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Research Article

ANALYTICAL STANDARDIZATION OF *RAJATA BHASMA*

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Keywords: *Rajata bhasma*, XRD, SEM, FT-IR, UV- Spectroscopy.

ABSTRACT

Bhasma Kalpana is a unique part of *Ayurvedic Rasa shastra*. It is a method of converting metals into metallic *Bhasma*. Metallic *Bhasmas* are well known for its quick effectiveness, smaller dose and a long shelf life. However if these *Bhasmas* are not well prepared and analyzed they can be toxic to human body. Therefore *Bhasma Pariksha* is given in Ayurveda to confirm the well prepared metallic *Bhasma*, but in this era we need to analyze the *Bhasmas* on modern parameters too to make it acceptable globally. So in this study prepared *Rajata* (Silver) *Bhasma* is analyzed on various parameters i.e. Ayuvedic parameters i.e., *Varitaratwa, Rekhapurnatwa, Slakshantwa* and *Laghutwa* and some modern parameters like, pH, Particle size, Zeta Potential, X-ray Diffraction (XRD), Scanning Electron Microscopy (SEM), Energy dispersive X-ray spectroscopy, Fourier Transform Infra red Spectroscopy (FTIR), U V – Spectroscopy.

Results: pH value is 5.6, UV- spectroscopy showed maximum absorption at 302nm, FT-IR of *Rajata bhasma* reveals the presence of C-H (alkyl), C-N (Aliphatic amine) groups at different wavelengths, Mean Particle size of *Rajata bhasma* is 2.7nm, Zeta Potential analysis reveals -19.3 mV, X-RD of *Rajata bhasma* reaveals the major peaks of HgS (Cinnabar), Ag_2S (Silver Sulphide), SEM analysis of *Rajata bhasma* showed small crystalline particles at 5KX & 10KX magnifications, EDX analysis of *Rajata bhasma* confirmed the presence of elements viz., Sulphur 20.34%, Oxygen 7.43%, Silver 26.72%, Mercury 45.51% by weight.

INTRODUCTION

Rasa Śhastra is a branch of Medicine, which deals with pharmacological aspect of metals & minerals and preparation of medicine having wide range of therapeutic efficacy, possessing innate qualities like quick action, less dose, tastelessness, prolonged shelf life and better palatability.[1] But, in present era there is change in mind set of patients. Safety of drug to be administered is at par with its efficacy. *Rajata* is mentioned as one of the *Shudha dhatu* by most of the *Ayurveda Rasa Shastra* texts. It was subjected to *Shodhana (Samanya & Vishesa*) and *Marana* by triturating *Visesha Shodita Rajata* with *Kajjali* by using *Kumari swarasa* as a *Bhavana dravya.*

Analytical study is the key part of any scientific research. It tells us about the correlation between pre-determined hypothetical values and actual results obtained. It gives us valuable information about safety, efficacy, stability, and contraindications etc. of any formulation. Hence highly sensitive modern parameters are employed for gaining information about identity, form, particle size, and structure of contents of the formulation. Considering this, an effort has been made to analyze classically prepared *Rajata bhasma*

through X-ray diffraction, Scanning electron microscopy, Energy dispersive X-ray spectroscopy and Zeta potential, UV- spectroscopy, FT-IR.

MATERIAL AND METHODS:

The process was carried out in two steps:

