OPEN ACCESS
Scholars International Journal of Traditional and Complementary Medicine
Abbreviated Key Title: Sch Int J Tradit Complement Med
ISSN 2616-8634 (Print) |ISSN 2617-3891 (Online)
Scholars Middle East Publishers, Dubai, United Arab Emirates
Journal homepage: https://saudijournals.com

Review Article

Modern Sophisticated Instrumental Techniques Used in the Characterization of *Bhasma*

Punam Kumari[1*], Dr. Yadevendra Yadav[2], Dr. Khem Chand Sharma[3]

[1]MD Scholar, P.G Department of Rasa Shastra & Bhaishajya Kalpana, Uttarakhand Ayurved University, Rishikul Campus Haridwar, India
[2]Assistant Professor, P.G Department of Rasa Shastra & Bhaishajya Kalpana, Uttarakhand Ayurved University, Rishikul Campus Haridwar, India
[3]Professor and H.O.D., P.G Department of Rasa Shastra & Bhaishajya Kalpana, Uttarakhand Ayurved University, Rishikul Campus Haridwar, India

DOI: 10.36348/sijtcm.2022.v05i06.003 | **Received:** 12.06.2022 | **Accepted:** 18.07.2022 | **Published:** 21.07.2022

***Corresponding author:** Punam Kumari
MD Scholar, P.G Department of Rasa Shastra & Bhaishajya Kalpana, Uttarakhand Ayurved University, Rishikul Campus Haridwar, India

Abstract

A nano-metallic drug called *Bhasma* is created from metal and minerals. It has long been regarded in Ayurveda as a trustworthy and quick-acting dose type. The following procedures—*Shodhana*, *Bhavana*, *Mardana*, and *Marana*—are used to prepare *Bhasma*. These processes assist in breaking down particles into their smallest components, get rid of undesirable traits, and give the substance new properties that make them more palatable to the body's tissues and organs. In the classic, various *Bhasma Parikshas*, such as *Varitaratwa*, *Unnama*, *Rekha purnatwa*, *Nirutha*, and *Apunarbhava*, are mentioned. However, these tests are insufficient to determine the safety and efficacy of *Bhasma.* For the examination of *Bhasma*, various contemporary factors are used to ensure its acceptance on a global scale like physical evaluation, chemical analysis, and instrumental analysis. *Bhasma* efficiency is reliant on its homogenous chemical composition, nanocrystalline structure, and biological activity. These highly sophisticated analytical tools, including XRD, SEM, TEM, NTA, ICP-MS, XRF, AAS, and FTIR, are required for the detection of crystal size, particle size, shape, surface area, dispersion state, and morphological features, in addition to chemical and physical properties like reactivity, optical behaviour, magnetism, zeta potential, and others. To understand the toxicity, pharmacokinetics, and pharmacodynamics of *Bhasma*, all of these methods are employed. As a result, the current paper's main focus is on characterizing *Bhasma* using modern sophisticated instrumental techniques.

Keywords: *Bhasma*, Nanocrystal, Nanomedicine, *Marana*, Crystal Size, Toxicity.

INTRODUCTION

The idea of employing nano metallic preparations has been widely accepted since the Charaka Samhita. For a metallic *Lauhadi Rasayana* preparation, iron is heated to a red-hot temperature and then quickly quenched in a liquid medium, resulting in the formation of a fine iron powder known as *Ayaskriti* [1]. In order to separate them from environmental metals and make them appropriate forms for oral treatment, metals and minerals are treated to form *Bhasma*. *Bhasma* is frequently used in combinations with other herbal or animal-based items. These herbo-metallic/herbo-mineral remedies have a long history of use in India and other countries treating a variety of ailments. Herbo-metallic interactions are thought to help medications reach their target more effectively, promote therapeutic effects, and reduce toxicity [2]. Due to the fact that these traditional remedies are based on plants or other "natural" substances and have been used for thousands of years in many cultural contexts, they are frequently seen as "safe." But toxicity frequently coexists with any medicine's positive effects. The usage of drugs that contain minerals or metals can have risks [3].

Recent years have seen an increase in cases of heavy metal toxicity linked to improper use of these medications, necessitating more pharmacovigilance.

Their toxicity and efficacy must be balanced. It is imperative to standardize *Bhasma* in order to establish its identity as well as its quality, purity, safety, effectiveness, and acceptability of the product. The advanced technologies are now utilized to characterize herbo-metallic preparations as Quality Assurance/Quality Control. These techniques reveal the secrets of these traditional treatments.

There are so many modern instrumental techniques but among them, XRD, XRF, SEM, TEM, AAS, FTIR, and ICP-MS are used widely. It is very important to select the proper instrument to carry out a fruitful analysis. A wrong selection at this point will lead to a meaningless analysis.

MATERIAL AND METHODS

Online search engines Google Scholar, Pubmed, DHARA, and AYUSH research portal were screened for the available references. Terms *Bhasma*, Nanocrystals, *Marana*, Crystal Size, Toxicity, XRD, XRF, SEM, TEM, FTIR, NTA, ICP-MS, AAS, and Nanomedicine were searched.

X-RAY POWDER DIFFRACTION (XRD)

X-ray powder diffraction (XRD) is a rapid non-destructive analytical technique primarily used for phase identification of a crystalline material and can provide information on unit cell dimensions. The analyzed material is finely ground, homogenized, and the average bulk composition is determined.

Max von Laue, in 1912, discovered that crystalline substances act as three-dimensional diffraction gratings for X-ray wavelengths similar to the spacing of planes in a crystal lattice. X-ray diffraction is now a common technique for the study of crystal structures and atomic spacing.

