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CHARACTERIZATION OF *SWARNMAKSHIKA BHASMA* USING STATE OF THE ART TECHNIQUES

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ABSTRACT

Bhasmas are unique therapeutics of *ayurveda*. They are metallo-medicines of nano to submicron particle size prepared through classical process of *shodhana* (purification) & *marana* (calcination). *Swarnmakshika bhasma,* a potent *rasayana* drug is also used in the management of *pandu* (anemia), insomnia, *apasmara* (~convulsion disorders), *kustha* (skin diseases) *mandagni* (*poor digestion*), etc. In the present study *Swarnmakshika bhasma* was prepared through classical method of *shodhana, marana* and the final product i.e. SM *bhasma* was characterized using ancient parameters of *bhasma* examination and modern state of the art techniques such as FTIR, XRD, ICP, PSA, SEM. It was observed that *bhasma* was mixture of organic, metallic and organo-metallic moieties having particle size in microns. The raw SM was chemically chalcopyrite (CuFeS2) whereas different phases identified in the XRD of final product include Fe_2O_3, FeS_2, copper sulphide (CuS) and SiO_2 which indicate changed chemical nature of final product. The particle size of *bhasma* ranged from1.82µm to 88.54 µm with a mean volumetric diameter of 16.58 µm. ICP and FTIR confirmed the presence of inorganic molecules, organic molecules, organic functional groups and various bonds including metal-carbon bonds in the *bhasma* indicating towards its organometallic nature.

KEYWORDS: *Bhasma, Swarnmakshika,* Nanoparticle, ICP, FTIR, XRD, Organometallic.

INTRODUCTION

Bhasma is a powdered metallo-medicine containing nano to submicron size particles and is unique to *ayurveda* system of medicine.[1] *Swarnmakshika* [SM] described as *maharasa*.[2] in *rasa shastra* texts has been widely used for management of *pandu* (anemia), insomnia, *apasmara* (~convulsion disorders), *kustha* (skin diseases) *mandagni* (*poor digestion*), etc., as well as a potent *rasayana* drug.[3] SM *bhasma* is used as a single constituent formulation or in multi-ingredient formulation. For preparation of *bhasma* raw metal/mineral is first purified through s*hodhana* (purification) which is proposed to reduce physical and chemical toxicities of the drug.[4] and then converted into therapeutic dosage form by classical process of *marana* (calcination) which is achieved through repeated lavigation, triturition and incineration.[5] After the process of *marana* the metal/mineral looses all its characteristic metallic properties and gets converted to some other compound. Finished product is then assessed through various physico-chemical parameters like appearance, odour, *varitaratva, rekhapurnatva, niruthatva* etc. to achieve a specific acceptable standard *bhasma*.[6] The process of *bhasmikarana* described in classical texts is very specific in terms of material, media, equipments and procedure. Quality of a drug depends upon its formulation, processing and application, so it is essential to fix some standards to ensure genuinity of drugs. Although general and substance specific guidelines are described in all the processes to maintain the quality of the product right from the selection of the raw drug upto finished product level, but considering the raising concerns regarding safety and efficacy of *ayurvedic* drugs of metal/mineral origin, the quality assurance system needs to be further strengthened. *Ayurveda*, a heritage science is often being criticized by scientific fraternity for its ambiguity and paucity of evidences required to bring strong scientific basis to its belief. Although experience, long term use and textual references form primary evidence base of *ayurveda*, still the primary evidences need to be supported by firm scientific evidences. Modern technological advancements should be used to explore the relevance of these centuries old concepts so that they may be interpreted in the light of contemporary scientific language to offer modern health care. In the present study the *Swarnmakshika* (SM) *bhasma* was prepared as per the classical textual guidelines and was analyzed for quality control parameters as described in *ayurvedic* texts as well as on the basis of modern parameters. The objective of this study was to analyze the physico-chemical changes in the SM after *shodhana* and *marana* processes.

MATERIAL AND METHOD

Material

Raw SM was procured from college's own pharmacy. Other materials used in the processing were procured from the local market.

