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ANALYTICAL STUDY OF TAMRA BHASMA

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ABSTRACT

Background: *Rasa Shastra* is a specialized branch of Ayurveda which mainly deals with the pharmaceutical preparations. *Bhasma* is a special dosage form mentioned in *Rasa Shastra* texts. *Tamra Bhasma* is an incinerated metal obtained after various *samskaras* (processing) like *Shodhana, Bhavana, Marana* and *Amrutikarana* for several times. *Bhasmas* are said to be properly prepared if they pass certain *Bhasma pariksha* enlisted in *Rasa Shastra* texts. But in the present era, only *Bhasma pariksha* are not enough to satisfy the modern scientific world. **Objective:** Hence the present study was carried out to assess *Tamra Bhasma* through various ancient and modern analytical parameters. **Materials and Methods:** *Tamra Bhasma* was prepared as mentioned in the classics and it was tested with both ancient parameters and modern parameters like X-ray diffraction (XRD), Scanning electron microscopy (SEM), Energy dispersive X-ray spectroscopy (EDS), Particle size analysis (PSA), Zeta Potential (ZP), UV-Spectroscopy and Fourier transform Infra-Red spectroscopy (FTIR). **Results:** XRD of *Tamra Bhasma* shows major peaks of HgS, CuS and a minor peak of Cu_4O_3. SEM micrographs showed distribution of particles as clusters of irregular shaped flakes at 3KX and 5KX magnifications; EDS analysis confirmed the significant presence of elements viz. O-21.74%, S-23.67%, Cu-32.93% and Hg-14.28%; Particle size was found to be 2.2 nm with Zeta Potential of -44.2 mV. UV- Spectrum of *Tamra Bhasma* showed maximum absorption at 574 nm and FT-IR analysis showed 27 peaks between the wavelengths 3956.11 – 883.46 cm^{-1}. **Conclusion:** Hence it can be concluded that both the parameters are essential for the qualitative and quantitative analysis of proper formation of the *Bhasma.*

Keywords: *Tamra Bhasma*, Ancient Parameters, Modern analytical Parameters.

INTRODUCTION

Ayurveda is the oldest medical system in the world that uses processed metals / minerals in the form of Bhasma for therapeutic purposes. Most of the preparations of Rasa Shastra are Herbo-mineral-metallic in nature, as they contain minerals and metals as an integral part of their formulations along with specified herbs. Innate qualities of these formulations are quick action, lesser dose, tastelessness and prolonged shelf life. Use of metals in medicine is often associated with toxicity, but Ayurveda made them into biocompatible form by certain detoxification processes like *Shodhana, Marana, Bhavana* etc. which removes the toxic potential from metals and imparts them with therapeutic efficacy of a high grade. The use of Bhasma as a potential drug is facilitated mainly because of two reasons. First, because these materials are being routinely used as effective drugs for centuries and second, these drugs do not show any noticeable side effects in recommended therapeutic doses. World Health Organisation has also accepted that traditional medicines may need less rigorous preclinical toxicological evaluations since their safety of use has been documented historically.

In spite of these facts, Bhasmas of metals are always under debate, not only in sense of its therapeutic excellence but also for unnecessary hue and cry about their toxicity. This is largely because of ignorance about the rationality of the methods of processing adopted in the preparation of these Bhasmas before they are actually used in therapeutics. These preparations pass through a series of laborious procedures of Shodhana, Bhavana, Marana, Amrutikarana etc. which have their impact in making these metals /minerals safe for therapeutics. Hence in the present study, an attempt has been made using various analytical tests like X-Ray Diffraction, Scanning Electron microscopy, Energy Dispersive X-Ray Spectroscopy, Particle size analysis; Zeta Potential, UV-Spectroscopy and Fourier transform Infra-Red spectroscopy to rule out the safety, toxicity and to gain knowledge of identity, form, particle size, surface morphology and structure, contents of Tamra Bhasma.