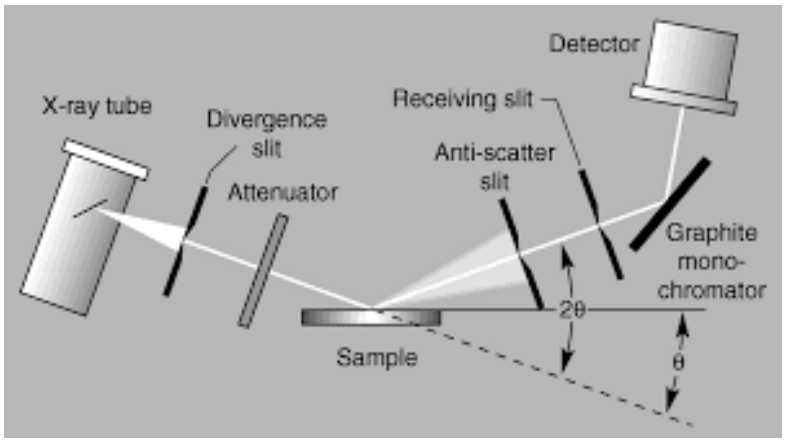

Fig 1: XRD Instrumentation

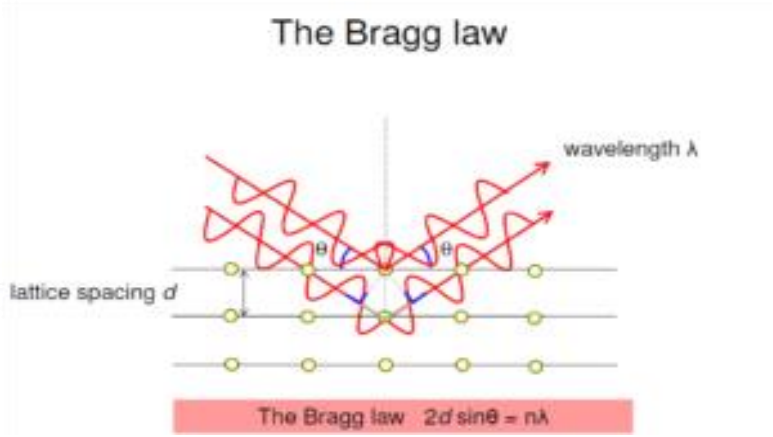

Fig 2: Bragg Law

PRINCIPLE

XRD works by irradiating a material with incident X-rays and then measuring the intensities and scattering angles of the X-rays that contain information about the atomic arrangement in the crystal. These X-rays are generated by a cathode ray tube, filtered to produce monochromatic radiation, collimated to concentrate, and directed toward the sample. The interaction of the incident rays with the sample produces constructive interference (and a diffracted ray) when conditions satisfy Bragg's Law ($n\lambda=2d \sin \theta$). These diffracted X-rays are then detected, processed, and counted. Conversion of the diffraction peaks to d-spacings allows identification of the mineral because

each mineral has a set of unique d-spacings. Typically, this is achieved by comparison of d-spacings with standard reference patterns.

Previous Research Study

Singh R. K. *et al*., (2018) carried out the crystallographic phase analysis of prepared *Abhrakh bhasma*. Several diffraction peaks were observed and the maximum intensity peak in the XRD pattern of *Abhrakh bhasma* is indexed to $Mg_2O_4Si_1$. Hence, it is assumed that the *Abhrakh bhasma* is mostly a byproduct of magnesium. The XRD analysis reveals that the *Abhrakh bhasma* contains various other elements also. The average crystalline size ~24±4 nm. Thus, the XRD study concludes the *Abharakh bhasma* is in nanocrystalline form [4].

Gupta R. K, *et al*., 2015, XRD study revealed that the strongest peaks identified in the raw material after comparing with JCPDS data was $CuFeS_2$. After *Shodhana*, the three highest peaks were identified as FeS_2, Fe_2O_3, and $FeSO_4$. Other peaks identified in *Shodhita Swarna Makshika* were Cu_2S and CuO. Strongest peaks identified in sample 1, sample 2, sample 3 of *Swarna Makshika Bhasma* is Fe_3O_4. In sample 4 of *Swarna Makshika Bhasma*, after *Kupipaka* the peaks of $CuFeS_2$ again reappear and in the same sample, after *Putapaka* strong peaks of Fe_2O_3, Cu_2O, and $FeSO_4$ were identified [5].

Kale B, *et al*., 2017, compared synthesized *Vanga Bhasma* with commercial sample using XRD. XRD shows that the peaks match the diffraction pattern of the tetragonal Tin Oxide (JCPDS 29-1484, 46-1088). The position of the main peak in final products Sn_4, Sn_5 and commercial sample Sn_3 is $2\theta = 26.67$, which is absent in Sn_1 and Sn_2. Other major peaks are observed at 2θ= 22.56, 34.200, 44.98, 52.04, 65.02, 51.88. This indicates that, the major planes 110, 101, 200, 211, 220, 002, 310, 112, 301 of SnO_2. XRD shows that *Vanga Bhasma* is crystalline in nature. The major component is Tin Oxide. The predominant peak in the sample comprises SnO_2. The crystallite size of *Bhasma* is calculated and it is observed that it is in nanometers. The study reveals that the synthesized *Bhasma* was converted into its nontoxic oxide form and had a highly reduced particle size. These studies reveal that *Vanga Bhasma* prepared by the traditional method of heating (Sn_1) has 50% nanoparticles (150-300 nm range) that prepared by using an electric muffle furnace (Sn_2) has 100% nanoparticles (50-100 nm range) while commercial samples (Sn_3) have 50% nanoparticles (100-300 nm range). Further examination shows that *Bhasma* prepared by incineration with electric muffle furnace has uniform particle size distribution, while *Bhasma* prepared by incineration with *Kukkutputa* (traditional method) shows trimodal particle distribution. In case of commercial *Bhasma* there is no uniform particle distribution but it is multimodal in nature [6].

Dubey S, *et al*., (2016) Yogaamruto Rasa was subjected to analysis with highly sensitive analyzers like XRD, SEM, EDX and ZP for checking its identity, crystalline structure, particle size, absorption power and stability. Through XRD It was clear in the report that major peaks are of Cu_2O (Cuprite) with cubic structure and minor peaks of HgS (Meta Cinnabar), Cu_2S (Cuprous Sulphide) with cubic and hexagonal structures respectively. The major peaks were sharp due to crystalline nature of Cu_2O [7].

X-RAY FLUORESCENCE (XRF)

XRF (X-ray fluorescence) is a non-destructive analytical technique used to determine the elemental composition of materials. XRF analyzers determine the chemistry of a sample by measuring the fluorescent (or secondary) X-ray emitted from a sample when it is excited by a primary X-ray source. Each of the elements present in a sample produces a set of characteristic fluorescent X-rays ("a fingerprint") that is unique for that specific element, which is why XRF spectroscopy is an excellent technology for qualitative and quantitative analysis of material composition.

