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A critical study to evaluate the importance and prospects of standardization of *bhasmas.*

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ABSTRACT

Ayurveda is the oldest surviving complete medical system in the world. *Rasashastra* and *Bhaishajya kalpana* is one of the branch of *ayurveda* that deals with the pharmaceutical processing, preparations of compound medicines and therapeutic utility of metals and minerals. There is always a necessity to properly define the identity of raw material as well as its consistency with respect to specifications. *Bhasmas* are unique *Ayurvedic* metallic preparations with herbal juices/fruits widely used for treatment of a variety of chronic ailments. Standardization of *bhasma* is utmost necessary to confirm its identity and to determine its quality and purity. It will also make sure the safety, effectiveness and acceptability of the product. But the most important challenges faced by these formulations are the lack of complete standardization by physicochemical, microbiological and analytical evaluation. An attempt has been made to summarize various methods available for standardization of *bhasma*.

Key words: Standardization, *Bhasma*, Physicochemical evaluation

INTRODUCTION

Metals play an important role in human body, the deficiency of which leads to various disorders. In *Ayurveda*, seven metals such as gold, silver, copper iron, tin, lead and zinc are described as Dhatu. These metals are essential elements for the body (Table 1). These metals are present in human body in different concentration and combination at various sites, and help the respective body tissues. to perform their normal activities. And perfect health is attributed to the state of equilibrium of these metals in body tissues. Any imbalance, whether excess or deficiency, disturbs the body metabolism. It has been de-scribed that metal based formulations, called *Bhasma*, are highly effective in prevention and cure of various diseases related to the organ where they are naturally found. *Bhasmas* are unique *Ayurvedic* metallic preparations with

herbal juices/fruits, used in the Indian subcontinent since the seventh century BC and widely recom-mended for treatment of a variety of chronic ailments.

The *Bhasmas* are in fact products of classical alchemy-inorganic compounds of certain metals and gems in a very fine powdered form, mostly oxides, made in elaborate calcinations process known as *bhasmikarana*. It is believed that *bhasmikarana* process converts the metal into its specially desired chemical compound which eliminates the toxicity of the metal and has the necessary medicinal benefits [1, 2]. Various minerals like iron pyrite, copper pyrite and bitumen; salts such as common salt, alkaline salt, black salt and fossil salt; certain compounds like *realgar*, iron sulfate, copper sulfate and antimony sulfide were used in the preparation of *Bhasmas* due to their medicinal value. Some of the commonly used Bhasmas are Kajjali, *Abhrak Bhasma*. *Naag Bhasma*, *Vang Bhasma*, *Jasad Bhasma*, *Tamra Bhasma*, *Mandoor Bhasma*, *Swarnamakshik Bhasma*, *Rasa Sindoor*, *Makardhwaj and Lauha Bhasma*. They will be available as nano-particles and are taken along with milk, butter, honey or ghee; thus making the metals easily *assimilable*, eliminating their harmful effects and enhancing their biocompatibility [3].

Aim:

Evaluation of importance and prospects of bhasma standardization.

Objectives:

- Literary study of Metals (Dhatu)
- Literary study of oxides of metals.
- Literary study of Standardization methods.

Materials and Methods:

Preparation of *Bhasma*:

The process of preparing *bhasmas* includes

***Shodhana*-**

By this process material becomes free from visible and invisible impurities, masses of minerals are converted to fine and brittle.

***Bhavana*:-**

It is a wet trituration process using mortar and pestle. By this materials are mixed uniformly and divided in to fine particles byrubbing and attrition that is the force applied which help to increase the surface area of the material and there by increases the rate of reaction.

***Jarana*:**

Small pellets are made and dried in sunlight. Their melting points are increased due to oxidation process. These pellets are arranged in earthen *sharava* andcovered with another *sharava*. Joint are sealed and dried again.

***Putpak*:**

Puta system of keeping – prepared sharava are heated, enumeration according o the nature of materials, inorganic part of plant material supplies trace elements to the materials. During putpaka material are formed on the surface of the particle.

***Maran*:**

The compound materials are converted to another compound where elements are gets reduced.

Need of standardization of *bhasma*:

Evaluation of a drug means confirmation of its identity and determination of its quality and purity and detection of its nature of adulteration. The analysis carried out on the formulations used for treatment show that raw metals used for their preparation

lose their metallic characteristics and turn into mineral complex.

