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Modern Instrumentation Techniques in the Characterization of *Bhasma* - A Review

Sakhitha K. S.[1]* and K. Shankar Rao[2]

[1,2]Dept.of Rasashastra & Bhaishajya Kalpana, National Institute of Ayurveda, Jaipur, Rajasthan, India

Abstract

Safety and efficacy of *Rasa* medicines has been a topic of discussion since a long time. Issues are also raised regarding the heavy metal contents in *rasa* preparations. No doubt, preparation of *rasa* medicines by stringently adhering to our classical methods has always resulted in quality products. *Bhasma* preparations are one of the unique dosage forms where metals and minerals are converted in to safe and effective remedies through a series of pharmaceutical processing methods envisaged by the ancient *Acharyas.* They had their own parameters which guaranteed quality, safety and efficacy of prepared *Bhasma*. Though these methods are excellent and time tested and the *bhasmas* passing all these tests are indeed of high quality, there is a dearth of knowledge regarding the structural and chemical aspects of various *bhasma* and also the complex reaction taking place between the metallic ions and the phytoconstituents of plants used in the pharmaceutics of *bhasma*. It is here that the importance of adopting modern technology can be looked up on. Modern analytical instrumentation methods play an important role in the characterization of *bhasma.* Utilizing XRD, advanced spectrometric techniques like AAS,ICP-MS etc for analysis of *bhasmas* can put aside some of the allegations faced by the *rasa* medicines and at the same time elevate this ancient science of metallo - therapeutics to the level of global acceptance. Present review highlights various instrumentation techniques that can be incorporated for the characterization of *bhasma* preparations.

Keywords

Bhasma, Characterization, Modern Technology, Instrumentation Techniques

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INTRODUCTION

Ayurveda, the holistic science of medicine, is practiced and utilized by Indians at large since centuries. *Ayurvedic* system of medicine is the only one out of all traditional medicine system of various civilizations where importance of metals for curing ailments was probably first recognized[1]. However their use has been flourished only after the development of *Rasashastra*. Recent health-seeking behavior studies suggests that any societal model of healthcare based on a single system of medicine will become obsolete in the next two decades, unless it broadens out to judiciously combine with complementary systems of medicine. This obsoleteness will occur on account of the insufficiency of a single system to offer on its own, effective treatment for curative and preventive healthcare[2].It is in this juncture that Ayurveda is regaining its lost momentum.

Development of *Rasashastra* which has incorporated metals and minerals into the therapeutics is undeniably a renaissance of *Ayurveda.* Ancient *Acharyas* knew various techniques to convert the metals and minerals in to safe and effective dosage forms. The pharmaceutics of *bhasma* preparation i.e., *bhasmikarana* or*marana* involves the incineration of the metals and minerals kept in closed crucibles *(sharavasamputa)* after levigating them with prescribed liquids like decoctions, juices etc. The technique of *marana* or incineration involves three procedures namely *shodhana* i.e., the purification of the metal which makes it nontoxic and at the same time help in the particle size reduction, *bhavana* i.e., levigation which helps in the reduction of material as well as add to the therapeutic efficacy of drug. The material is then made in to pellet form and is incinerated. The end product called *bhasma* is expected to be a nontoxic material.

Analysis and standardization of*bhasma*

Safety and efficacy of *Rasa* medicines has been a topic of discussion since a long time. Issues are also raised regarding the heavy metal contents in *rasa* preparations. No doubt, preparation of *rasa* medicines by stringently adhering to our classical methods has always resulted in quality products, but lack of standardization has degraded the quality of medicines compromising on its safety and efficacy. At one instance *bhasma* is regarded as the nano medicine[3] .of the ancient times but at the same time questions are raised regarding its safety profile. Thus standardization of *bhasma* has become

imperative to break away from this dilemma. A lot of work is being carried out all over the world regarding the standardization of herbals, but when it comes to herbomineral preparations there is dearth of initiatives undertaken by the scientific community all over.