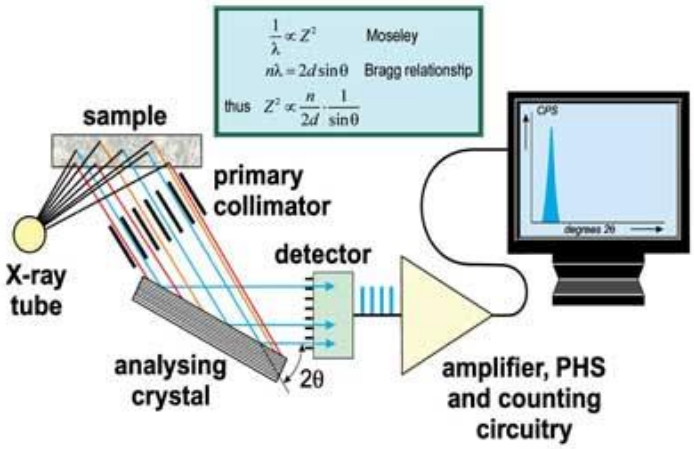

Fig 3: XRF Instrumentation

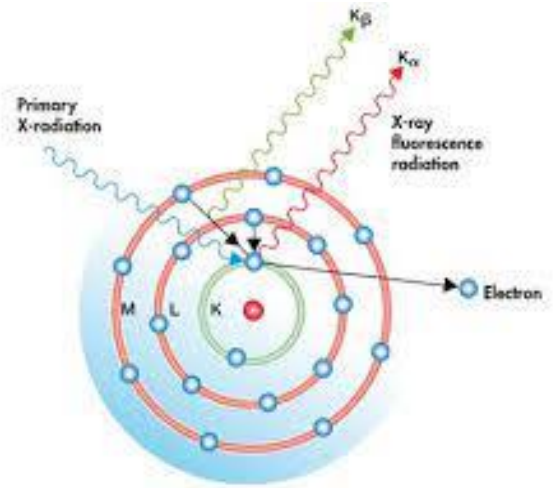

Fig 4: Fluorescent X-rays

PRINCIPLE

X-ray fluorescence is based on the excitation of atoms in the sample. A primary X-ray typically generated in an X-ray tube, hits an inner shell electron of the atom. This void is filled by an electron from an outer shell and fluorescence radiation is emitted. The fluorescence energy is equal to the energy difference between the two shells. Therefore, the energy of this radiation is characteristic of the atom and indicates, what atom is present in the sample. As many atoms are present in the sample, it will emit various x-rays with different energy. The fluorescence radiation is collected by a semiconductor detector. The X-rays create signals in the detector, which are collected in a multi-channel analyzer. This process handles each X-ray one by one but at a high speed. A detector of a modern XRF machine can handle 1 million counts per second. Even in a short period of time, sufficient information to calculate intensities can be used to determine the composition of the sample.

Previous Research Study

Preet R *et al*., in the year 2017, analysed the total mineral content of both the cytotypes from different plant parts of *Physalis angulata* L. using the XRF technique. The analysis led to the identification and concentration analysis of 27 elements in diploid and 25 in tetraploid cytotype. The percentage value of potassium (5.52%); iron (0.50%) and selenium (0.0042%) are reported to be higher in diploid cytotype and the amount of calcium (2.15%); magnesium (0.75%) and zinc (0.0075%) are higher in tetraploid cytotype. The amount of most of the minerals is higher in the tetraploid cytotype as compared to the diploid cytotype. This information is helpful in the standardization of herbal plants having medicinal benefits [8].

Sumedhan V, *et al*., in the year 2018, prepared *Mahagandhakam* in a single *Gaja Puta*. XRF detects that Calcium oxide (CaO) was found to be the major ingredient (87%) and mercury was present only at 20.23 ppm levels. From these analytical data, it was assessed that the product contains more calcium compounds rather than mercury and sulphur. As *Mukta Sukti* is basically $CaCO_3$, on incineration, the CO_2 portion may have escaped and the CaO part remained [9].

Shah N. D *et al*., in year 2018, did a comparative XRF study of *Hingula* purified in two different media i.e *Nimbuka swarasa* (lemon juice) and *Ardraka swarasa* (ginger juice) separately. XRF analysis was done for *Ashuddha Hingula*, after *1st Bhavana* (trituration), 3rd *Bhavana* and 7th *Bhavana* and finally again after *Prakshalana* (wash). Arsenic levels were seen reducing with subsequent *Bhavana*. Iron, Lead, Zinc, Calcium, and Copper which were initially absent in Raw *Hingula* were found in small quantities in subsequent samples as trituration with the above media was done and finally showed a reduction after *Prakshalana* [10].

Shakya N. K *et al*., in year 2020, analysed Raw *Shilajatu* and *Shilajatu Vatika* by using modern parameter XRF, and established the presence of many trace elements in *Shilajatu* as well as in *Shilajatu Vatika* like K, Ca, Cl, Si, Fe, S, P, Mn, Mg, Sr, Mo, Rb, Ru, Br, Zn, Ti, Cu, Ni and Na. It was observed that the *Shilajatu Vatika* contains mercury (Hg) (3.64%) whereas raw *Shilajatu* does not contain mercury. Raw *Shilajatu* contains 6.97% potassium whereas *Shilajatu Vatika* contains 4.87% of potassium. The percentage of Sulphur is found more in *Shilajatu Vatika* (2.08%) in comparison to Raw *Shilajatu* (0.33%) [11].

SCANNING ELECTRON MICROSCOPE (SEM)

A scanning electron microscope (SEM) produces images of a sample by scanning the surface with a focused beam of electrons. The electrons interact with atoms in the sample, producing various signals that contain information about the surface topography and composition of the sample. The electron beam is scanned in a raster scan pattern, and the position of the beam is combined with the intensity of the detected

signal to produce an image. In the most common SEM mode, secondary electrons emitted by atoms excited by the electron beam are detected using a secondary electron detector (Everhart-Thornley detector). SEMs can achieve resolutions better than 1 nanometer.

Specimens are observed in high vacuum in a conventional SEM, or in low vacuum or wet conditions in a variable pressure or environmental SEM, and at a wide range of cryogenic or elevated temperatures with specialized instruments.

PRINCIPLE

Unlike the Transmission Electron Microscope which uses transmitted electrons, the scanning electron Microscope uses emitted electrons. The Scanning electron microscope works on the principle of applying kinetic energy to produce signals on the interaction of the electrons. These electrons are secondary electrons, backscattered electrons, and diffracted backscattered electrons which are used to view crystallized elements and photons. Secondary and backscattered electrons are used to produce an image. The secondary electrons are emitted from the specimen play the primary role of detecting the morphology and topography of the specimen while the backscattered electrons show contrast in the composition of the elements of the specimen.