MATERIALS AND METHODS

Parada, Gandhaka and Tamra were obtained from the local market of Vijayawada. Entire preparation of Tamra Bhasma was carried out in Department of Rasa Shastra and Bhaishajya Kalpana, TTD's S.V.Ayurvedic College, Tirupati. Requirement for XRD: Model- Powder X-ray Diffractometer D8 advance, Manufacturer-Bruker Germany. SEM and EDS: Model- EVO MA 15, Manufacturer- Carl Zeiss – Germany; PSA and ZP: Model- Horiba scientific Partical Size and Zeta Potential Analyzer, Manufacturer - Horiba instruments, Irvine, CA 92618 USA; UV- Spectroscopy: Model- Nano drop 8000 Spectrophotometer, Manufacturer- Thermo Scientific, India.

Pharmaceutical process

The pharmaceutical procedures adopted in this study are Shodhana, Bhavana, Marana and Amrutikarana. Shodhana of Parada was done by mardana with Kshara traya (Sarja Kshara, Yava Kshara, Tankana) for three days[1]. Shodhana of Gandhaka was performed by puta method using cow's milk[2]. Equal quantities of Shodhitha Parada and Gandhaka were taken and made into Kajjali[3]. Tamra Patras were subjected to *Samanya shodhana* by *nirvapa* in *Taila, Takra, Gomutra, Aranala* and *Kulattha Kwatha* for seven times[4]; Visesha shodhana was done by *dola yantra swedana* in *Gomutra* for three hours[5]. Equal quantities of Kajjali and Shodhitha Tamra Patras were triturated in a khalwa yantra using Nimbu Swarasa. Chakrikas of uniform size were prepared and placed in a Sharava and subjected to Sharava samputikarana. This was subjected to Laghu puta and the entire procedure was performed for 18 times[6]. Then, the *Tamra Bhasma* having all the *Bhasma laxanas* have been attained. Then the obtained *Tamra Bhasma* was triturated with *Kumari Swarasa* and subjected to Amrutikarana procedure

by Laghu puta for 7 times[7]. In this way entire preparation of Tamra Bhasma was carried out.

Analysis of *Tamra Bhasma* using ancient parameters (Bhasma Pariksha)

The final Bhasma was analyzed for quality control as described in the ancient texts and the following observations were made:

- Rekhapurnatva[8]: After proper trituration, small amount of bhasma was taken in between thumb and index finger. It filled into the fine lines of fingers. Rekhapurnatwa was obtained after 14th puta.
- Varitaratwa[9]: After proper trituration, small amount of bhasma was sprinkled on the surface of water. Bhasma being light floated on the surface of water. This was obtained after 18th puta.
- Nischandratwa: Small quantity of bhasma was observed under bright sunlight for presence of any free shiny metal particle. There was no shining particle observed in the Bhasma after 3rd puta.
- Niswadu Pareeksha: When a small amount of the bhasma was kept on tongue, there was not any feeling of taste / untoward sensation.
- Dantagre na kach kacha bhavati: When a small amount of the bhasma was placed between the teeth, no sandy feeling was appreciated.
- Anjana sadrishya sukshmatva: The bhasma prepared was fine like collyrium.
- Avami: Ingestion of small amount of the bhasma did not produce any nausea / vomiting.
- Amla Pareeksha: Tamra Bhasma was taken in little quantity and sprinkled over the curd taken in a watch glass and kept undisturbed for 24 hours. No bluish discolouration was seen after 24 hours.
- Nimbu Swarasa Pareeksha: Very little quantity of Tamra Bhasma was added to the fresh Nimbu Swarasa taken in a test tube and kept aside for 24 hours. On the next day there was no colour change in the lemon juice.
- Discolouration was not found in Dadhi Pareeksha and Amla Pareeksha after 16th Puta.

Analysis of Tamra Bhasma using modern parameters

X-Ray Diffraction (XRD)

Tamra Bhasma was subjected to XRD at Department of Physics, Yogi Vemana University, Kadapa, Andhra Pradesh.

