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SWARNA BHASMAS DO CONTAIN NANOPARTICLES?

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ABSTRACT

Traditional medicine has maintained its popularity in every region of the developing world. Its practice is increasingly adopted by people worldwide. Indian traditional system of medicine Ayurveda makes use of unique metallic-herbal preparations (called *Bhasmas*) These bhasmas are prepared as per procedures described in ancient textsThese typically involve iterative processes involving calcination of metal in presence of herbal products.The *Swarna bhasma* has been used in treatment of many diseases. In the context of novel properties attributed to nanoparticles, in recent times, the present project aims at chemical characterization of Bhasma preparations. Such characterization would be useful to gain insight into its therapeutic properties. *The Swarna bhasma* samples from three different manufacturers were procured and physiochemical characterization was carried out. Chemically synthesized, commercially available gold nanoparticles were used as control. Particle size and surface area analysis of the samples indicated the presence of micron-sized particles in the range of 3µm-50µm. Size of gold nanoparticles ranged from 88nm-206nm. Gold content in the bhasma samples,ranges from 87% to 98%. The studies indicate that commercially available *Swarna Bhasmas* do not seem to contain nanosized particles.

KEY WORDS: ***Swarna Bhasma*, Gold Nanoparticles, Scanning Electron Microscopy, Atomic absorption spectroscopy**

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INTRODUCTION

In the recent years there is a resurgence of interest in ancient traditional Indian medicine and its therapeutic benefits. For thousands of years formulations of Ayurvedic agents and their therapeutic effectiveness, has stood the test of time.The mechanisms of action of many of these compounds however remain as yet poorly understood. Hence there are serious gaps in understanding of physiological basis of their action.[1] Since ancient times, *Swarna bhasma* , prepared from Gold is used in Ayurvedic treatment of T.B., infertility, asthma, tissue wasting, poisoning etc. [2-4] Last few years have witnessed incredibly rapid development of nanotechnology which seamlessly integrates many disciplines including biotechnology, medicine, chemistry, engineering, materials science and physics. As the size of matter decreases from micrometric to nanomeric dimensions, it exhibits novel physical and chemical properties because of increase in surface to volume ratio. Gold nanoparticles, have found wide ranging applications for diagnosis, targeted drug delivery in nanomedicine because of their chemical stability, surface chemistry and unique optical properties.[5,] [6]Some reports suggest that Bhasmas that are metallo medicines in powder form contain nanoparticles.[7] It is suggested that because of their nanometric dimensions these particles display unusual properties which may provide physiological basis of their action. Possible therapeutic use of nanoparticles has attracted intense attention to their associated toxic effects. There is concern about alleged toxicity associated with matello particles in many traditional drug formulations. Studies indicate that many nanoparticles exhibit size and shape dependent toxicity. [8, 9] Nanoparticles could have many adverse effects at the cellular level. Adverse outcomes could include organelle or DNA damage, oxidative stress, apoptosis (programmed cell death), mutagenesis, and protein up/down regulation. Interestingly, according to some reports Gold nanoparticles have been claimed to be “nontoxic” [10,11]. Therefore, present studies have been carried out to determine content and size distribution of particles of Suvarna *bhasmas* available commercially. Most of these products are claimed to be prepared through traditional procedures. There is no unique procedure to produce these particles. As such it is not clear if various preparations conform to specific size distribution and gold content. Three commercial preparations of *Swarna Bhasma* and one chemically synthesized gold nanoparticle preparation have been analyzed in this study.

MATERIALS AND METHODS

Commercially available formulations of *Swarna bhasma*, prepared and marketed by three reputed Ayurvedic pharmaceutical companies were used (designated as XB1, XB2 and XB3 respectively). In addition, Gold nanoparticles (size >100nm) prepared by chemical synthesis (designated as XB4) was purchased from Sigma Aldrich (USA).

Gold content Analysis

Gold content in these preparations was measured by ICP-AES (Inductively Coupled Plasma-Atomic Emission Spectroscopy (ICP-AES) (Instrument No-iCAP 6200 Duo) with the wavelength range of 175-847nm. -Merck (Germany).

Morphological Analysis; Scanning Electron microscopy

The size distribution and surface topology of particles from each commercial sample of *swarna bhasma* was qualitatively assessed using a cold field emission scanning electron microscope (JSM 6701F, JEOL, Japan). Scanning Electron Microscopy (SEM) was performed in the University Dept. of Chemical Technology, Mumbai.