The source of the electrons and the electromagnetic lenses are from tungsten filament lamps. The electrons are emitted after thermal energy is applied to the electron source and allowed to move in a fast motion to the anode, which has a positive charge. The beam of electrons activates the emission of primary scattered (Primary) electrons at high energy levels and secondary electrons at low-energy levels from the specimen surface. The beam of electrons interacts with the specimen to produce signals that give information about the surface topography and composition of the specimen. The samples are mounted and coated with thin layer of heavy metal elements to allow spatial scattering of electric charges on the surface of the specimen allowing better image production, with high clarity. When the secondary electrons reach and enter the detector, they strike a scintillator (a luminescence material that fluoresces when struck by a charged particle or high-energy photon). This emits flashes of light which get converted into an electric current by a photomultiplier, sending a signal to the cathode ray tube. This produces an image that looks like a television picture that can be viewed and photographed. The quantity of secondary electrons that enter the detector is highly defined by the nature of the specimen i.e raised surfaces receive high quantities of electrons, entering the detector while depressed surfaces have fewer electrons reaching the surface and hence fewer electrons enter the detector. Therefore, raised surfaces will appear brighter on the screen while depressed surfaces appear darker.

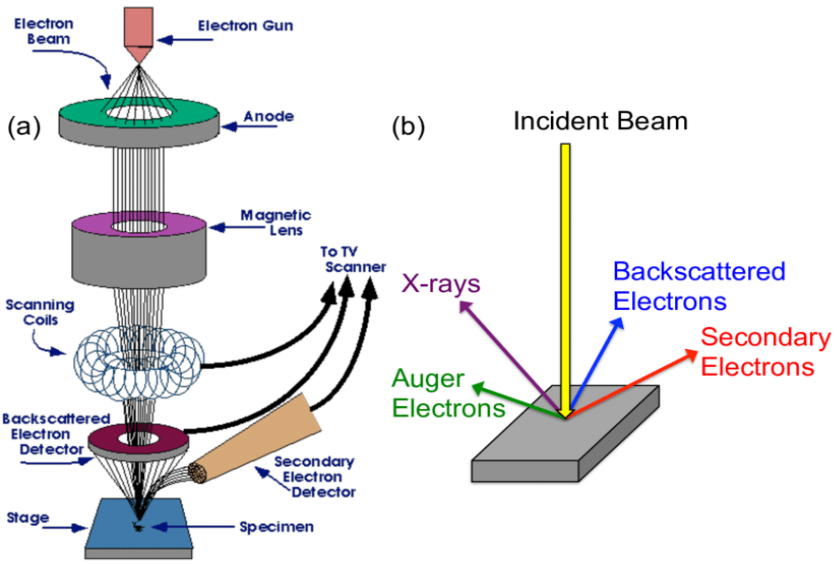

Fig 5: SEM Instrumentation & Principle

Previous Research Study

Badekila S *et al*., in year 2018, pharmaceutico-analytical standardization of *Rasa Bhasma* prepared as per Rasa Manjari by using *Bhudhara Yantra*. SEM photomicrograph of *Kajjali* and *Rasa Bhasma* samples show the appearance of particles of 10 µ and less than 5 µ sized particles in all the samples. SEM images of the drug samples show cubic shape like structure with the particle size lying in the micro range. Particles with Rhombohedral features are also observed [12].

Kale B *et al*., in year 2018, revealed that the synthesized *Bhasma* was converted into its nontoxic oxide form and had a highly reduced particle size. The images of *Vanga Bhasma* particles show granular appearance and porous morphology. These

nanoparticles have spherical morphology with the size smaller than 100 nm. The Initial sample shows the rough surface morphology. The *Jarana* method helps to convert sample into nanometer size [6].

Vadnere G. P *et al*., in year 2013, analyzed *Muktashukti Bhasma* to scrutinize their chemical composition. The raw material, intermediates obtained during synthesis and the final products have been characterized by various instrumental techniques. The particle in raw material and in the sample of intermediary process were not uniformly arranged while in the final product the particles seen uniformly arranged. The particle size even found lesser in the final product. In this process it was found that spongy and relatively compact microcrystalline aggregates of calcite were observed after the first calcinations cycle, which were covered by small dusty crystalline. The second calcinations cycle resulted into a spongy nature of the crystallites with increased agglomeration as indicated by the increased particle size. A distinct change in the morphology was also observed with last calculations cycle as several well-defined-shaped particle was seen in the SEM of *Muktashukti bhasma*. This simply means that repeated calcination cycles are necessary to stabilize the particle to a minimum particle size [13].

INDUCTIVELY COUPLED PLASMA MASS SPECTROMETRY (ICP-MS)

ICP (Inductively coupled plasma) mass spectroscopy is an analytical method used to detect and measure trace elements to analyze chemical samples. Inductively coupled plasma mass spectrometry (ICP-MS) is a type of mass spectrometry that uses an inductively coupled plasma to ionize the sample.The process is based on the ionization of a sample by an extremely hot plasma usually made from Argon gas. It atomizes the sample and creates atomic and small polyatomic ions, which are then detected. It is known and used for its ability to detect metals and several non-metals in liquid samples at very low concentrations. It can detect different isotopes of the same element, which makes it a versatile tool in isotopic labeling. Compared to atomic absorption spectroscopy, ICP-MS has greater speed, precision, and sensitivity.

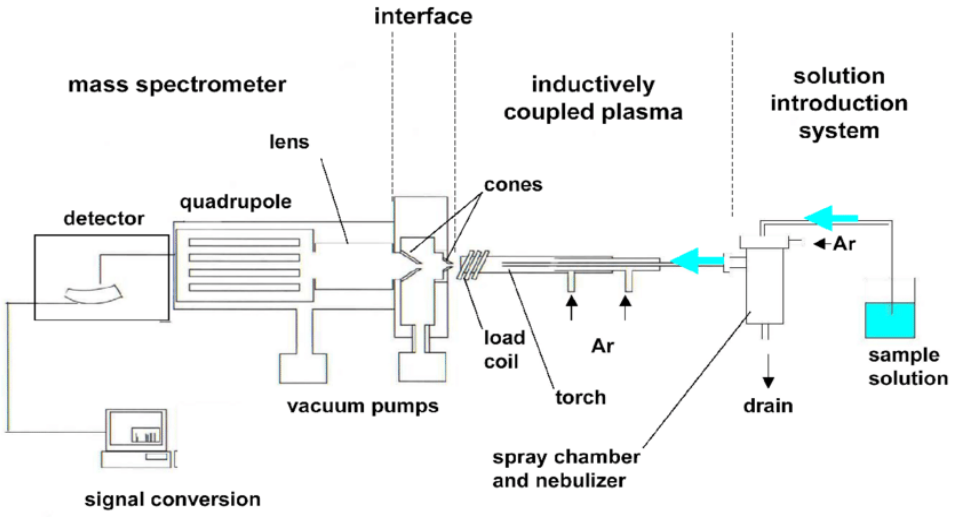

Fig 6: ICP-MS Instrumentation

PRINCIPLE

There are six fundamental compartments of a single quadrupole ICP-MS: the sample introduction system, inductively coupled plasma (ICP), interface, ion optics, mass analyser and detector. The Liquid sample is introduced into the device by means of a peristaltic pump. There it becomes nebulized in a spray chamber. The resulting aerosol is injected into an argon plasma that has a temperature of 6000-8000 K. Inside the plasma torch atomization and ionization of the sample occur. The ions from the plasma are extracted through a series of cones into a mass spectrometer, usually a quadrupole. The ions are separated on the basis of their mass-to-charge ratio and a detector receives an ion signal proportional to the concentration.