Procedure: Sample was powdered in agate mortar to very fine powder and it was mounted in sample tray of machine. X-Ray beam bearing a wavelength of 1.5418740 A° from copper source is passed on the sample. Detector was set to identify diffracted beams between 10 -70 degrees of 2 range. Obtained soft files of XRD consisting values of 2θ and intensity are plotted on a graph (2θ on X-Axis and Intensity of Y-Axis) using "Origin Pro 8.5 SR2" Data Analysis Software. Various compounds consisting similar diffraction pattern were identified by matching their peaks with corresponding JCPDS Crystallographic cards. For even better accuracy and precision, XRD soft files were also analyzed for corresponding phase/entry matching with Crystallographic Open Data base (COD - 20120320) – USA, after plotting values in PANalytical X'pert high score plus software 3.0.0.123, UK.

Scanning Electron Microscopy and Energy dispersive X-Ray spectroscopy

The practical was performed at Department of Physics S.V University, Tirupati.

Procedure of SEM: Specimen of the sample to be analyzed was directly kept on the specimen holder for visualization. As the sample employed has non-conductive nature, the sample surface was coated by carbon using arc melting technique. Then the dried powder was observed under the microscope at 1,000X to 10,000KX and the micrographs were taken with the inbuilt camera.

Procedure of EDS: Electron beam excitation is used in scanning electron microscopes (SEM). A detector is used to convert X-ray energy into voltage signals;

this information is sent to a pulse processor, which measures the signals and passes them on to an analyser for data display and analysis. The detector used in EDS is often the Lithium drifted Silicon detector which is operated at liquid nitrogen temperatures. Sample of Tamra Bhasma was placed on the specimen holder and subjected to Energy-Dispersive X-ray spectroscopy (EDS). When the sample was bombarded by the SEM's electron beam, electrons are ejected from the atoms comprising the sample's surface. The resulting electron vacancies are filled by electrons from a higher state, and an X-ray is emitted to balance the energy difference between the two electron's states. The X-ray spectrum thus acquired gives the information on the elemental composition of the material under examination.

Particle Size Analysis and Zeta Potential

The practical was conducted at Department of science and Technology, PURSE, S.V. University, Tirupati.

Procedure of PSA: The sample was mixed in water and shaked for 10 minutes. Then it was poured into the sample chamber, where it passes through the laser beam as homogeneous stream of particles. The scattering of light occurs over a wide range of angles upon interacting with the particles in the suspension which are moving by Brownian motion. Based on this scattering pattern of sample, particle size distributions are calculated comparing with appropriate optical model.

Procedure of ZP: A 1% concentration of Tamra Bhasma was prepared in distilled water. The particles were well dispersed before analysis and the sample was taken in a 1ml syringe and injected slowly into the capillary cell (cuvette) through the sample port. Care was taken to see that air bubbles are not formed during this process. As the sample comes out from the second port of the capillary cell, the injection process is stopped. This indicates complete filling of the sample into the capillary cell. The sample ports are then covered with lids and the capillary cell was then placed into the sample holder of the zeta sizer instrument for analysis.

UV- Spectroscopy

Practical was performed at Department of science and Technology, PURSE, S.V. University, Tirupati.

Procedure: 5gm of Tamra Bhasma was macerated with 100 ml of solvent in a closed flask for twenty-four hours, shaking frequently during six hours and allowed to stand for eighteen hours. It was filtered, taking for UV spectroscopic study. The Spectra was taken at 200-800 nm from the peak obtained, the λmax value was calculated.

Fourier Transform Infrared Spectroscopy (FT-IR)

This practical was conducted at Padmavathi Mahila University, Tirupati.

Procedure: Sample was placed in the Potassium bromide plate of FTIR spectrometer and the interference pattern was detected by the infrared detector as variations in the infrared energy level, and the obtained spectral information was calculated.

RESULTS

X-Ray Diffraction Studies (XRD):

Table 1: Showing the details of matching peaks of XRD data for Tamra Bhasma:

S.No	Element/Molecule	JCPDS Ref.No	2θ	Intensity	FWHM	h	K	L
1.	HgS (Cinnabar)	00-042-1408	26.49	100	0.336	1	0	1
			31.19	93	0.192	1	0	2
			43.59	21	0.432	1	1	0
			45.75	19	0.12	1	0	4
			51.74	13	0.192	2	0	1
2.	CuS (Copper Sulphide)	00-006-0464	29.27	65	0.264	1	0	2