RESULTS

Content of gold *in bhasma* and Sigma preparations was measured with the help of ICP-AES. Gold content in *Bhasmas* ranged from 87% - 98%. Of these three preparations highest

degree of purity was observed for XB2 (98%).The lowest of degree of purity for XB1 sample (shown in Table-1). Standard gold particles (Sigma)-XB4 displayed 99.9% gold content. The nature of impurities was not assessed.

Table 1
Gold Content of Three Different Ayurvedic Preparations and standard Gold Nanoparticles by ICPAES.

SAMPLE NAME	GOLD CONTENT
XB1	87%
XB2	98%
XB3	97%
XB4	99.9%

SEM images indicated differences in size and pattern of agglomeration of the particles. A difference in patterns of agglomeration of the particles was also observed this could be due to the repeated cycles of calcinations involved in preparation.

***XB1*:** SEM image of XB1 gold preparation in Fig-I show particle sizes range from 1 µm -6.79µm, at different magnifications from 2000X, 5000X and 20000X. Particles were found to be highly heterogeneous in different shapes (elongated, oval and spherical).

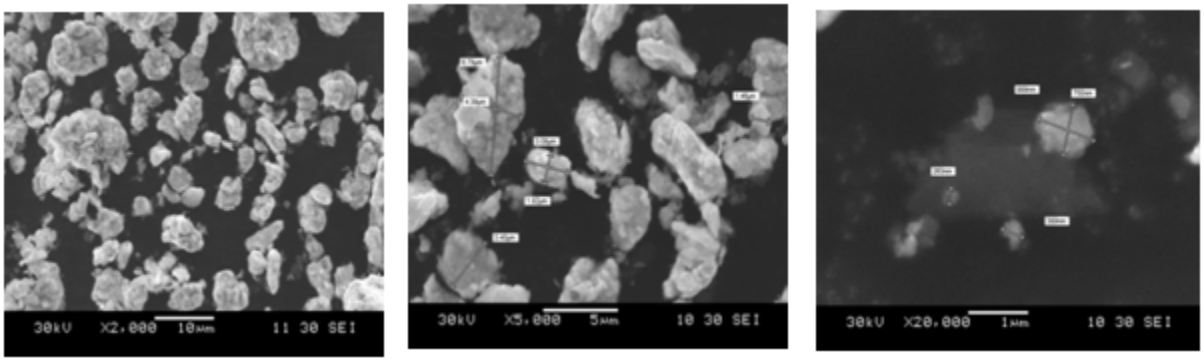

Figure I
Morphology of Swarna bhasma preparations XB1 by scanning electron microscopy

XB2: SEM image of XB2 gold preparation shown as Fig-II shows particle sizes range from 3µm -6µm, at different magnifications from 2000X, 5000X and 30000X. Particles were found to be spherical, with smooth surfaces and were relatively homogenous.

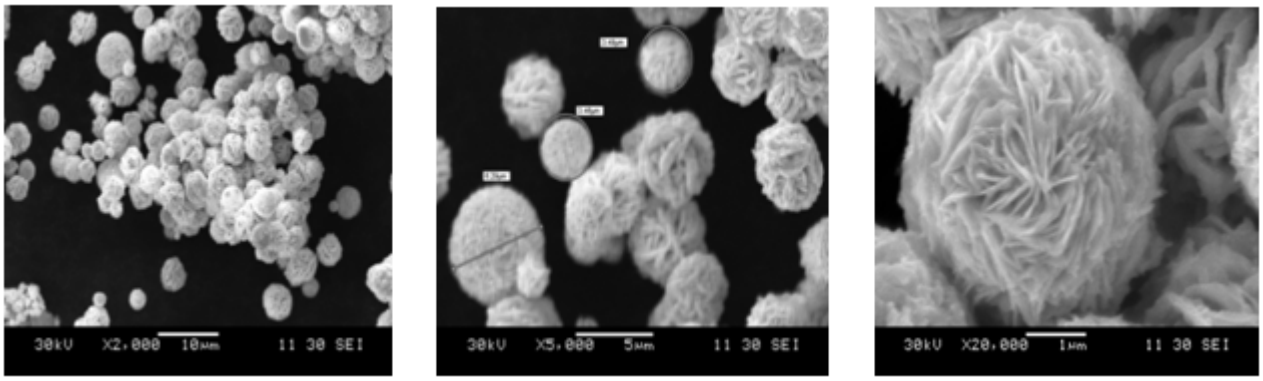

Figure II
Morphology of Swarna bhasma preparations XB2 by scanning electron microscopy

XB3: SEM images of XB3 gold preparation are shown as Fig-III. Particle size ranges from 9μm - 50μm at different magnification from 500X. , 2000X and 5000X and Particles were elliptical and circular in shape and their surface topology was generally smooth.