Previous Research Study

Saraswathy A, *et al*., ICP analysis of the sample revealed the content of calcium was found to be high among the trace elements analyzed whereas aluminium and iron were comparatively in moderate amount. Presence of the trace elements such as calcium, aluminium, iron, arsenic, phosphorus and silicon along with tim might be attributing for the therapeutic activity of the *Bhasma* [14].

TRANSMISSION ELECTRON MICROSCOPE (TEM)

The transmission electron microscope is a very powerful tool for material science. A high energy beam of electrons is passed through a very thin sample, and the interactions between the electrons and the atoms can be used to visualize specimens and generate a highly-

magnified image. TEMs can magnify objects up to 2 million times. Specimens must be very thin so that electrons are able to pass through the tissue. This may be done by cutting very thin slices of a specimen's tissue using an ultramicrotome.

The TEM operates on the same basic principles as the light microscope but uses electrons instead of light. Because the wavelength of electrons is much smaller than that of light, the optimal resolution attainable for TEM images is many orders of magnitude better than that from a light microscope. Thus, TEMs can reveal the finest details of internal structure.

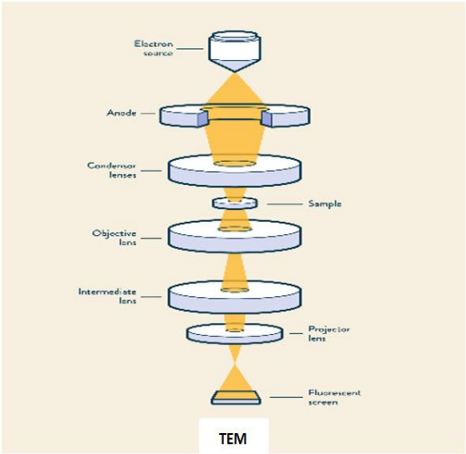

Fig 7: TEM instrumentation

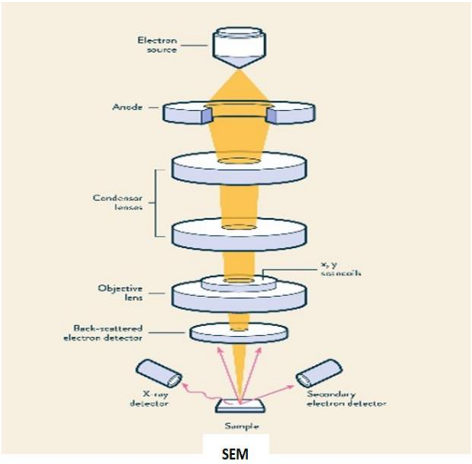

Fig 8: SEM Instrumentation

PRINCIPLE

TEM employ a high voltage electron beam in order to create an image. An electron gun at the top of a TEM emits. Magnetic condensing lens is used to condense the electrons and is also used to adjust the size of the electron that falls on to the specimen. The specimen is placed in between the condensing lens and the objective lens. The magnetic objective lens is used to block the high angle diffracted beam and the aperture is sued to eliminate the diffracted beam (if any) and in turn increases the contrast of the image.

This beam then passes through the specimen, which is very thin, and the electrons either scatter or hit a fluorescent screen at the bottom of the microscope The magnetic projector lens is placed above the fluorescent screen in order to achieve higher magnification. An image of the specimen with its assorted parts shown in different shades according to its density appears on the screen. The image can be recorded by using a fluorescent (Phosphor) screen.

The tissue must first be put in a chemical solution to preserve the cell structure. The tissue must also be completely dehydrated (all water removed). Once preserved and dehydrated, tissue samples are placed in hard, clean plastic. After sections are cut and mounted on grids, (tiny circular disks with openings,) a solution of lead is used to stain the tissue. The lead provides contrast to the tissue by staining certain cell parts. When placed in the electron microscope, the electrons are scattered by the lead. They do not penetrate the tissue or hit the fluorescent screen, leaving those areas dark.

Previous Research Study:

Singh S. K, *et al*., in his study Synthesis, characterization, and Histopathological study of a lead based Indian traditional drug: *Naga Bhasma*, found, the TEM image of the drug sample shows spongy like structure with the irregular particle size in the submicron range. The reason is the use of the organic materials. Due to the organic materials from the herbal source in the preparation of the *Bhasma* and heat treatments, the nano size crystallite gets agglomerated and give rise to the micro sized particles. These studies confirm that the *Bhasma* are nano-crystallite with submicron size particle [15].

Kale B, *et al*., TEM study reveals that particle sizes of bhasma prepared by using traditional method of heating and using electric muffle furnace heating were 20 nm. It is observed that particles of *Bhasma* are uniformly distributed, more clustering. The study also reveals that the particles are uniformly scattered. TEM images clearly show the morphological variation and the nanocrystallite nature of sample. The SAED pattern of diffraction shows that products formed in both the methods are polycrystalline in nature. Similarly commercial sample also polycrystalline particles are aggregated and having smooth surface. Some rod-shaped structures also present in the commercial sample, this is may be due to incomplete burning of ingredients used for its preparation [6].

Table 1: Difference between SEM and TEM

FEATURES	SEM	TEM
Electron stream	Fine, focused beam	Broad beam
Image taken	Topographical/surface	Internal structure
Resolution	Lower resolution	Higher resolution
Magnification	Up to 2,000,000 times	Up to 50,000,000 times
Image dimension	3-D	2-D
Sample thickness	Thin and thick samples okay	Ultrathin samples only
Penetrates sample	No	Yes
Sample restriction	Less restrictive	More restrictive
Sample preparation	Less preparation required	More preparation required
Cost	Less expensive	More expensive
Speed	Faster	Slower
Operation	Easy to use	More complicated; requires training

NANOPARTICLE TRACKING ANALYSIS (NTA)

Nanoparticle tracking analysis (NTA) is a method for visualizing and analyzing particles in liquids that relates the rate of Brownian motion to particle size. The rate of movement is related only to the viscosity and temperature of the liquid; it is not influenced by particle density or refractive index. NTA allows the determination of a size distribution profile of small particles with a diameter of approximately 10-1000 nanometers (nm) in liquid suspension. Analysis of particles at the lowest end of this range is possible only for particles composed of materials with a high refractive index, such gold and silver. The upper size limit is restricted by the limited Brownian motion of large particles; because a large particle moves very slowly, accuracy is diminished. The viscosity of the solvent also influences the movement of particles, and it, too, plays a part in determining the upper size limit for a specific system.

