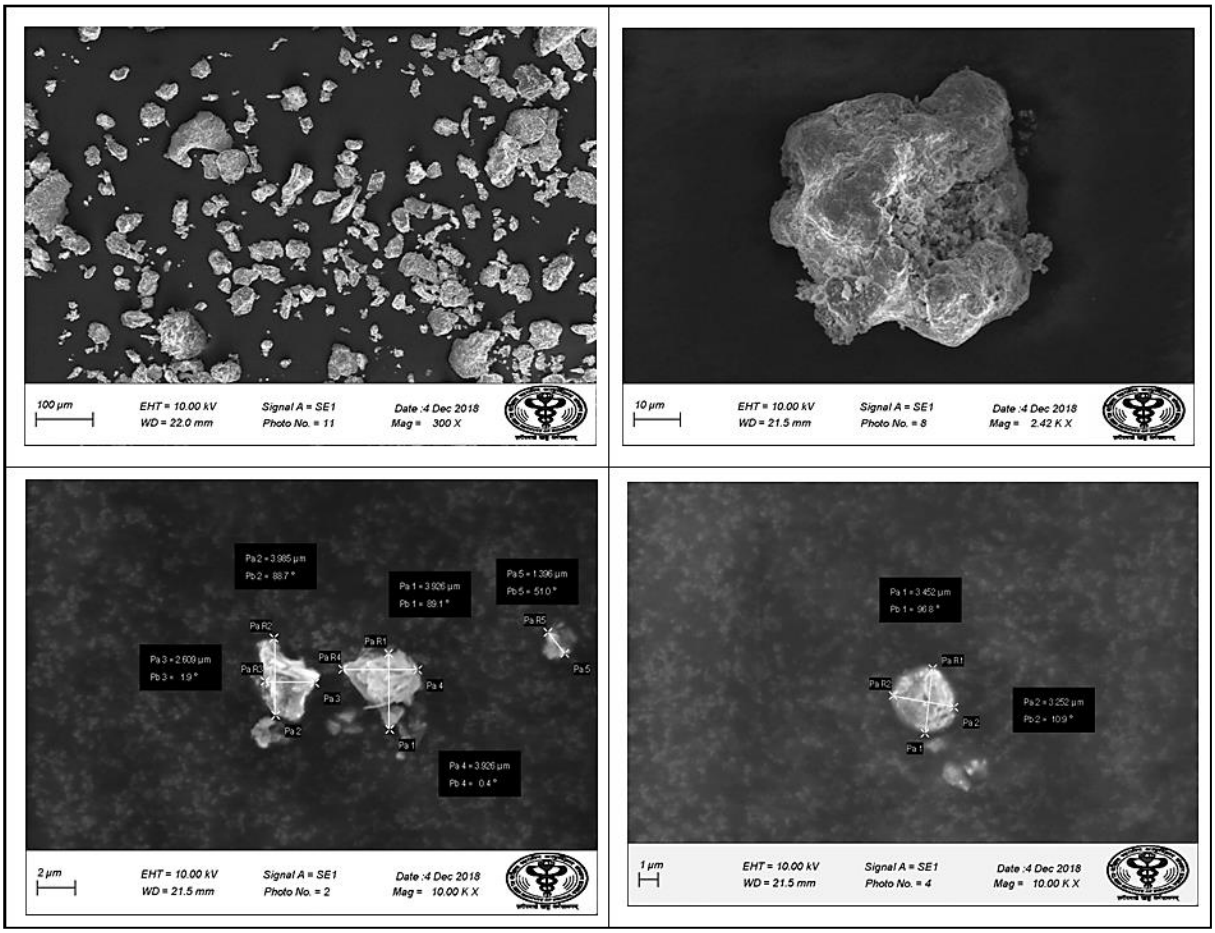

Fig.2: SEM photomicrographs of *Swarna Bhasma* (SB)

EDX

Elemental composition analysis of SB showed that it contains gold 97.40% w/w. The other elements present in trace amounts were Na, Fe, Al, K, Ag and Ca.

PSA

The mean particle size of gold in SB was found to be 2.633 μm with Poly Dispersity Index of 25.0%. The mean particle size measurement by PSA was consistent with particle size results of SEM (Figure 3).