1. Pharmaceutical Study
2. Analytical Study.

Pharmaceutical Study

Shodhana

- *Samanya Shodhana* of *Rajata Patra* was carried out by heating to red hot and quenched subsequently into the *Tila Taila, Takra, Gomutra, Kanji* and *Kulattha Kwatha* for seven times in each. After every *Nirvapa*, the liquid medium was changed. [2]
- *Visesha Shodhana* of *Samanya Shodita Rajata patra* was carried out by placing it in an iron ladle and heated up to red hot. Then dipped in a vessel containing *Agastya patra Swarasa*. This process was repeated for 2 more times by taking fresh *Agastya patra swarasa* each time. [3]
- *Shodhana of Parada was carried out by doing mardana with equal quantity of Sarja kshara, Yava Kshara* and *Tankana* with sufficient quantity of *Ardraka swarasa* and *Nagavalli swarasa* for 3 days. [4]
- After trituration for three days, the mixture was washed with hot water to obtain *Shudha Parada*.
- *Shodhana* of *Gandhaka* was done by pounding in a *Khalwa yantra* to form coarse powder. Cow's milk was poured in the wide mouthed earthen pot.
- The mouth of pot was covered with double layered cotton cloth and *Gandhaka* was spread evenly over it. Earthen lid was placed over the pot and sealed with fuller's earth. The pot was buried up to the neck level in a pit and 8 Cow dung cakes arranged above it.
- After ignition of Cow dung cakes, *Gandhaka* melted and dropped into milk through the cloth. After self-cooling, the apparatus was removed out of the pit and opened. Purified *Gandhaka* was collected at bottom of the pot in form of small pellets and washed in hot water and dried.[5]
- Equal quantity of *Parada* and *Gandhaka* are taken and triturated till it attains the properties (*Siddha Lakshanas*) of *Kajjali.* [6]

Marana

- *Marana* of *Visesha Shodhita Rajata Patras* was done by triturating in a *Khalwa yantra* with equal quantity of *Kajjali* and sufficient quantity of *Kumari Swarasa* as bhavana dravya. [7]
- *Chakrikas* of uniform size were prepared and dried well. They were kept in *Sharava* and subjected to *Sandhibandhana*. *Sharavasamputa* was kept in sunlight for drying.
- After drying it was subjected to *Laghu Puta* (8 *Upalas*). Whole procedure was repeated untill it attains *Bhasma lakshanas*. Totally 25 *Putas* were done during the whole procedure to attain *Rajata bhasma*.

ANALYTICAL STUDY

pH Value

The pH value of a liquid is determined by means of a glass electrode and a pH meter. Suitable glass electrode and pH meter of both potentiometer and deflection type are available. The pH meter is an electronic digital voltmeter, scaled to read pH directly, and may range from a comparatively simple hand held instrument, suitable for use is the field, to more elaborate bench models, often provided with a scale expansion facility, with a resolution of 0.001 pH unit and an accuracy of +0.001 unit.

Materials: Glass electrode, pH meter, Buffer tablets, Beakers

Sample

Rajata Bhasma – 0.5g

Procedure

- Instrument is switched on and allowed to warm up.
- As the instrument is equipped with a manual temperature control, the temperature of the solutions is taken and the control is set to this value. The electrode assembly is inserted into the same beaker, the selector switch of the instrument is set and pH is noted.
- The "Set buffer" control is adjusted until the meter reading agrees with the known pH of the buffer solution.
- The electrode assembly is removed, rinsed in distilled water and placed into a small beaker containing the second buffer solution.
- Now 0 .5 gram of *Rajata bhasma* sample was put in 5 ml. of water and pH is determined for the solution.

X-Ray Diffraction (XRD)

The *Rajata Bhasma* was subjected to XRD at Yogi Vemana University, Kadapa.

Principle of XRD

X-ray diffraction is based on constructive interference of monochromatic X-rays and a crystalline sample. 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 ($n\lambda = 2d \sin\theta$). This law relates the wavelength of electromagnetic radiation to the diffraction angle and the lattice spacing in a crystalline sample. These diffracted X-rays are then detected, processed and counted. By scanning the sample through a range of 2θ angles, all possible diffraction directions of the lattice should be attained due to the random orientation of the powdered material. Conversion of the diffraction peaks to d-spacing allows identification of the mineral because each mineral has a set of unique d-spacing. Typically, this is achieved by comparison of d-spacing with standard reference patterns.

Procedure

Sample is powdered in agate mortar to very fine powder. It is 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 2θrange. Obtained values are plotted on graph with the help of inbuilt "Reyflex Software" for further analysis.

Scanning Electron Microscopy and Electron Dispersive X-ray Spectroscopy (SEM &EDX):

The *Rajata Bhasma* was subjected to SEM and EDX at Department of physics, S.V. University, Tirupati.