Method

Pharmaceutical Process

Raw SM was subjected to s*hodhana* (purification) and m*arana* (calcination) processes to convert it into *bhasma* form. For *shodhana*, powdered raw SM was mixed with 1/3rd part by weight of s*aindhava lavana* (rock salt) in an iron pan and was roasted at high temperature with repeated addition of sufficient quantity of lemon juice till the mixture became red hot. Then the pan was covered with lid and continuous high heat was applied for 3 hours. After complete cooling the roasted material was washed 3 times with water to remove the traces of s*aindhava lavana* and was sundried to obtain purified SM.[7]

Marana (calcination) of purified SM was done with *Hingul* (HgS) using lemon juice as *bhavna dravya*. Ten *gajaputta* (conventional heating system) were given to achieve the SM *bhasma* of desired quality.[8]

After completion of the pharmaceutical process the SM *bhasma* was subjected to classical quality control parameters followed by analysis through modern scientific parameters.

Analysis using ancient quality control parameters

The final product i.e. SM *bhasma* was analyzed on the basis of paramaters as described in *ayurveda* texts for *bhasma* testing. Following observations were made.

1. ***Gandha*(Odour)** – The *bhasma* was taken in a petridish and was smelt for any detectable odour. As described in the texts that *bhasma* should have no detectable odour and final product complied with the same.
2. ***Varna*(Colour)**- *Swarnmakshika bhasma* was observed for its colour which was red.
3. ***Rasa*(Taste)**- *Bhasma* was tasted for any specific taste by putting little amount over tongue and was found to be tasteless as described in classical texts.
4. ***Avami*-** Ingestion of *bhasma* should not produce any nausea, vomiting and the final product complied with the same.
5. ***Sparsh*-** *Bhasma* was found to be *slakshan* (fine/soft) on touch.

6. ***Varitaratva***- A small amount of *bhasma* was sprinkled over still water in a beaker. It was observed that the *bhasma* particles floated over the surface of water.
7. ***Rekhapurnatva***- A pinch of *bhasma* was rubbed in between the thumb and index finger, it was observed that the *bhasma* entered into pattern of ridges on the fingertips and was not easily washed out with water as described in texts.
8. ***Nishchandratva***- *Bhasma* should not exhibit its original shining lusture after incineration. *Bhasma* was taken in a petridish and was observed for any lusture in daylight. No lusture was observed in the final product.
9. ***Amla Pariksha***- A little amount of prepared *bhasma* mixed with curd was kept for 12 hours in a petridish and was observed for any colour change. The test was found to be negative as per textual reference.
10. ***Apunarbhava***- The *bhasma* was thoroughly mixed with *mitrapanchak dravyas*, heated at high temperature using *gajputa* and was observed for any change in its physical properties. No change was observed in *bhasma's* physical appearance.
11. ***Nirutha***- When measured quantity of silver metal was heated with *Swarnmakshika bhasma*, no change in the weight of silver was observed.

After the final product complied with all the quality control parameters mentioned in *rasa* texts, it was further subjected to analysis through modern parameters to provide strong scientific evidence.

Analysis using modern parameters

To analyze the present drug on the basis of modern parameters, analysis of drug was carried out at two different centres, one at Drug Testing Laboratory (DTL), Joginder Nagar (Deptt. of Indian system of medicine and Homeopathy, Himachal Pradesh) and at Sophisticated Instrumentation Centre for Applied Research and Testing (Sponsored by Deptt. of Science and technology (SICART), Govt. of India) located at Anand, Gujarat.