PRINCIPLE

Particles in liquid suspension are loaded into a sample chamber, which is illuminated by a specially shaped laser beam. Particles in the path of the beam scatter the laser light which is easily collected by the 20x microscope objective and is viewed with a digital camera. The camera captures a video of the particles moving under Brownian motion. The Nanoparticle Tracking Analysis (NTA) software analyses many particles individually and simultaneously (particle-by-particle), and by using the Stokes Einstein equation, calculates their hydrodynamic diameters. Nano Sight instruments provide high resolution nanoparticle size, count-based concentration and aggregation measurements while a fluorescence mode provides specific results for suitably labelled particles. With real time monitoring, subtle changes in the characteristics of particle populations are provided with all of these analyses confirmed by visual validation.

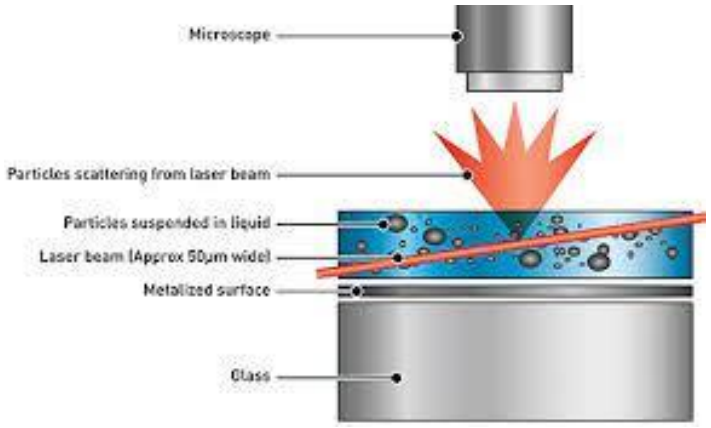

Fig 9: NTA Instrumentation

Previous Research Study

Sawant R. S *et al*., The Raw pearl powder, prepared samples of *Bhasma* and *Pishti* were subjected to analysis using NTA (Nano-particle Tracking Analyzing) methods. MB achieved particle size as 156 nm by rigorous trituration, levigation and heat treatment, MP achieved average particle size as 62 nm only by rigorous trituration [6 hours daily] for 21 Days which was confirmed by NTA [16].

Wavare R, *et al*., Two types of *Manikya bhasma* were prepared i.e *Hartaladi Manikya Bhasma* (MB1), and Vanaspati (gulab jal marit) *Manikya Bhasma* (MB2) as per texual reference with the help of

8 puta (ancient heating procedure). Prepared Bhasma was subjected to ancient as well as modern physico-chemical analysis and results were compared. Mean Particle Size of Raw Ruby is 1071.58 nm. NTA results shows that the particle sizes of MB1 and MB2 were respectively 43 nm and 63 nm. So, it can be concluded that both MB1 and MB2 are nano-medicine, hence both will have better assimilation in human body [17].

FOURIER TRANSFORM INFRARED SPECTROSCOPY (FTIR)

Fourier Transform Infrared Spectroscopy, also known as FTIR Analysis or FTIR Spectroscopy, is an analytical technique used to identify organic, polymeric, and, in some cases, inorganic materials. The FTIR analysis method uses infrared light to scan test samples and observe chemical properties. It is an established technique for quality control when evaluating industrially manufactured material, and can often serve as the first step in the material analysis process. A change in the characteristic pattern of absorption bands clearly indicates a change in the composition of the material or the presence of contamination. If problems with the product are identified by visual inspection, the origin is typically determined by FTIR microanalysis. This technique is useful for analyzing the chemical composition of smaller particles, typically 10-50 microns, as well as larger areas on the surface.

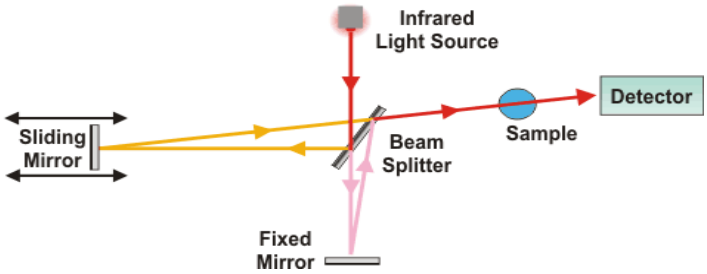

Fig 10: FTIR Instrumentation

PRINCIPLE

The FTIR instrument sends infrared radiation of about 10,000 to 100 cm^{-1} through a sample, with some radiation absorbed and some passed through. The absorbed radiation is converted into rotational and/or vibrational energy by the sample molecules. The resulting signal at the detector presents as a spectrum, typically from 4000 cm^{-1} to 400cm^{-1}, representing a molecular fingerprint of the sample. Each molecule or chemical structure will produce a unique spectral fingerprint, making FTIR analysis a great tool for chemical identification.

Previous Research Study

Garg *et al*., 2012, FTIR spectral analysis was used to ascertain the presence of organic matter in the finally prepared drug. IR spectra of all the samples selected for comparative purpose were recorded in KBr pellets in the region 4000-400 cm^{-1} on Perkin-Elmer FTIR spectrophotometer model 1600. FTIR studies conducted on these five sample formulations revealed the presence of various organic functional groups viz. tertiary alcohol, dialkyl ketones, secondary amides and 2-substituted pyridines common in all samples whereas some other variable functional groups were also present in some formulations like halogens, aldehydes, carboxylic acids and alkyl amine. It is important to mention here that presence or absence of these different functional groups affect the quality and efficacy of a particular formulation [18].

Swapnil Y *et al*., 2015, Raw *Tamra*, intermediate samples obtained during purification, incineration and *Amritikarana* were analyzed using FTIR. It was observed that *Shodhana* procedure leads in the formation of bonds between surface particles of *Tamra* and *Shodhana* media. These formed bonds on the surface of *Shodhita Tamra* samples gave various sharp peaks representing presence of many functional groups. The FTIR spectra revealed that both Bhasma samples contained organic compounds probably in the form of a complex with common functional groups like alkyl, methyl, etc., [19].