Preparation of SEM specimen

Specimen of the sample to be analyzed is directly kept on the specimen holder for visualization. As the sample employed has nonconductive nature, the sample surface is coated by carbon by arc melting technique.

Materials needed

1) Small amount of powder sample. 2) Small round piece of metals specimen holder. Generally it is made of aluminum or copper. 3) Double side cello tape. 4) Conducting paste of aluminum powder. 5) Spreading and vapor sputtering unit.

Principle of SEM

In a scanning electron microscope, the specimen is exposed to a narrow electron beam from an electron gun, which rapidly moves over or scans the surface of the specimen. This causes the release of a shower of secondary electrons and other types of radiations from the specimen surface. The intensity of these secondary electrons depends upon the shape and the chemical com-position of the irradiated object. These electrons are collected by a detector, which generates electronic signals. These signals are scanned in the manner of a television system to produce an image on a cathode ray tube (CRT).The image is recorded by capturing it from the CRT. Modern variants have facility to record the photograph by digital camera. This microscope is used to observe the surface structure of microscopic objects.

Procedure:

The dried powder was placed over the specimen holder and observed under the microscope at 5,000X to 7,000X. Microphotographs were taken with the inbuilt camera.

Principle of EDX

The excess energy of the electron that migrates to an inner shell to fill the newly created hole can do more than emit an X-ray. Often, instead of X-ray emission, the excess energy is transferred to a third electron from a further outer shell, prompting its ejection. This ejected species is called an Auger electron, and the method for its analysis is known as Auger electron spectroscopy (AES).

Procedure

Electron beam excitation is used in electron micro-scopes, scanning electron microscopes (SEM) and scanning transmission electron microscopes (STEM). 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 onto an analyzer for data display and analysis. The most common detector now is Si (Li) detector cooled to cryogenic temperatures with liquid nitrogen; however newer systems are often equipped with silicon drift detectors (SDD) with Peltier cooling systems. The detector used in EDX is often the Lithium drifted Silicon detector. This detector must be operated at liquid nitrogen temperatures. When an X-ray strikes the detector, it will generate a photoelectron within the body of the Si. As this photoelectron travels through the Si, it generates electron-hole pairs. The electrons and holes are attracted to opposite ends of the detector with the aid of a strong electric field. The size of the current pulse thus generated depends on the number of electron-hole pairs created, which in turn depends on the energy of the incoming X-ray. Thus, an X-ray spectrum can be acquired giving information on the elemental composition of the material under examination.

Zeta Potential (ZP)

The *Rajata bhasma* was subjected to ZP at Department of Science and technology, PURSE S.V.University, Tirupati.

Principle of ZP

The most widely used technique for determining the ZP of colloidal-sized suspensions is particle electrophoresis or micro electrophoresis i.e. the movement of charged particles suspended in a liquid under the influence of an applied electric field. This offers the possibility of measuring the complete mobility spectrum. ZP is measured by applying an electric field across the dispersion. Particles within the dispersion with a ZP will migrate to-ward the electrode of opposite charge with a velocity proportional to the magnitude of the ZP. The Zetasizer Nano series instrument uses micro-electrophoresis and electrophoretic light scattering technology to measure ZP and electrophoretic mobility by determining the electrophoretic mobility and then applying the Henry equation. The electrophoretic mobility is obtained by per-forming an electrophoresis experiment on the sample and measuring the velocity of the particles using Laser Doppler Velocimetry (LDV).

Sample preparation

A 1% concentration of Rajata bhasma sample was prepared in distilled water. The particles were well dispersed before analysis.

Procedure

The sample is taken in a 1ml syringe and injected slowly into the capillary cell (cuvette) through the sample port. Care should be 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. The capillary cell is then placed into the sample holder of the zeta sizer instrument for analysis.

Particle Size Analysis

The *Rajata bhasma* was subjected to Particle size at Department of Science and technology, PURSE S.V.University, Tirupati.

Dynamic Light Scattering (DLS) is a commonly used term to describe a non-invasive, well-established technique which measures the particle size and estimated distribution of submicron particulate systems. The technique is widely recognized throughout the pharmaceutical and industrial world.