3.	**CuS (Copper Sulphide)**	01-074-1234	31.80	100	0.336	1	0	3
4.	**CuS (Copper Sulphide)**	00-024-0060	47.88 32.81 52.67 59.27	62 44 23 34	0.288 0.192 0.24 0.24	- - - -	- - - -	- - - -
5.	**Cu_4O_3 (Copper Oxide)**	00-033-0480	28.09	100	0.12	1	1	2

Table 2: Showing Crystal details of JCPDS entries:

Name	HgS
Space group	P3221
Crystal System	Hexagonal
Cell Parameters	a = 4.1495 A˚ b= 4.1495 A˚ c = 9.4970 A˚
Z	3.0

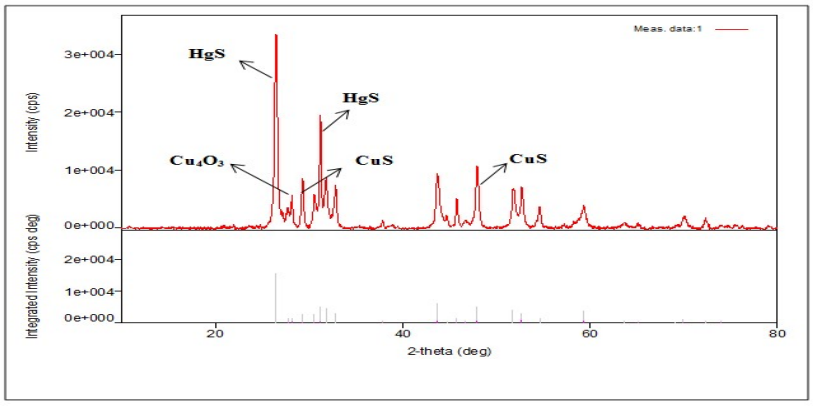
Name	CuS (00-006-0464)
Space group	P63/mmc
Crystal System	Hexagonal
Cell Parameters	a = 3.7920 A˚ b= 3.7920 A˚ c = 16.3440 A˚
Z	6.00

Name	CuS (01-074-1234)
Space group	P63/mmc
Crystal System	Hexagonal
Cell Parameters	a = 3.7900 A˚ b= 3.7900 A˚ c = 16.3400 A˚
Z	1.00

Name	CuS (00-024-0060)
Space group	P63/mmc
Crystal System	Hexagonal
Cell Parameters	a = 3.7960 A˚ b= 3.7960 A˚ c = 16.3600 A˚
Z	6.00

Name	Cu_4O_3 (00-033-0480)
Space group	I41/amd
Crystal System	Tetragonal
Cell Parameters	a = 5.8370 A˚ b= 5.8370 A˚ c = 9.9320 A˚
Z	4.00

Figure 1: Showing XRD graph of Tamra Bhasma



XRD of Tamra Bhasma shows major peaks of Hgs (Cinnabar) and CuS (Copper Sulphide) compounds with hexagonal structure. A minor peak showed the existence of Cu_4O_3 (Copper Oxide) compound with tetragonal structure. The presence of sharp peaks indicates the highly crystalline nature of Bhasma. The HgS peaks were detected at a diffraction angle of 26.49, 31.19, 43.59, 45.75, 51.74, CuS peaks were detected at a diffraction angle 29.27, 31.80, 47.88, 32.81, 52.67, 59.27 and Cu_4O_3 was detected at a diffraction angle of 28.09.

Scanning Electron Microscopy

Figure 2: Showing SEM result of Tamra Bhasma (Mag. 3KX)