In order to prove the effect of processing in the elimination of toxicity of metal based formulation various toxicological studies were carried on raw, partially processed & processed copper, mercury and sulfur metals. The parameters included various liver function tests, hematological and *histo-pathological* studies. Based on the results obtained it can be inferred that processing indeed has profound influence in the elimination of toxicity as maximum deviation from normal values of various studies was found in rats treated with raw metal and then followed by partially processed copper.

Methods:

The methods of *bhasma* preparation vary so much for each metal such that bhasma with different colors are produced. The resultants are considered to be same medicinal substances with the ascribed indications even though these may differ in the composition between them and should ideally be addressing different ailments. In short, there is no standard *bhasma* of a metal as such. *Ayurveda* provides a list of tests for the efficacy of the *bhasmikarana* process. The tests are essentially qualitative and ensure that the resulting drug is very fine (small grains), has no metallic shine and does not alloy with silver even at higher temperature to which it was subjected [4-7]. However, these qualitative tests do not provide any quantitative information about the composition and the structure of the final drug. For any drug containing heavy metals (for example lead, mercury), such structural information is an absolute necessity. In view of such ambiguity and the risk due to their inconsiderate use, there is an urgent need to bring about a standardization of the preparation-process and the end product, as also to resolve the prospective indications and strengthen the regime to monitor the manufacturing, and administration of these preparations.

Metals and their presence in human body:

Sr. No.	Metal	Presence in human body
1	**Gold**	Present in trace amount in blood, semen, eyes, heart.
2	**Silver**	Present in bone marrow, upper layer of bones, gall bladder, pancreas, lungs and meninges.
3	**Copper**	Present in mucous of soft tissue, large glands, eye pupil, hair, pleura and pericardium.
4	**Iron**	Present in villi of intestine, eye pupil, hair and all tissues in body.
5	**Tin**	Present in every tissue, more in abdominal muscles, blood and blood vessels.
6	**Zinc**	Present in blood, brain, sensory issue, and flesh.
7	**Lead**	Present in blood and lymphatic tissue.

2. CORRESPONDENCE BETWEEN BHASMA AND OXIDES OF METALS

A correspondence between a few metal *bhasma* and oxides and sulfides of the same metals has been drawn (Table 2). A plurality of *bhasma* of some metals has been noticed. This is not surprising since for a given metal, more than one method is available to make its *bhasma* while treated respectively with different drug materials. The resulting *bhasma* show up with different colours and with different physical and chemical characteristics. This can be attributed to any of the following or some combination among them such as formation of oxides where a metal takes on different valence states; these compounds have different crystal

structure and physical and chemical properties; compounds formed by the metal with the accompaniments, and, drug intermediates; doping of the *bhasma* crystals with impurities (foreign atoms) present even in very minute proportions; in doping foreign metal atoms substitute in the structure of a crystal for atoms of similar size [8].

Correspondence between bhasma and oxides of metals:

Metals	***Bhasma* Colour**	**Corresponding metal compound**	**Compound colour**
Suvarna (Gold)	Red	*Aurous oxide* (Au_2O)$_2$ *Auric oxide* (Au_2O_3)	Reyish-violet Red or brown
Rajat (Silver)	Black	*Argentous oxide* (Ag_2O)$_2$ *Argentic oxide (AgO) Argentic sulfide (AgS)*	Black brown
Tamra (Copper)	Black	*Cupric oxide (CuO) Cupric sulfide (CuS)*	Black
Loha (Iron)	Red dark brown	*Ferric oxide* (Fe_2O_3)	Red
Vang (Tin)	Grayish white	*Stannic oxide* (SnO_2)	White
Yashad (Zinc)	White reddish yellow	*Zinc oxide (ZnO)*	White
Naag (Lead)	Light red	*Plumbous oxide* (PbO)$_2$	Light red

3. RELATION BETWEEN BHASMA AND NANOMEDICINE

Bhasmas are biological *nanocrystals*. In terms of nanotechnology nanocrystalline materials are solids composed of crystallites with size less than 100 nm in at least one dimension. Milling parameters like milling temperature and nature of product influence the attainable grain size. *Ayurvedic* concepts of *mardana* (trituration) and *bhavana* (levigation) are used to reduce particle size. The various methods that are used to detect *nanoparticles* in bhasma are Scanning electron microscopy, Transmission electron microscopy, Fast freeze fracture, Fluorescence microscopy, X-ray photoelectron spectroscopy, Atomic absorption *spectros*-copy, Gel electrophoresis and Enzyme expression.