Parameters and Methods adopted

The SM *bhasma* (final product) was analyzed using following techniques

- Physico-chemical analysis
- Qualitative/Quantitative tests
- SEM (Scanning Electron Microscope)
- ICP (Inductively Coupled Plasma Emission Spectroscopy)

- XRD (Phase Indentification of Diffractogram using X-ray Diffraction)
- FTIR (Fourier Transform Infrared Spectrometry)
- PSA (Particle size Distribution)

Swarnmakshika bhasma was analyzed for its appearance, colour, odour, taste, pH, total ash value & acid insoluble ash. For revealing the information about external morphology, structure and orientation of materials making up the sample, high resolution images of SM *bhasma* were taken using SEM at different magnifications(200x-5000x). ICP Emission Spectroscopy was carried out for quantitatively analyzing the elements present in raw SM and SM *bhasma* as it is regarded as the most reliable method available for quantitative elemental analysis. It was performed using instrument ICP-OES, Perkin Elmer, model no. 3300RL. *Swarnmakshika bhasma* was further subjected to XRD analysis to identify the compounds and phases present in it. The powder X-ray diffraction of the sample was carried out in the range (2θ):3º to 136º using Xpert MPD Philips diffractometer.

The FTIR spectrum analysis was used to ascertain presence of organic matter in prepared drug and was recorded using FTIR spectrometer Perkin Elmer spectrum by using KBr pellet technique over a range of 400-4000 cm^{-1}. Particle size of SM *bhasma* was determined by particle size analyzer.

RESULTS AND OBSERVATIONS

Organoleptically SM *bhasma* was found to be maroon coloured, tasteless, soft, fine powder with no specific odour or taste. SM *bhasma* also complied with various *bhasma parikshas* described in the classical texts further confirming the quality of the *bhasma*. Physico-chemically SM *bhasma* contains 98.64% total ash out of which 72.47% is acid insoluble. High ash value indicates high inorganic content. [Table 1] Qualitative tests further confirmed the presence of iron and copper in SM *bhasma*.

Table 1: Physico-chemical analysis of SM *bhasma*

S.No.	Tests	SM *Bhasma*
1	Appearance	Fine Powder
2	Colour	Maroon
3	Odour	Odourless
4	Taste	Tasteless
5	pH	6.88
6	Total ash	98.64%
7	Acid insoluble ash	72.47%

SEM

Among advanced analytical techniques SEM provides the crystallographic information. It reveals the information about external morphology, crystalline structure and orientation of material making up the sample. High resolution images of sample at different magnification revealed the particles of size ranging between 1-5µ forming regular and uniform clusters. [Fig. 1]

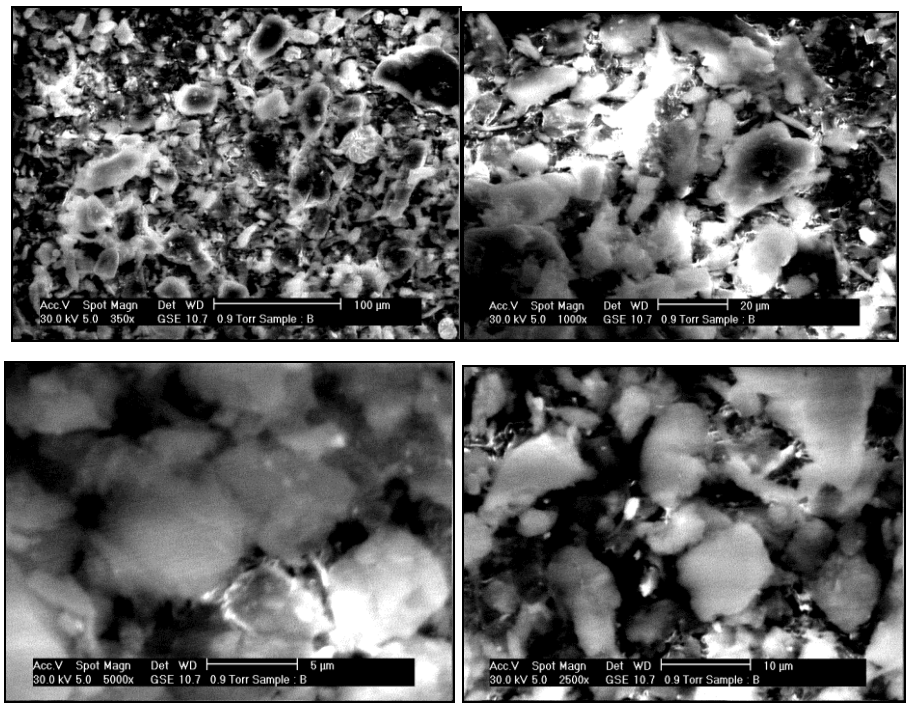

Fig. 1: SEM micrographs of *Swarnmakshika bhasma* at different magnifications.