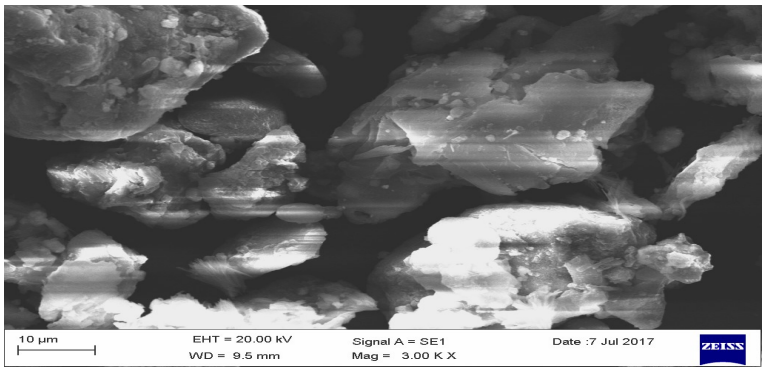
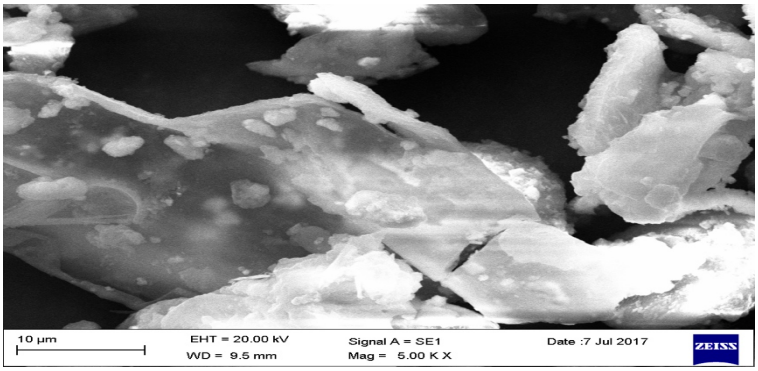


Figure 3: Showing SEM result of Tamra Bhasma (Mag. 5KX)



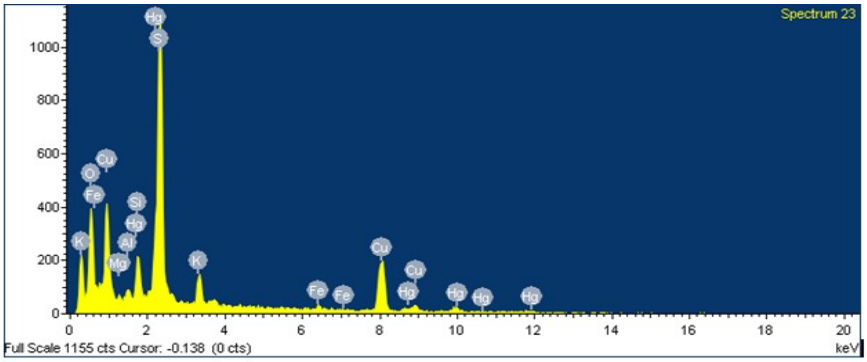
SEM micrograph of Tamra Bhasma showed distribution of particles as clusters of irregular shaped flakes at 3KX and 5KX magnifications.

Energy Dispersive X-Ray Spectroscopy

Table 3: Showing the quantity of all the elements in Tamra Bhasma

Element	Weight%
O K	21.74
Al K	1.11
Si K	4.21
S K	23.67
K K	0.88
Ca K	1.19
Cu K	32.93
Hg M	14.28
Total	100.00

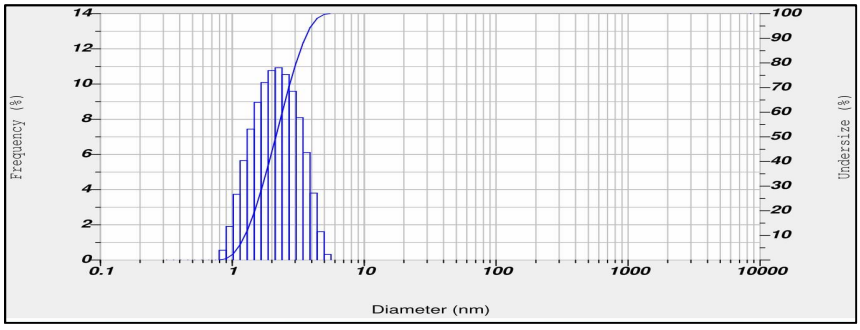
Figure 4: Showing EDS graph of Tamra Bhasma



EDS analysis of Tamra Bhasma confirmed the presence of elements viz. O- 21.74%, Al- 1.11%, Si- 4.21%, S-23.67%, K-0.88%, Cu-32.93%, Hg-14.28%.