Speaking of standardization, it is the 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. Classics of *Rasa shastra* have mentioned various methods for analyzing the quality of *bhasma* preparations. Starting from the selection of raw material to the final product ancient texts have described stringent quality control measures. Raw materials of superior quality are identified by the *grahyalakshana* (characteristics of superior quality) mentioned for each drug. Standard Operating Procedures are mentioned for individual *bhasma* preparations regarding the method of *shodhana,* levigation material, quantum of heat required for incineration etc. Regarding the final product various tests are enumerated to make sure that the *bhasma* is safe, effective assimilable and non toxic. Colour of a particular *bhasma* and flame test for specific metals are already mentioned in the classics[4]. A few tests like *varitaratwa* (ability of bhasma to float over water), *rekhapurnatwa* (ability of *bhasma* to get in to the fine ridges of thumb) ensure that the particle size of *bhasma* is in the finest range while *apunarbhavatwa, nirutha* etc ensure the absence of free metal in the *bhasma*[5] . Though these methods are excellent and time tested and the *bhasmas*passing all these tests are indeed of high quality, there is lack of knowledge regarding the structural and chemical aspects of various *bhasma* and also the complex reaction taking place between the metallic ions and the phyto constituents of plants used in the pharmaceutics of *bhasma* preparations. Thus the ancient methods become inadequate for the characterization of *bhasma* for its global acceptability.

Adopting modern techniques

Science and technology is ever expanding. Invention of modern instrumentation techniques has contributed a lot to modern pharmaceutics. Incorporation of these modern techniques in to *Rasashastra* can play a major role in the characterization and standardization *bhasmas*. Many research institutions have taken up various projects to develop standardization parameters of

bhasmas using state of the art instruments and many concepts has been cleared. Still there is a long way to go.

Some of the sophisticated instrumentation techniques that are used in the characterization of *bhasma* are detailed below.

1. **XRD (X RayDiffraction)[6]**

It is an efficient analytical technique used to identify and characterize unknown crystalline materials. Monochromatic X rays are used to determine the inter planar spacing of the unknown materials. Samples are analyzed as powders with grains in random orientations to ensure that crystallographic directions are covered by the beam. This is generally a non destructive method of analysis. Rapid determination of peak position, relative intensities, and calculation of d spacing are some of the features of this technique. In addition, changes in peak position that represent compositional variation can be easily detected. Thus XRD emerge as an important tool in phase analysis of various *bhasmas.*

2. XRF(X Ray Fluoroscence Spectroscopy)[7]

XRFis used to identify and measure the concentration of elements in a sample .It is versatile, accurate, reproducible and fast. X-rays are used to irradiate the specimen and to cause the elements in the specimen to emit their characteristic x rays. A detection system is used to measure the peak of x rays for quantitative and qualitative measurement. XRD and XRF a- highly complementary material analysis methods which, when used together, greatly improve the accuracy of phase identification and quantitative analysis. The combination of both methods provides an increase in the numbers of measured parameters, this in turn provides not only greater accuracy of results, but also increases the range of samples that can be measured.

3. SEM (Scanning Electron Microscope)[8]

It is a type of electron microscopy that produces images of a sample by scanning it with a focused beam of electrons. The electrons interact with atoms in the sample, producing various signals that can be detected and that contain information about the sample's surface topography and composition.

4. FESEM(Field Emission Scanning Electron Microscopy)[9]

It is used for quantitative elemental analysis of the bulk material.Advantages include fast elemental mapping, compositional and other

information, detection of small variations of trace element content and analysis and imaging of samples in their natural, hydrated state.

5. TEM(Transmission Electron Microscope)[10]

It is a microscopy in which a beam of electrons is transmitted through an ultra-thin specimen, interacting with the specimen as it passes through. An image is formed from the interaction of electrons transmitted through the specimen; the image is magnified and focused on to an imaging device, such as a fluorescent screen, on a layer of photographic film, or to be detected by a sensor. TEMs are capable of imaging at a significantly higher resolution than light microscopes.TEM find application in material science nanotechnology etc.

6. **AFM(Atomic Force Microscopy)[11]**

Also known as scanning force microscopy (SFM), AFM is a very high-resolution type of scanning probe microscopy (SPM), with demonstrated resolution on the order of fractions of a nano-meter. Using an AFM, it is possible to measure a roughness of a sample surface at a high resolution, to perform a micro-fabrication of a sample.