ICP

It is a type of emission spectroscopy that uses ICP to excite atom/ions which emit electromagnetic radiations at wavelengths characteristic of a particular element. Iron, copper and sulphur were detected in the sample in different proportions expressed in mg/kg. [Table.2]

Table 2: Quantitative status of elements detected by Inductively Coupled Plasma Emission Spectroscopy (ICP).

Sample ID	Element	Wavelength	Instrument Detection Limit (mg/L)	Sample results (mg/kg)
Sample B Swarnmakshika Bhasma	Iron (Fe)	238.204	0.0046	391110
	Copper (Cu)	327.393	0.0097	77.324

(Chalcopyrite)	Sulphur (S)	181.975	-	5490.1
	Mercury (Hg)	253.652	0.0610	Not Detected

XRD

The pxrd pattern of final drug is shown in figure. The strongest peaks identified in the diffraction pattern of final drug suggest the formation of Fe_2O_3 and FeS_2 with low peak intensity. The peaks attributing to CuS & SiO_2 formation are also identified in the diffraction pattern. [Fig. 2]

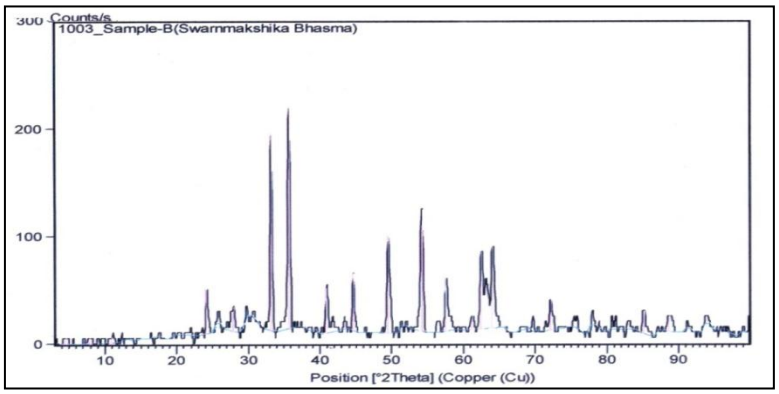

Fig. 2: XRD analysis of *Swarnmakshika bhasma.*

FTIR

FTIR spectrum of SM *bhasma* was analyzed in the region from 4000-400cm^{-1} as shown in figure. In the SM *bhasma* sample, stretching at 3854.50 cm^{-1} 3640.64 cm^{-1}, 3376.73 cm^{-1}, 1633.51 cm^{-1}, 1533.43 cm^{-1}, 1053.22 cm^{-1}, 775.98 cm^{-1}, 728.99 cm^{-1}, 579.72 cm^{-1} & 474.35 cm^{-1} were observed.[Fig. 3]