ATOMIC ABSORPTION SPECTROSCOPY (AAS)

AAS is an analytical technique used to determine the concentration of metallic elements in different materials. As an analytical technique, it uses electromagnetic wavelengths, coming from a light source. Distinct elements will absorb these wavelengths differently. It gives a picture of what concentrations of a specific element there is in whatever material, or liquid, is being tested. In some cases, metal content in a material is desirable, but metals can also be contaminants (poisons). Therefore, measuring metal content is critical in many different applications. It finds a purpose in quality control, toxicology and environmental testing.

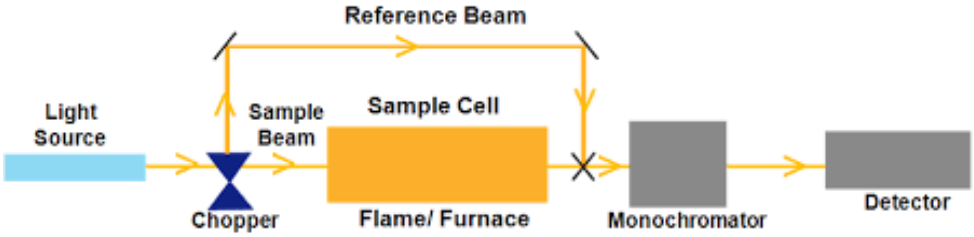

Fig 11: AAS Instrumentation

PRINCIPLE

The basic principles of AAS can be expressed as follows. Firstly, all atoms or ions can absorb light at specific, unique wavelengths. When a sample containing copper (Cu) and nickel (Ni), for example, is exposed to light at the characteristic wavelength of Cu, then only the Cu atoms or ions will absorb this light. The amount of light absorbed at this wavelength is directly proportional to the concentration of the absorbing ions or atoms. The electrons within an atom exist at various energy levels. When the atom is exposed to its own unique wavelength, it can absorb the energy (photons) and electrons move from a ground state to excited states. The radiant energy absorbed by the electrons is directly related to the transition that occurs during this process. Furthermore, since the electronic structure of every element is unique, the radiation absorbed represents a unique property of each individual element and it can be measured. An atomic absorption spectrometer uses these basic principles and applies them in practical quantitative analysis.

Previous Research Study

Kerur B. R *et al*., 2017, detected accuracy of essential elemental concentration in ppm level in *Loha Bhasma* (calx of Iron) of four brands by AAS method. Total of nine elements were found in *Loha Bhasma* in various proportions viz., Mg, Al, K, Ca, Cr, Mn, Cu and Zn along with Fe element. Except Iron element in all *Bhasmas* all other elements are available in trace amount and found to vary from 5 to 62 % [20].

Singh S. K *et al*., 2010, characterize the *Naga Bhasma* on elemental and structural point of view. AAS method detect the trace metal composition of *Naga Bhasma*. *Bhasma* is found to be rich in Mg, Ca and Fe and electrolytic elements Na and K. Zn is also found in sufficient concentration. All these elements taken from herbs act as the essential elements to increase the efficiency of the drug. Other heavy metals Cd, Cr, Cu and Ni found are in small amount and well within the limit. Lead was found to be high enough but in the lead sulfide form (least toxic). It is clear that medicinal herbs used in preparation of *Bhasma* are very rich in essential nutrients such as Ca, Fe, Mg, and Zn. Thus, elemental analysis shows that the nutrient element present in *Bhasma* sample are due to herbal sources [15].

Garg *et al*., 2012, it was observed that all marketed *Naga Bhasma* sample formulations are rich source of essential elements Mg, Ca, Zn, Cu and Al. Percentage of lead in formulations A, B, D, G and U are 1.5, 3.0, 2.0, 4.6 and 5.5% w/w, respectively which are quite variable and sufficient to affect the quality of a drug. However, previous studies suggest that *Naga Bhasma* contains approximately 5% w/w of lead (Pb) when prepared in laboratory [18].

Table 2: Modern Sophisticated Instrumental Techniques

Technique	Purpose
XRD	Phase Identification
XRF	Elemental Composition
EDX-SEM	Chemical nature, Size, Morphology
TEM	Particle size, Distribution
ICP-MS	Measure & Identify trace elements in fluids
AAS	Concentration of metallic element in sample
FTIR	Reveals composition of solid, liquid, gas using infrared
NTA	Nanoparticle size in liquid suspension

DISCUSSION

Bhasma Pariksha signifies – the genuinity of *Bhasma*. In the classic, there is mention of different types of *Bhasma Pariksha*, like *Varitaratwa, Unnama, Rekha Purnatwa, Nirutha, Apunarbhava*, etc. But after confirming *Bhasma Pariksha* through the classical method, one needs to perform the modern method for global acceptance. Through the modern Instrumental techniques, qualitative and quantitative analysis, as well as structural analysis, is possible which helps to analyze the changes that take place from raw drugs to the final product. Sometimes metal content in a material is desirable, but metals can also be contaminants. Therefore, measuring for metallic content is a critical part of many different processes. These modern sophisticated techniques have greater **sensitivity** than

other classical methods. Various industries and sectors are dependent on these testing to ensure their products (the materials they are processing) are sufficiently free from contamination or contain the right degree of certain metallic elements to support their intrinsic value. Using the advanced capabilities of modern instrumental techniques, samples can be tested rapidly and accurately.

For quality and process control many modern elemental analysis techniques are available. Each of these techniques has a number of advantages and disadvantages giving the analyst the flexibility to choose which technology suits best. XRD helps to study the nature of the crystalline substances, atomic spacing, information on unit cell dimension, provides vital information regarding the arrangement of atoms. XRF is a non-destructive method of solid or liquid samples. Most of the elements in the periodic table respond to this and limit is between 10-100 ppm. SEM helps to detect the particle size and is also applied to the surface related physical properties such as topography, depth profiling studies. SEM images are created by electrons that bounce off or are ejected from the sample, because of this the SEM gets surface images of the sample, whereas TEM is a special type of microscope that uses electrons to visualize sample and generate highly magnified image up to a level of about 2 million times. The image producing system of TEM is what distinguishes it from SEM. ICP-MS is known and used for its ability to detect metals and several non-metals in liquid samples at very low concentrations. Compared to AAS, ICP-MS has greater speed, precision, and sensitivity. One of the largest uses for ICP-MS is in the medical and forensic field, specifically, toxicology. For a number of reasons, metal assay is done in cases such as suspicion of heavy metal poisoning, metabolic concerns, and even hepatological issues. In the pharmaceutical industry, ICP-MS is used for detecting inorganic impurities in pharmaceuticals and their ingredients. FTIR method uses infrared light to scan test samples and observe chemical properties. AAS, is a technique which uses electromagnetic wavelengths coming from a light source. It is an older technique used to quantify metal content in a sample. It is very important to select the proper instrument to carry out a fruitful analysis. A wrong selection at this point will lead to a meaningless analysis.