7. EPMA(Electron Probe Micro Analyser)[12]

It work similarly to SEM. It is also known as electron micro probe (EMP) / EMPA. The technique is commonly used for analyzing the chemical composition or composition of individual particles, high detection sensitivity for trace elements, high accuracy of quantitative analysis, high accuracy of light elements analysis.

8. ICP-AES(Inductive Coupled Plasma-Atomic Emission Spectroscopy)[13]

ICP-AES analytical technique is used for the detection of trace elements. Also referred as ICP-MS (Inductive Coupled Plasma-Atomic Emission Spectroscopy), it is a type of emission spectroscopy that uses the ICP to produce excited atoms and ions that emit electromagnetic radiation at wavelengths characteristic of a particular element. It is a flame technique with a flame temperature in a range from 6000 to 10000 K. It is also a solution technique & standard silicate dissolution methods are employed. The intensity of this emission is indicative of the concentration of the element within the sample. It detects the various elements up to ppm and ppb level.

9. FTIR(Fourier transform Infrared Spectroscopy)[14]

Infrared (IR) spectroscopy is one of the most common spectroscopic techniques used by organic and inorganic chemists. Simply, it is the absorption measurement of different IR frequencies by a sample positioned in the path of an IR beam. The main goal of IR spectroscopic analysis is to determine the chemical functional group in the sample. Different functional groups absorb characteristic frequencies of IR radiation .IR spectroscopy is an important and popular tool for structural elucidation and compound identification.

10. EDX(Energy Dispersive X ray Analysis)[15]

It is a technique used for identifying the elemental composition or chemical characterization of a sample. It relies on an interaction of some source of x-ray excitation and a sample. Its characterization capability are due to the fundamental principle that each element has a unique atomic structure allowing unique set of peaks on its X-ray emission spectrum. It provide a good estimate of concentration of main elements in the sample in a significantly faster way and provides useful information on the distribution of element forming the sample and their possible chemical form.

11. **AAS(Atomic Absorption Spectroscopy)[16]**

The technique is a spectro analytical procedure for quantitative determination of chemical elements using absorption of optical radiation. It is used for determining the concentration of particular element in a sample. More than 70 different elements can be determined in solutions or in solid samples by AAS.

12. XPS(X ray Photoelectron Spectroscopy)[17]

The technique uses highly focused monochromatic x rays to probe the material of interest. The energy of the photo emitted electrons ejected by the x rays are specific to the chemical state of elements and compounds present ie bound state or multivalent states of individual elements can be differentiated.XPS provide the information regarding elemental identification and quantification, chemical functional group identification and quantification, chemical state imaging, surface sensitivity, layer by layer depth profiling etc. All these are carried out with minimal sample damage.

13. PIXE(Particle Induced X ray Emission)[18]

It is a technique used in the determining of the elemental make-up of a material or sample. Compared to EDX, PIXE offers better peak to noise ratios and much higher trace element sensitivities. Absolute trace sensitivity for a given trace element depend s on factors like matrix composition, detector efficiency etc.

14. NMR(Nuclear Magnetic Resonance)[19]

NMR spectroscopy is an analytical chemistry technique that provides detailed information about the structure, reaction state and chemical environment of molecules. It can quantitatively analyze known compounds. For unknown compounds it can be used to match against the spectral libraries or to infer basic structure directly.

Instrumental analysis of few *bhasma* preparations

1. Swarnamakshikabhasma

An XRD study carried out on the raw material revealed all major peaks of $CuFeS_2$ while for finished products peaks were indicating many compounds viz Cu_2O_3, $FeSO_4$, Fe_2O_3 ,SiS_2 and Cu_2S .TEM study showed the particle size of *bhasma* in the range of 50-200nm[20].

2. Vangabhasma

XRD analysis of the sample after *shodhana* was carried out which revealed peaks corresponding to untransformed tin metal evident by the strongest Sn peaks. In *jaritavanga* sample the peaks were identified as SnO_2, Sn, K_2SnO_2, which showed untransformed stage of tin after *jarana* stage. However the *vangabhasma* showed peaks of SnO_2 only[21].