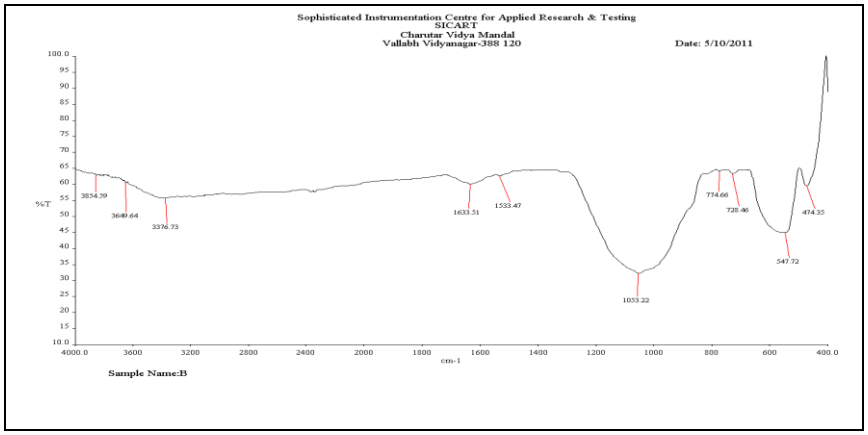

Fig. 3: Fourier transform infrared spectrometry (FTIR) pattern of *Swarnmakshika bhasma.*

PSA

PSA report shows the distribution of the particles around a mean. 10% of SM *bhasma* particles were found to be below 1.82µm, 16% below 2.6µm, 50% below 8.18µm, 84% below 33.77µm, 90% below 38.83µm and 99% particles were below 88.54µm in size. The volumetric mean diameter was 16.58µm as summarized in the table. [Fig. 4] [Table 3] Raw SM was also subjected to PSA test & volumetric mean diameter of raw SM was 258.87µm. [Fig. 5][Table 4]

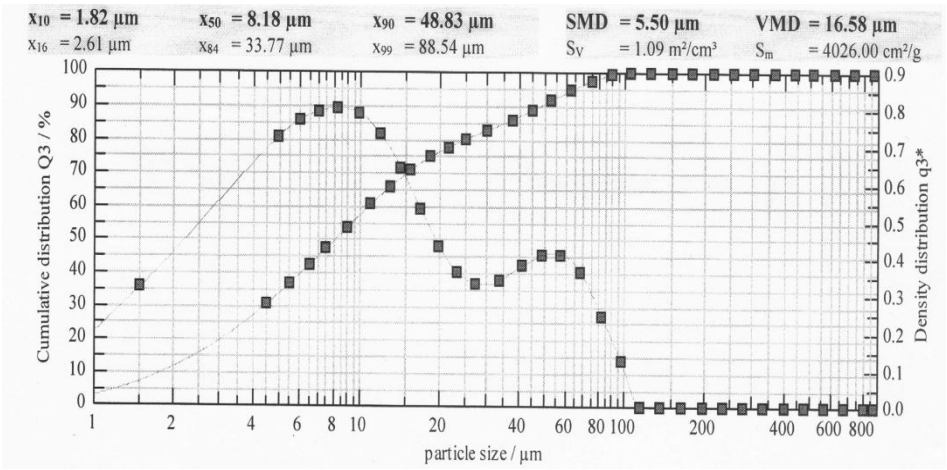

Fig.4: Particle Size distribution of *Swarnmakshika bhasma.*

Table 3: Particle size distribution of *Swarnmakshika bhasma*

Sr. No.	% Below	Size (in µm)	Volumetric mean diameter (in µm)
1.	10	1.82	16.58
2.	16	2.61	
3.	50	8.18	
4.	84	33.77	
5.	90	38.83	
6.	99	88.54	

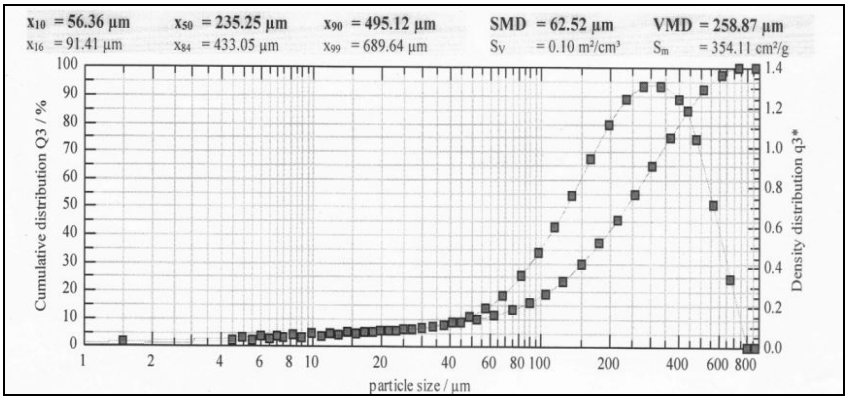

Fig. 5: Particle Size distribution of Raw *Swarnmakshika.*

Table 4: Particle size distribution of Raw *Swarnmakshika*

Sr. No.	% Below	Size (in µm)	Volumetric mean diameter (in µm)
1.	10	56.36	258.87
2.	16	91.41	
3.	50	235.25	
4.	84	433.05	
5.	90	495.12	
6.	99	689.64	