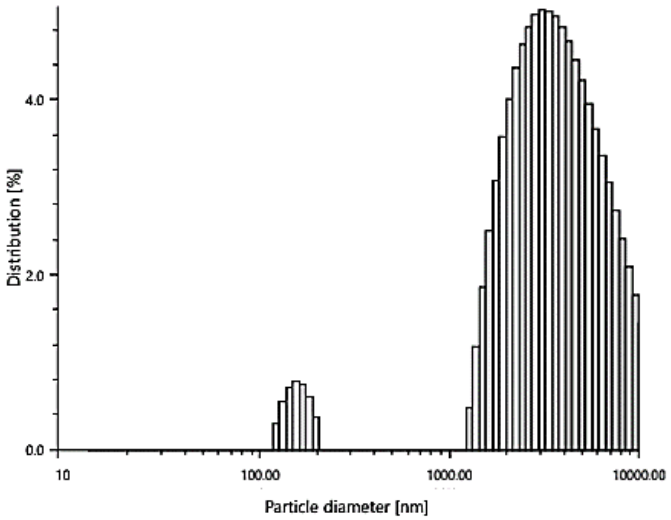

Fig.3: Particle size distribution histogram

DISCUSSION

In the preparation of *Bhasmas* metals and minerals are processed (*samskaras*), purified (*shodhana*), roasted (*jarana*), incinerated and crushed (*marana*) in the presence of plant products including decoctions. During these stages of preparations, the metal's particle size gets reduced and converts into *Bhasma* nanomaterials. Conversion of metal in nano-size particles results in an increase in its surface area and enhanced adsorption of the phyto-constituents. The increase in the surface area also leads to enhanced absorption, bioavailability and therapeutic potential.[10] As per ancient Ayurvedic texts, the ideal *Bhasma* are characterized based on *Varitara* (floats on water), *Varna* (colouration), *Unama* (floating of grain on *Bhasma* already floating on water), *Rekhapurnata* (filling furrows of fingertips when taken between the thumb and index finger), *Anjana Sanniba* (soft and smooth like collyrium), *Sukshamatva* (very fine), *Slakshnatvam* (smooth), *Nishchandratvam* (lack of luster), *Apunarbhava/iruttha* (stays in non-metallic state), *Avami* (do not produce nausea on administration), *Gatarasatvam* (tasteless), and *Ketaki Rajah Samah* (nano-sized as pollen grains of *Pandanus odoratissimus*).[15] Since the evaluation of *Bhasma* based on these parameters may require a great deal of expertise and may vary from expert to expert, hence physico-chemical methods have been developed to ensure accuracy, repeatability and reliability in the evaluation of their characteristics. The use of lower grades of raw materials, adulterations and anomalies in manufacturing processes, whether deliberately or accidentally, contributes to the manufacture of poor quality *Bhasma*, which not only poses questions about effectiveness but also causes toxicity.[16]

Swarna Bhasma, has been used for thousands of years for its charismatic therapeutic potential in many chronic ailments such as rheumatoid arthritis and diabetes mellitus.[4] The process of preparation of SB as per Ayurvedic Pharmacopeia and other classical texts resembles the top-down method of preparation of nanoparticles, as it is reduced to gold particles from a gold bar. The SB also has been considered to be large aggregates of smaller nanoparticles.[17]

The particle size of the SB in SEM analysis ranged from 3-4 μm in our study. In a similar study, the particle size of the SB procured from two different pharmaceutical companies (name of the companies not mentioned) was found to be 3-50 μm.[18] In previous studies, the size of gold particles in SB has been reported in the range of 3-6 μm,[2] 1-10 μm[19] and 10 nm-60 μm.[20] In comparison, the results of this study with the earlier reported literature; it is evident that the SB manufactured by Baidyanath is having a uniform gold particle size with very low variability. It indicates that the standardized manufacturing process has been followed very meticulously in preparation of SB by Baidyanath. This resulted in the smaller size of particles which may be attributed to grinding of raw material for a long duration (similar to top-down approach of formation of nanostructure) as well as heat treatment which causes the change in the chemical nature of raw material. The SEM showed that the particles were spherical and irregular in shape and found to be agglomerated which corresponds to a larger size in SEM analysis. The agglomeration of particles may be due to the repeated calcination process adopted at the time of preparation of SB.[21]

The mean particle size of gold particles in SB is 2.633 μm. The results of PSA were concomitant with the result of SEM which

showed that the mean particle size was 3-4 µm. Because of its hydrophobic nature, the gold particles tend to aggregate on the dispersion in an aqueous medium.[22] Previously, SB purchased from the Indian Medical Practitioners Co-operative Pharmacy and Stores Limited, Chennai, India characterized for mean particle size was found to be in the range of 0.6 to 0.7 µm.[4] In another similar study, the mean size of gold particles in SB manufactured as per Ayurvedic text Bharat Bhaishajya Ratnakar 5/8357 (BBR) has been reported in a range of 7.55 to 9.97 µm.[2] Similarly, a mean particle size of 4.7 µm was reported in incinerated gold taken from Jaya Indian Medicine, Pharmaceutical Pvt Ltd, Chennai.[17] The lower and uniform particle size of the Baidyanath brand SB is an indicator of its good quality.