The process of *nanoparticles* testing in *bhasma* involved 5 steps:

1. To establish presence of *nanoparticle* in test sample.
2. To ascertain whether chemical compound is homogenous.
3. Whether *nanoparticles* are crystalline of amorphous.
4. Nature of defects in the sample.
5. Sample has to be biologically tested to check their bioactivity.

Finally convergence of all these factors in mechanism of action for particular application needs to be tested as well.

Permissible limits of heavy metals:

Heavy metals	**Permissible limit in ppm**
Lead	10.0
Cadmium	0.30
Mercury	1.00
Arsenic	10.0

4. STANDARDIZATION

Standardization is a measurement for ensuring the quality and is used to describe all measures, which are taken during the manufacturing process and quality control leading to a reproducible quality. For herbals formulations, it place major role from birth of a plant to its clinical application. It also means adjusting the herbal drug preparation to a defined content of a constituent or a group of substances with known therapeutic activity respectively by adding *excipients* or by mixing herbal

drugs or herbal drug preparations. Standardization is not an easy task as numerous factors influence the bio efficacy and reproducible therapeutic effect. In order to obtain quality oriented herbal products, care should be taken right from the process of preparation [9, 10].

For standard *bhasma* preparations, there is dearth of scientific analytical studies carried out, and even the existing ones suffer from incomplete analysis. Thus there is an imperative need for a scientific approach, which includes the following steps

a. Physical standardization and elemental analysis of raw material and finished products.
b. [illegible]
c. Pharmacokinetics of the prominent metallic component of *bhasma* using tracer tech-niques or by metal extraction from tissues.
d. Metal accumulation studies in different tissues and organs.
e. Acute and chronic toxicity.
f. Expression of heat shock proteins.
g. Effect of *bhasmas* on normal physiological and antioxidant parameters.
h. Therapeutic response of *bhasmas* on the recommended disease model at cellular and molecular level (based on claims written in *ayurvedic* texts).
i. The role of *bhasmas* as drug carriers, and
j. The role of *bhasmas* in body *immunomodulation* and physiology of gastrointestinal tract (GI) (site of *jataragani*).

These studies will provide evidence for the safety behind the use of bhasmas and also provide knowledge *regarding* their mechanism of action.

4.1. Standardization techniques

The standardization process include following methods:

4.1.1. Preliminary tests according to *ayurveda*

(i) *Varitaratva* (Floating test): If a small quantity of bhasma is sprinkled on water surface it should float on the surface.
(ii) *Rekha purntva* (Fineness test): On rubbing a small quantity of the sample between the fingers it should enter into the lines on the fingers.
(iii) *Nishchandratva* (Loss of metallic luster): When visually examined preferably in presence of sun light no metallic luster should be observed.

(iv) *Nirutthha* (Loss of metallic state): This involves heating of a very thin silver sheet (600 nm thickness) along with a small quantity of bhasma to red hot for about 5 min. After cooling the sheet to room temperature, no traces of this sample should permanently stick to the silver sheet indicating no alloy formation takes place, thus confirming the metal has totally transformed into bhasma, its oxide form [11].

4.1.2. Physicochemical evaluation

The various physicochemical evaluation include colour, odour, pH, taste, fineness, loss on drying at 105^0C, total ash, acid insoluble ash, water soluble ash and particle size mesh test [12-15]. Tests for heavy/toxic metals should be carried out for standard formulation and their permissible limits as per WHO / FDA is given in (Table 3).

4.1.3. Microbiological evaluation

The various microbiological evaluation includes total viable aerobic count, total E*ntero-bacteriaceae* and total fungal count, test for specific pathogen: *E. coli*, *Salmonella spp.*, *S. aureus*, *Pseudomonas aeruginosa* [16]. The permissible limits of microbial load and pathogens according to WHO/FDA are given in (Table 4) [17].