Particle Size Analysis

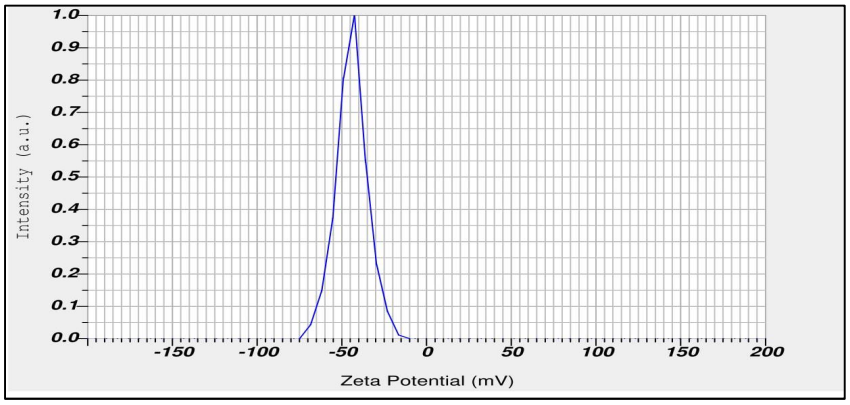
Figure 5: Showing the result of Particle size analysis of Tamra Bhasma



The mean particle size of Tamra Bhasma is 2.2 nm.

Zeta Potential

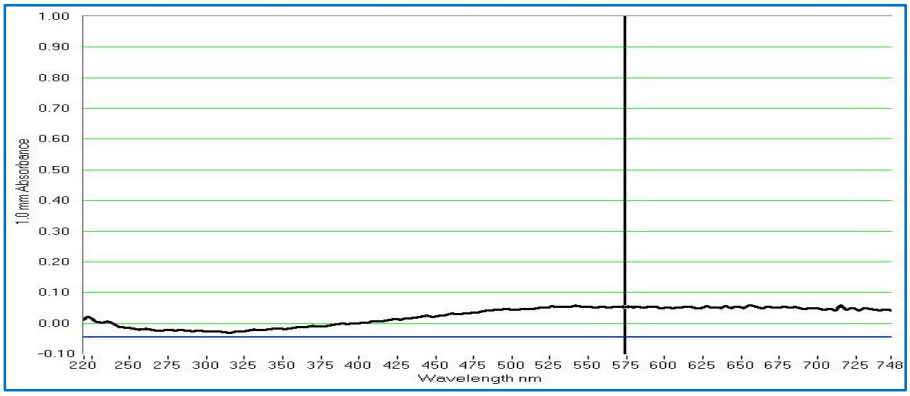
Figure 6: Showing Zeta potential distribution of Tamra Bhasma



The Zeta Potential (mean) value of Tamra Bhasma found to be **-44.2 mV** which indicates its high colloidal stability.

UV- Spectroscopy

Figure 7: Showing UV-Spectrum of Tamra Bhasma



UV- Spectrum of Tamra Bhasma showed maximum absorption at 574 nm.

Fourier Transform Infrared Spectroscopy (FT-IR)

Figure 8: Showing various peaks obtained in FTIR analysis of Tamra Bhasma

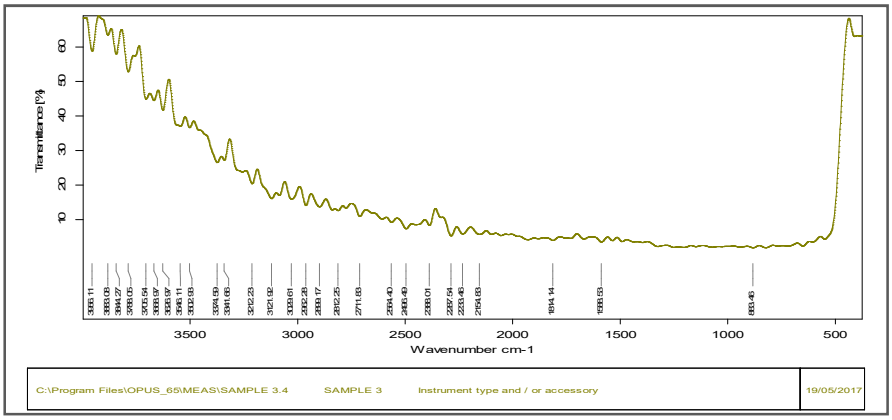


Table 4: Showing details of Peaks obtained in FTIR analysis of Tamra Bhasma.