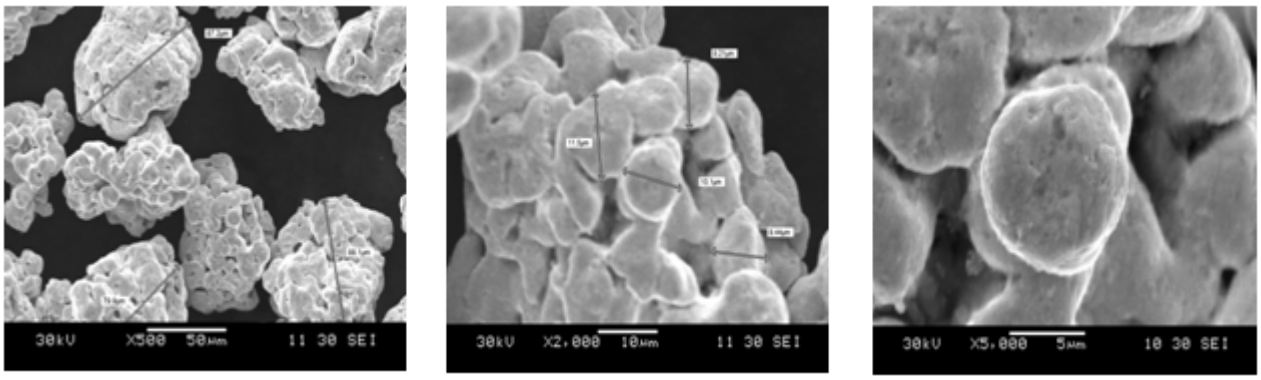

Figure III
Morphology of Swarna bhasma preparations XB3 by scanning electron Microscopy

XB4: Scanning Electron Microscopic images of XB4 gold nanoparticles are shown in [Fig. IV (a)] and [Fig. IV (b)]. In these preparations, particles were found to be homogenous and aggregated, the size as estimated at 50,000X magnification ranged from 88nm to 206nm.

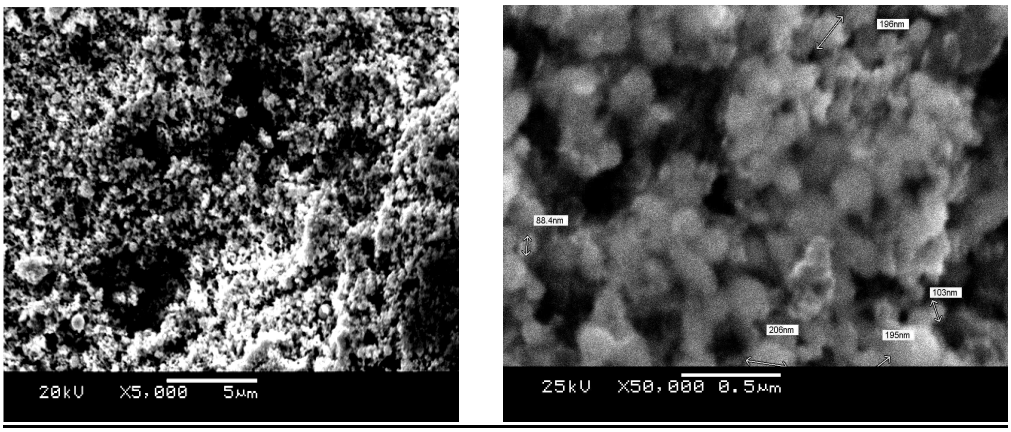

Figure IV
(a) SEM image of XB4 SP showing gold nanoparticles with average size ≈ 157.68 nm and (b) SEM image of XB 4 SP showing standard gold nanoparticles

Table 2
Size distribution of four different gold particle preparations at different magnifications