Permissible limits of microbial load and pathogens:

Microbial load	For the contamination in crude plant materials	For plant materials that have been pre treated	For other plant materials for internal use
Total Viable aerobic count	-	$<10^7 cfug^{-1}$	$<10^5 cfug^{-1}$
E Coli	$10^4 g$-1	$10^2 g^{-1}$	$10 g^{-1}$
Total yeast mould count	$10^3 g^{-1}$	$10^4 g^{-1}$	$10^3 g^{-1}$
Total *Entrobacteriaceae*	-	$10^4 g^{-1}$	$10^3 g^{-1}$
Salmonella spp	-	None	None
S. aureus	Absent		
Pseudomonas Aeruginosa	Absent	Absent	Absent
coliforms	Absent	Absent	Absent

4.1.4. Analytical evaluation

The various modern analytical evaluation include **A**tomic Absorption Spectroscopy (AAS), Atomic Force Microscopy (AFM), X-Ray Diffraction (XRD), X-Ray Fluorescence (XRF), X-Ray photo electron microscopy, Scanning Electron Microscopy (SEM), Transmission Electron Microscopy (TEM), Energy Dispersive X-Ray Analysis (EDAX), Infrared *spectroscopy* (IR), Inductively Coupled Plasma-Optical Emission Spectroscopy (ICP-OES), FT-IR and Thermal Gravimetric Analysis (TGA) [18-20]. The various analytical instrument used and their purpose of analysis are given in Table 5.

Analytical instruments and their purpose of analysis

Sr. No.	Instrument	Purpose
1	EDX-SEM	Chemical nature, size and morphology of particles
2	TEM, AFM	Particle size, size distribution
3	EPMA	Distribution of individual elements
4	XRD	Phase analysis
5	XRF, PIXE Analysis	Bulk chemical analysis after making pellets

		Detecting metal as element
6	ESCA	Electronic Nature and Oxidation state of metal
7	Single crystal XRD	To confirm exact molecular structure of crystalline intermediates or products
8	Extraction and Chromatography	To extract out organic matter if any
9	HPLC,NMR, IR, MALDI & ESI-MS	Characterization of organic matter (if >20%wt/w)
10	Wet inorganic analysis,AAS or Iron chromatography	Anion and cation analysis

5. SOME RECENT STUDIES

Gold in traditional *ayurvedic* medicine as *swarna bhasma* has been characterized as globular particles of gold (avg. 56-57 nm). *Swarna bhasma* and gold *nanoparticles* are prepared by transmission electron microscopy analysis. *Nano* sized gold particle have been proven to be effect in ameliorating symptoms of *mycobacterial* collagen. Typical features of *ayurvedic swarna bhasma* have been demonstrated through transmission electron microscopy and atomic force spectroscopy. *Nanoparticles* are responsible for its fast and targeted action. Subsequent action upon DNA/RNA molecule and protein synthesis with in the cell is further hypothesized as possible mechanisms for rapid onset of therapeutic action of *bhasma* preparations. *Ayurvedic* pharmaceutics are receiving new thrust through *bhasma* preparations as novel technological applications [21].

Jasada Bhasma is a unique preparation of zinc and has been used for the treatment of diabetes and age-related eye diseases. Comprehensive physicochemical characterization of *Jasada Bhasma* using modern state-of-the-art techniques such as X-ray photoelectron spectroscopy (XPS), inductively coupled plasma (ICP), elemental analysis with energy dispersive X-ray analysis (EDAX), dynamic light scattering (DLS), and transmission electron microscopy (TEM) were carried out for the first time and a clearly identifiable fraction of particles were found to be in the nanometer size range [22].

Tamra bhasma, the copper based Indian traditional drug, is administered for different ailments of liver and spleen, abdominal pains, colitis, heart problems, *anaemia*, tumors, loss of appetite, dropsy, eye troubles and tuberculosis. Its synthesis involves treating metallic copper with plant juices and then repeated *calcination* in presence of air so that the metallic state is transformed into the corresponding oxide form. In this work, a systematic characterization of this traditional drug using various techniques like X-ray diffraction (XRD), scanning electron microscopy (SEM)–energy dispersive X-ray analysis (EDX), X-ray photoelectron *spectros-copy* (XPS), infrared spectroscopy (IR), *thermogravimetry* (TG) and surface area measurement were carried out and compared the results with those of standard copper oxide. The results were found to match very well with those of a standard copper oxide confirming the composition of the drug sample and some specific findings of the study provide useful hints on its therapeutic properties [23].

6. CONCLUSION

In view of high demand for the use of *bhasma*, the *herbo*-mineral *ayurvedic* formulation, there is an urgent need to bring about standardization of their preparation process and the end product. In this article, an attempt has been made to bring forth the importance of standardization of *bhasma* and the various physicochemical, microbiological and analytical methods available for standardization.

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