DISCUSSION

The final product was studied with the objective of analyzing physico-chemical changes after various steps of *shodhana* and *marana*. The phenomenon of *rekhapurnatva* and *varitaratva* indicate the smaller particle size of *bhasma*. *Bhasma* was *nishchandra*(without lusture) indicating loss of crystalline nature of the material. *Bhasma* was also found to be *nirutha* and *apunarbhava* which clearly suggest the irreversible changes in the metallic properties of the compound.

The raw SM which was subjected to the processing was chemically chalcopyrite ($CuFeS_2$) whereas different phases identified in the final product including Fe_2O_3, FeS_2, Copper sulphide (CuS) and SiO_2 indicate changed chemical nature of final product. The low peak intensity of the final drug suggests the loss of crystalline nature of the material.[9]

FTIR is an effective analytical tool for profiling and screening of an unknown sample. Sample is eradiated by the infrared (IR) light and the frequencies of absorbance are plotted resulting in IR spectrum. This spectrum is characteristic of the molecules present in the sample. The band observed at 1633.51 cm^{-1} confirms the presence of metal carbonyl complexes and primary and secondary amides in the sample. Although the frequency for free carbonyl complexes lie at 2143 cm^{-1} but with each charge added to metal centre CO stretching frequency decreases. Better the sigma donating capability of other ligands on the metal, lower is the CO stretching frequency. So the stretching frequency to triply bridging (mu-3) carbonyl complexes lie between 1675-1600 cm^{-1} .[10] which is here detected at 1633.51 cm^{-1}. The broad band observed at 775.98 cm^{-1}& 728.99 cm^{-1} is due to out of plane C-H bonding. The peak observed at 3376.73 cm^{-1} indicates the presence of alkenes, aromatic compounds and O-H/N-H bonds.[11] The distinct sharp peak observed at 474.35 cm^{-1}can be assumed to attributing to the presence of Iron sandwiched between cyclodiene complexes.

The SM *bhasma* consist of particles of varying sizes ranging from 1.82µm to 88.54µm with mean diameter of 16.58µm. Size of SM was reduced from 258.87µm to 16.58 µm in the final *bhasma* sample. Change in particle size can be attributed to the *marana* process i.e. levigation, triturition & incineration resulting in mechanical breakdown of the particles as well as change in the chemical nature of the *bhasma*.[12] Lesser particle size results in enhanced bioavailability and efficacy of the drug.

SM is made up of iron, sulphur and copper elements and all of these were also detected in the final compound. Although the *marana* was done with the help of *Hingul*(HgS) but mercury was not detected in the final sample. It can be attributed to the fact that in last two *puttas* the *samputta* was not smeared and sealed with fuller's earth.

CONCLUSION

From the present study it is evident that the raw SM containing iron, copper and sulphur is converted into a mixture of organic, metallic and organo-metallic moieties during the manufacturing process of *bhasma*. The manufacturing process comprising of *shodhana* and *marana* processes also play a specific role in changing the physical as well as chemical nature of *Swarnmakshika*. Although the actual biological action of SM *bhasma* is yet to be experimentally proved but several possibilities can be chalked out. From present study it can be projected that SM *bhasma* as a dosage form might be acting as a carrier for different metallic and organometallic molecules of micron and submicron particle sizes formed during the process of *bhasmikarana*. The smaller particle size of the *bhasma* also facilitates absorption and assimilation of the drug into the biological system.

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