S.No.	Code	Size at 500X	Size at 2000X	Sizeat5000X	Size at50,000X	Size Range
1	XB1	-	10 µm	5µm	-	3 µm-6 µm
2	XB2	-	10 µm	5µm	-	3 µm-6 µm
3	XB3	50µm(clump)	10 µm	5 µm	-	9 µm- 11µm
4	XB4	-	-	-	88nm-206nm	88nm-206nm

DISCUSSION

In an era of evidence-based medicine, traditional Indian practices, like Ayurveda and Unani medicine, are being compared with allopathic practices. They need to be open to examination and analysis like modern medicine. These faith based systems need to become science based practices. Such analysis can be ultimately helpful to the patients. As per WHO, 80% of the population living in developing countries relies, almost exclusively on traditional medicines (Ayurvedic) for their primary health care needs. In Ayurveda structural and chemical transformation of metal into metal compounds (*bhasma*) which are bioabsorbable is the main objective of *marana*. Ayurvedic practices aim to avoid toxicity and adverse effects of these products. Hence complete transformation of base metal into *bhasma* form is of prime importance. T*Rasashastra* texts have laid down certain *bhasma parikshas* (tests), to determine whether the *bhasma* is properly formed or not. [1] One of the major problems of these ancient practices is that numerous procedures are documented for production of a single medicine. Thus there is no SOP for their production. Characterization of *bhasmas* using scientific techniques is therefore necessary to determine the effect of the processes which employ addition of unusual, exotic materials and to judge its safety and efficacy. These *bhasmaparikshas*are is qualitative in nature and neither offer guideline for characterization nor any insight into its mechanism of action hence for physical characterization, modern analytical parameters likeSEM, and ICPAES etc. are very helpful. preparations used in these studies were procured commercially. These were shown to contain about 87%-98%% gold content by ICP-AES (Inductive Coupled Plasma-Atomic Absorption Spectroscopy) (Table-1). The “impurities”, which were not identified, could be due to ingredients used during “ayurvedic process” of preparations. SEM studies of the

three different ayrurvedic preparations coded XB1, XB2 and XB3 showed varying size distributions of their constituents. Interestingly they did not contain significant proportions of nano particles. Nano sized particles were not found in any of the three. On the other hand, expectedly these were found in standard gold nanoparticles procured from sigma as a control. The range of particle sizes in the bhasmas in all three was found to be 1-50 µm. Thus *Swarna bhasmas* consist of gold particles of any size of over a micron much higher than nanometric dimensions (4100nm). Whether these differences are related to respective method of preparation is not known. Thus, modern techniques can assist in proper characterization of *Ayurvedic* dosage forms and standardization of *Ayurvedic* medicines. These observation underlines need to standardize the procedure of *bhasma* preparation also. Christopher L Brown *et.al* had claimed that their preparations of s*warna bhasma* did contain nanoparticles of average diameter 57 nanometers. These preparations starting from gold film employed elaborate procedures outlined in the ancient texts. It is not clear whether this was an isolated case or the procedure if repeated, always yields nanoparticles of the same size as in case of chemical synthesis[7]. The traditionally prepared *Swarna bhasmas* also seem to contain impurities like Cu, Fe, Si, etc. Further, it is not clear if clinical response to these particles prepared by traditional procedures was studied [12].The studies reveal that the particles of commercial *Swarna bhasma* prepared as per the ayurvedic procedures have micrometric dimensions, with average size of few microns. Paul and PC Sharma also found that the two commercial preparations they examined had an average size of about 800nm and these preparations displayed considerable heterogeneity of size and surface morphology[13]. On the basis of X-ray diffraction studies it was suggested that though the average size of particles in *Bhasmas* was several hundred nanometers they could be aggregates of crystallites which have nanometric (few nms)dimensions .However there is no evidence that nanoparticles disintegrate into constituent crystallites during their interaction with biological system [14]. In the three preparations examined, particles of nanometric range were not observed. Considerable heterogeneity in size distribution, surface topography was also observed. Of the three preparations two displayed considerable heterogeineity in size distributions (XB1 and XB3). It is not clear if this was because of different processes employed to prepare the *Bhasmas* by the respective companies as the companies do not reveal that information. However these characteristics could be significant as Size and morphology play an important role in the therapeutic and pharmaceutical value of *Swarna bhasma.* The development of nanostructure materials has generated many new expectations in science and their clinical applications. The studies thus indicate that commercially available preparations of *swarna bhasma* do not seem to contain nanoparticles of Gold. The clinical response to these bhasmas thus is related to micrometric dimensions and not to novel properties of nanometic dimensions.

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