Sample Name	No. of Peaks	Wavelength
Tamra Bhasma	27	3956.11, 3883.08, 3844.27, 3788.05, 3706.54, 3608.97, 3626.97, 3546.11, 3502.93, 3374.59, 3341.66, 3212.23, 3121.92, 3029.61, 2962.28, 2890.17, 2812.25, 2722.83, 2564.40, 2496.49, 2388.01, 2287.54, 2233.46, 2154.83, 1814.14, 1588.53, 883.46.

Table 5: Various peaks obtained in FTIR analysis of Tamra Bhasma and their correlation with compounds

Si. No.	Peak	Actual peak	Bond	Type of bond	Appearance
1.	3610-3670 cm^{-1}	3608.97 3626.97	O – H	Alcohols, phenols	Broad
2.	3500-3560 cm^{-1}	3546.11 3502.93	O – H	Alcohols, phenols	Strong, Broad
3.	3200-3400 cm^{-1}	3374.59 3341.66 3212.23	O – H	Alcohols, phenols	Strong, Broad
4.	2400-3200 cm^{-1}	3121.92 2962.28 2812.25 2890.17 3029.61 2722.83 2564.40 2496.49 2388.01	N – H	Ammonium ions	Multiple broad peaks
5.	2100-2260 cm^{-1}	2233.46 2287.54 2154.83	C=C	Alkynes	Medium
6.	1670-1820 cm^{-1}	1814.14	C = O	Carboxylic acids / derivatives	Strong
7.	1550-1640 cm^{-1}	1588.53	N – H	Amide	Weak to strong
8.	860-900 cm^{-1}	883.46	C – H	Aromatic	Strong

Tamra Bhasma showed 27 peaks between the wavelengths 3956.11-883.46 cm^{-1}. Absorption peaks of Alcohols, Phenols, Ammonium ions, Alkynes, Carboxylic acids and Amides were observed.

DISCUSSION

Analytical study plays an important role in the standardization of the drugs. Ayurveda, the ancient system of medicine is gaining recognition throughout the world and many herbal, metal and mineral drugs are now clinically tested and accepted. However, one

of the impediments in the acceptance of the ancient systems of medical preparation is the lack of standard quality control profiles. The quality of the drugs, that is, the profile of the constituents in the final product has implication in efficacy and safety.

X-ray diffraction has been in use in two main areas, for the finger print characterization of crystalline materials and the determination of their structure. Each crystalline solid has its unique characteristic X-ray powder pattern, which may be used as a "fingerprint" for its identification. Once the material has been identified, X-ray crystallography may be used to determine its structure, i.e. how the atoms pack together in the crystalline state and what the interatomic distance and angle etc. X-ray diffraction is one of the most important characterization tools used in solid state chemistry and material science. Size and the shape of the unit cell for any compound can be detected most easily using the diffraction of X-rays. Major peaks of HgS and CuS, a minor peak of Cu_4O_3 were seen in the XRD of Tamra Bhasma. The presence of sharp peaks indicates the highly crystalline nature of Bhasma. The presence of minor peak as Copper Oxide may be due to repeated incinerations performed during the preparation of Tamra Bhasma. HgS occurs in two forms cinnabar and metacinnabar. Formation of cinnabar requires a temperature of more than 270°C, while metacinnabar forms at temperature ranging from 20°C - 90°C. Hence, we can justify the formation of Cinnabar from the heat produced due to laghu puta (514^0C). The shape of crystals was found to be hexagonal. Higher temperatures (>165°C) are required like that of in Bhasma making, to enhance reaction and yield pure CuS in the solid-state reaction. This indicates that Copper and sulphur might have reacted at higher temperatures in the absence of oxygen resulting in the formation of Copper Sulphide (CuS).