3. Naga bhasma

Xray diffraction pattern showed high crystalline nature of the drug .Different peaks corresponds to single lead sulphide phase and the crystalline size calculated from the XRD pattern concludes the presence of nanocrystalline structure of the drug.TEM image also supports the nanocrystalline nature of the drug.XPS survey spectrum showed presence of carbon and oxygen peaks on the surface of drug in addition to lead and sulphur peaks. EDAX and AAS showed that the *bhasma* was rich in sodium, potassium, zinc, manganese ,copper and iron while these elements were not present on the surface of bhasma and was not detected by XPS.FTIR showed large number of well defined peaks which concluded that the final product bhasma is associated with macromolecules derived

from the herbal drugs used in their processing[22].

4. Yashadabhasma

Physiochemical characterization of bhasma using XPS, ICP, EDAX, SEM etc reveals the particles are in nano range. Yashada *bhasma* particles were found to be in an oxygen deficient state which may have imparted particular therapeutic property to the drug[23].

5. Lohabhasma

The XRD graphs of *Lauhbhasma* and *Lauhbhasma 100 puti* showed that all the major peaks of the two patterns correspond to Fe2O3 crystal structure. The peak widths for *Lauhbhasma*100 *puti* were sharper than the corresponding peaks of *Lauhbhasma* showing that repeated heating results in grain growth. The sample *Lauhbhasma* has iron in α-Fe2O3. FTIR spectra of these two samples show small intensity bands in the frequency range 400-500 cm^{-1} characteristic of iron in oxide phase. Apart from this, FTIR spectra also exhibit presence of broad bands beyond 1000cm^{-1}. Intensity distribution in these higher wave number bands is quite different for the two samples. It appears that these bands correspond to the base material probably organic fraction (herbs) which is mixed in the drug during heating process. The FTIR results thus show that iron is mainly present in oxide phase in both types of *Lauhbhasma*, consistent with the findings with XRD .It appears that iron attains its final chemical phase, that is Fe2O3, in one cycle of heating only but the matrix in which this oxide is dispersed evolves continuously as more heating cycles are given. The fact that the pattern of absorption of the medicine in the body depends on the number of heating cycles of the *bhasma*, is then related to the role of the base material in response of the body with different kinds of diseases[24]. SEM show particle size of sample reduced prominently to 200-500nm,which has further reduced to 50-100nm in further putas. TEM results confirmed the formation of nanoparticles after 20^{th}puta[25].

6. Tamrabhasma

Systematic characterization of drug using various techniques like XRD, SEM, IR etc were carried out and compared with those of standard copper oxide. Particle size less than 2μm were seen in SEM. High resolution images of the sample clearly showed particle size in nanometers. Analysis by ICP-AES showed 56.24 wt% of copper and 23.06 wt % of sulphur in the sample[26].

7. Swarnabhasma

Nano Particle size of *swarnabhasma*has been confirmed by various instrumental analysis where it is reported that NP size is responsible for its fast and targeted action. The size of gold crystallites in swarnabhasma when calculated from XRD pattern was found to be 28nm.The XRD peaks of swarnabhasma were identical with those reported for standard gold. From the EDAX result it was confirmed that 90% of bhasma contain pure gold[27].

CONCLUSION

The main aim of therapeutics is to serve mankind to make them free from potential illness or prevention of the disease. For the medicine to serve its intended purpose they should be safe effective and non toxic. This review aimed at focusing the role of various analytical instruments in the analysis and characterization of *bhasma.*Though these advance techniques have succeeded in the structural elucidation of *bhasma* a lot more things have to be done. It should be kept in mind that while adopting modern technology principles of ancient science must not be compromised. Currently, spectroscopy processes herbometallics to excite the electrons to a state that they can be measured. This changes the covalent bonding that was painstakingly created via *Rasa shastra* to make the metal safe and therapeutic to the body. Thus the picture obtained is incomplete. Many times the report of instrumentation analysis confirms the presents of elements which were not used during the processing of metals and the experts are unable to explain its source. This shows that mere analysis using the state of art instruments is not enough. A strenuous effort from multidisciplinary experts in various sciences is necessary in bringing the *bhasma* preparations to the level of universal acceptance.

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