CONCLUSION

Modern instrumental techniques offer unlimited advantages such as it is time saving, provide improvement in accuracy of results by elimination of errors introduced due to personal bias, improvement in sensitivity leading to trace analysis, these sensitivities could not be imagined using the conventional classical approach and provide benefit of fast decisions during manufacturing operations. The distribution, effectiveness, and toxicity of metals in *Bhasma* are significantly influenced by their chemical forms. The main causes of Pb, Hg, and As poisoning instances from the use of traditional remedies containing minerals include improper use (high dose and long-term administration) and improper manufacturing techniques. Mineral toxicity frequently coexists with therapeutic effects. The right assessment and balance of their efficacy and toxicity are crucial. These modern techniques are useful in quality assurance and determine the levels of trace elements which is being consumed by patient in the form of powder or *Bhasma* of which patients are not aware. But one should always bear in mind that basic analytical operational skills cannot be overlooked due to over dependence on instrumental techniques.

REFERENCES

1. Shastri, K. N. (2006). Vidyotini hindi commentary on Charaka Samhita of Agnivesa, Chaukhambha Sanskrit Sansthan, Varanasi, Edition 2006, Part 2, Chikitsa Sthana, Chapter 1, *Kara Prachiteeya Rasayan Pada Verse*, 15-23, P 37.
2. Liu, J., Zhang, F., Ravikanth, V., Olajide, O. A., Li, C., & Wei, L. X. (2019). Chemical compositions of metals in Bhasmas and Tibetan Zuotai are a major determinant of their therapeutic effects and toxicity. *Evidence-based complementary and Alternative Medicine*, *2019*. https://doi.org/10.1155/2019/1697804
3. Reimer, K. J., & Koch, I. (2012). Metals and metalloids in complementary and alternative medicines. *Maturitas*, *72*(4), 267-268.
4. Singh, R. K., Kumar, S., Aman, A. K., Kumar, S., & Kar, M. (2018). Study on physical properties of Indian based ayurvedic medicine: Abhrakh bhasma as nanomaterials by employing modern scientific tools. *GSC Biological and Pharmaceutical Sciences*, *5*(2), 041-047.
5. Gupta, R. K., Lakshmi, V., & Jha, C. B. (2015). X-ray diffraction of different samples of swarna makshika bhasma. *Ayu*, *36*(2), 225-229.
6. Kale, B., & Rajurkar, N. (2019). Synthesis and characterization of Vanga bhasma. *Journal of Ayurveda and Integrative medicine*, *10*(2), 111-118.
7. Dubey, S., Gubrele, D., & Rao, R. M. (2016). Standardization of Yogaamruto Rasa by Using Modern Analysis Techniques, *International Journal of Ayurveda and Pharma Research*, 4(7), 27-33.
8. Preet, R., Gupta, R. C., & Pradhan, S. K. (2017). Elemental Analysis and Biological Studies of Physalis Angulata L. Using Wave Length-Dispersive X-Ray Fluorescence Technique, Wavelength Dispersion X-Ray Fluorescence, From Rajasthan. *Asian Journal of Pharmaceutical and Clinical Research*, 220-224.
9. Sumedhan, V. (2018). Pharmaceutico -Analytical evaluation of Mahagandhakam, *J Res Tradit Med*, 4(2), 58-69.

10. Shah, N. D., Vaity, P. C., Pargunde, S. D., Khobragade, V. M., & Punde, A. A. Comparative XRF Study of Hingula Purified in Two Different Media. *Ayurpub*, 3(2), 830-837.
11. Shakya, N. K., & Mohapatra, S. (2020). Physico-Chemical Characterization of Shilajatu Vatika(Herbo-Mineral Compound Formulation)-An Approach to Compound Formulation Standardization, *World Journal Of Pharmacy And Pharmaceutical Sciences*, 9(11), 1308-1320.
12. Badekila, S. (2018). Pharmaceutico Analytical Standardization of Rasa Bhasma Prepared as Per Rasa Manjari by Using Bhudhara Yantra. *Journal of Ayurveda and Holistic Medicine (JAHM)*, *6*(3), 1-17.
13. Vadnere, G. P., Pathan, A. R., & Singhai, A. K. (2013). Characterization of indigenous traditional medicine-Muktashukti Bhasma. *Indian Journal of Traditional Knowledge*, 12(3), 483-488.
14. Saraswathy, A., Ruckmani, S., Devi, A. M., & Ariyanathan, S. (2013). Chemical analysis of Vanga Bhasma, *Indian Journal of Traditional Knowledge*, 12(3), 483-488.
15. Singh, S. K., Gautam, D. N. S., Kumar, M., & Rai, S. B. (2010). Synthesis, characterization and histopathological study of a lead-based Indian traditional drug: Naga bhasma. *Indian Journal of Pharmaceutical Sciences*, *72*(1), 24-30.
16. Sawant, R. S. (2015). Comparative study of mukta bhasma & mukta pishti With reference to their particle size. *International Journal of Ayurvedic Medicine*, *6*(1), 122-128.
17. Ramesh, W., Reena, Y., Suchita, S., & Ranjeet, S. (2014). A pharmaceutical approach of Manikya Bhasma towards its standardization. *Journal of Pharmaceutical and Scientific Innovation (JPSI)*, *3*(1), 82-86.
18. Garg, M., Das, S., & Singh, G. (2012). Comparative physicochemical evaluation of a marketed herbomineral formulation: Naga bhasma. *Indian Journal of Pharmaceutical Sciences*, *74*(6), 535-540.
19. Chaudhari, S. Y., Rajput, D. S., Galib, R., & Prajapati, P. K. (2015). Fourier transform infrared analysis of Tamra Bhasma at different levels: A preliminary study. *Ayu*, *36*(1), 77-82.
20. Ashwini, A. (2017). Estimation of heavy and trace elements in ayurvedic drug (loha bhasma) alternative medicine for anemia by AAS and ICP-OES. *Int J Res Ayurveda Pharm*, 8(5), 81-85.