Scanning electron microscopy (SEM) is an analytical technique to know the surface morphology of the drug. It uses electron beam rather than light to form a figure. It is capable of producing high resolution figures of a sample surface, which means that closely spaced features can be examined at a high magnification. Due to the manner in which the figure is created, SEM figures have a characteristic three dimensional appearance and are useful for determining the surface structure of the sample i.e. topography. It can magnify objects to extreme levels where even structure of nano particles could be clearly visible. Distribution of particles as clusters and irregular sharped flakes were seen in the micrographs of Tamra Bhasma. This may be due to hexagonal and tetragonal shaped structures of compounds found in the XRD. The surface area of Tamra Bhasma was observed to be smooth, may due to involvement of procedures like Shodhana, Bhavana and Marana in the preparation of Tamra Bhasma.

Energy-Dispersive X-ray spectroscopy (EDX) is an analytical technique used for elemental analysis or chemical characterization of a sample. It relies on the investigation of an interaction of some source of X-ray excitation and a sample. EDS of Tamra Bhasma revealed the significant presence of elements like Oxygen, Sulphur, Copper and Mercury. The presence of other elements like Aluminium, Silica, Potassium and Calcium may be due to addition of herbal ingredients during the preparation of Tamra Bhasma.

The size of the particles in the drug plays major role in its therapeutic action and efficacy. Particle size and surface area of solid drug are inversely related to each other. The mean particle size of Tamra Bhasma is 2.2 nm. This shows Shodhana, Bhavana and Marana procedures employed in the preparation of Tamra Bhasma have reduced the particle size. The nano size of drug is indicative of its quick absorption and faster dispersion into body resulting into better therapeutic efficacy. Zeta potential is a measure of the magnitude of the electrostatic or charge repulsion or attraction between particles, and is one of the fundamental parameters known to affect stability. The Zeta Potential (mean) value of Tamra Bhasma found to be -44.2 mV which indicates its high colloidal stability. High zeta potential indicates easy dispersion, whereas less zeta potential indicates strong ag-

gregation of particles in suspension. High colloidal stability of Tamra Bhasma indicates its easy dispersion in the body fluids by reaching the target site quicker resulting in high therapeutic potential.

UV-Spectroscopy refers to absorption spectroscopy or reflectance spectroscopy in the ultraviolet-visible spectral region. Different molecules absorb radiation of different wavelengths. An absorption spectrum will show a number of absorption bands corresponding to structural groups with the molecule. Electromagnetic spectrum of U.V region is from 190 to 400 nm whereas for visible region it is 400-800 nm. UV-Spectrum of Tamra Bhasma showed maximum absorption at 574 nm which shows its absorbency in visible region.

FTIR was performed to detect the presence of functional groups or organic legends in Tamra Bhasma. Infrared spectroscopy deals with the infrared region of the electromagnetic spectrum that is light with a longer wavelength and lower frequency than visible light. When infrared light or radiation hits a molecule, the bonds in the molecule absorb the energy of the infrared and respond by vibrating. Tamra Bhasma showed 27 peaks between the wavelengths 3956.11- 883.46 cm^{-1}. Absorption peaks of Alcohols and phenols, (O – H) stretching bonds were observed in between 3610-3670 cm^{-1} , 3500-3600 cm^{-1} , 3200-3400 cm^{-1}. Two peaks of C = O stretching vibrations indicate Carboxylic acids, were observed between 1670-1820 cm^{-1}, multiple peaks were obtained due to N-H stretching vibrations between 2400-3200 cm^{-1}. C=C stretching vibrations at 2100-2260 indicates presence of alkynes. A peak at 883.46 cm^{-1} represents C – H stretching vibrations having aromatic structure. N-H stretching vibrations containing amides was observed at a peak of wavelength 1588.53 cm^{-1}. This indicates that there are no complex structures in Tamra Bhasma.

CONCLUSION

From the present study, it can be confirmed that Tamra Bhasma is a nano sized particle with high dispersion rate to the target site proving its high therapeutic efficacy. The presence of major peaks of HgS, CuS and a minor peak of Cu_4O_3 shows the complete transformation of elemental Tamra into bioavailable compound form (Bhasma). Free metal were not found in the Tamra Bhasma, representing the absence of toxicity and adverse effects. All these modern analytical tests help in bringing the hidden facts said by our Acharyas to the contemporary scientific world evidencing its safety.

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