The size of the metal particle in *Bhasma* is important as it affects its rate of dissolution and absorption. *In vitro* studies have shown that incinerated gold particles of around 60 nm in size, forming large aggregates of an average diameter of 4.7 µm can enter HFF-1 (human foreskin fibroblast cells) and HeLa (human cells derived from cervical cancer) cells.[17] *Bhasma* absorption and internalization have been studied, where the particle size >1 µm can be taken up by micropinocytosis and ~200 nm by caveolin mediated endocytosis. In order to obtain entry into the systemic circulation, smaller particles at <20 nm can cross the intestinal barriers along the paracellular pathway. They can circulate across the body and accumulate in different organs such as the liver, kidney, spleen depending on whether the NP is functionalized with particular protein receptors (e.g.transferrin, G-protein) to target specific sites.[12] In the present study, the small size of gold particles in SB may confer better cellular uptake and bioavailability.

EDX is a useful technique to estimate the composition of elements in a sample. From the EDX results, it was confirmed that SB contains 97.40% of gold. The other elements present in trace amounts were Na, Fe, Al, K, Ag and Ca. The presence of a very sharp and characteristic peak for gold in XRD has been proven by the EDX results of the high purity of gold in the Baidyanath brand SB in our study. There is no consistency in the gold percentage in SB as per the earlier reports. In a sample of SB purchased from Indian Medical Practitioner Cooperative Pharmacy and Store Ltd. Chennai, 90 % of gold (Au) element has been reported.[4] Dhootapapeshwar, a leading Ayurvedic Drug manufacturing company in India prepared SB as per the procedure of Bharat Bhaishajya Ratnakar 5/8357 and reported 98 % of elemental gold.[2] 56.88 % gold has been reported in an SB sample purchased from Jaya Indian Medicine, Pharmaceutical Pvt Ltd, Maduravoyal, Chennai, Tamil Nadu, India has been reported which was characterized by ICPMS technique.[17] The previous studies have shown gold content in SB ranging from 50 to 98%, along with the various other elements.[12] Variation in the content of gold in the different sources of SB maybe because of the different methods adopted for preparation. The EDAX report showed that the SB characterized presently does not constitute any toxic element and thus safe for human consumption.

The XRD diffraction of SB at 2θ showed characteristic diffraction pattern peaks at 37.17^0, 44.35^0, 64.53^0 and 77.50^0 in our study. The peaks were identical to those of standard gold metal (Au), reported in ICDD reference. A similar kind of diffraction pattern was observed in XRD analysis of Gold nanoparticles[23] and *Swarna Bhasma*.[2]

The level of gold in Baidyanath SB was higher than the earlier reported samples of other brands and unbranded SB. The EDX analysis of SB prepared by Baidyanath showed the presence of Na, Fe, Al, K, Ag and Ca, which suggests that it does not contain any other toxic metals. The various metals detected in trace amounts are required by the body as a source of micronutrients. To explain the mechanism of remedial action of various herbo-metallic formulations like *Bhasma*, it is believed that it is because of plant extract used in the preparation as it acts as both reducing and stabilizing agents. Also, many of the plants that have been used for preparation confer antioxidant and anticancer activity because of various phytoconstituents present that may be absorbed into the surface of *Bhasma* and confers for the various therapeutic effects. But it is still controversial whether the medicinal property of plants is transferred to *Bhasma* or not because high temperature is used for the incineration of SB.[12]

In the present study, we observed that the *Swarna Bhasma* manufactured by Shree Baidyanath Ayurved Bhawan Ltd. Jhansi, India contains 97.40% gold (w/w) along with Na, Fe, Al, K, Ag and Ca in trace amounts. The mean particle size of gold is 2.633 µm with 25% of the polydispersity index. The particle size by SEM is in the range of 3-4 µm. The morphological characteristics of the particles were spherical and mostly irregular in shape and seen to be agglomerated. The SB is prepared through a special process involving incineration that result in the formation of smaller sized particles and thereby being used as nanomedicine. Recently, there has been great interest in studying them using modern characterization techniques like electron microscopy, XRD, as well as various cell & animal models to acquire greater knowledge about their fabrication & therapeutic efficiencies. The heterogeneity in various physical properties like size, shape, composition of SB, prepared by various companies may result in contradictory particle-cell interaction. Since conclusive evidence regarding the SB particle size based uptake by human cells and toxicity are not available, more studies on sub-cellular behavior required to accurately expound their impact at different levels which will confer for its worldwide acceptance & scientific validation.

CONCLUSION

The *Swarna Bhasma* considered as a wonder drug of Ayurveda prepared by various processes, thereby converting raw gold metal to therapeutic form. Physio-chemical profiling, standardization and biological study of Ayurvedic medicine are very important for quality assessment as well as for the maintenance of scientific documentation for validation and its worldwide acceptance. The SB characterized in the present study is safe as it doesn’t constitute any toxic elements. However, a lot of variation has been observed in the various

properties of SB manufactured by different companies. To rule out all the variations during manufacturing practices a certain benchmark to be made for ensuring its therapeutic efficacy and safety. Also despite of the wide variety of medicinal applications of SB, research about its pharmacological action, safety and efficacy is lacking. It will be interesting to correlate the physio-chemical characters of SB with that of the pharmacological activity and safety.

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CONFLICTS OF INTEREST

There are no conflicts of interest.

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Nil

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