Principle

Particles, emulsions and molecules in suspension undergo Brownian motion. This is the motion induced by the bombardment by solvent molecules that themselves are moving due to their thermal energy. If the particles or molecules are illuminated with a laser, the intensity of the scattered light fluctuates at a rate that is dependent upon the size of the particles as smaller particles are kicked further by the solvent molecules and move more rapidly. This technique measures the diffusion of particles moving under Brownian motion and convert this to size distribution using the Stokes-Einstein relationship.

Procedure

The sample was mixed in water and sonicated 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.

The scattered light is captured by a detector over the course of the analysis to determine the rate of diffusion (i.e. how fast the particles move within a system due to Brownian motion) and thus the average Hydrodynamic particle size (referred to as the Z-Average) is calculated on an intensity weighted basis using the Stokes-Einstein equation. In simple terms, small particles move/diffuse more rapidly than larger particles.

Fourier Transform Infrared Spectroscopy (FT-IR)

FTIR (Fourier Transform Infrared Spectroscopy) was performed to detect the Presence of functional groups or organic legends in *Rajata Bhasma.*

Principle of FTIR

FTIR relies on the fact that the most molecules absorb light in the infra-red region of the electromagnetic spectrum. This absorption corresponds specifically to the bonds present in the molecule. The frequency range is measured as wave numbers typically over the range 4000 – 600 cm-1.

The background emission spectrum of the IR source is first recorded, followed by the emission spectrum of the IR source with the sample in place. The ratio of the sample spectrum to the background spectrum is directly related to the sample's absorption spectrum. The resultant absorption spectrum from the bond natural vibration frequencies indicates the presence of various chemical bonds and functional groups present in the sample. FTIR is particularly useful for identification of organic molecular groups and compounds due to the range of functional groups,

side chains and cross-links involved, all of which will have characteristic vibrational frequencies in the infra-red range.

UV-Spectroscopy

Rajata bhasma was subjected to UV-Spectroscopy at Department of Science and technology, PURSE S.V.University, Tirupati.

Spectroscopy is the measurement and interpretation of electromagnetic radiation (EMR) absorbed or exited when the molecular atoms or ions of the sample move from the one energy state to another energy state or excited stage from ground stage. At ground state the energy of a molecule is sum of total of rotational, vibrational, and electronic energies. In another words spectroscopy measure the changes in rotational, vibration and electronic energies.

When light (monochromatic or heterogeneous) falls upon a homogeneous medium, a portion of the incident light is reflected, a portion is absorbed within the medium, and the remainder is transmitted. If the intensity of the incident light is expressed by I_o the adsorbed light by I_a, that of transmitted light by I_t, and that of the reflected light by I_r, then: $Io = I_a + I_t + I_r$. Many molecules absorb ultraviolet or visible light. The wavelength range for the UV light is 200 - 400nm and for visible light it is from 400 - 800 nm. The absorbance of a solution increases as attenuation of the beam increases. Absorbance is directly proportional to the path length, b, and the concentration, c, of the absorbing as stated in to the Beer's Lambert Law. Different molecules absorb radiation of different wavelengths. An absorption spectrum will show a number of absorption bands corresponding to structural groups within the molecule. An attempt has been made to find out another UV spectra can be useful for its analysis. The UV spectra of the different extracts of the drug were recorded in an UV visible double beam spectrophotometer.

Procedure

5gm *Rajata Bhasma* was macerated with 100 ml of solvent in a closed flask for twenty-four hours separately, 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 λ max value was calculated.

OBSERVATIONS AND RESULTS

- *Nischandratva* (lusterless) was attained after whole process.
- *Laghutva* (lightness) and *Mrudutwa* were attained after the whole process.
- *Varitaratva* attained partially after 12th *Puta* and completely after 23rd *Puta*.
- *Rekhapurnatwa* was attained after 8th *Puta*.
- pH Value of *Rajata bhasma* is 5.6.
- UV-Spectrum of *Rajata Bhasma* showed maximum absorption at 302nm.

Image No. 1 showing the UV – Spectroscopy of *Rajata Bhasma*

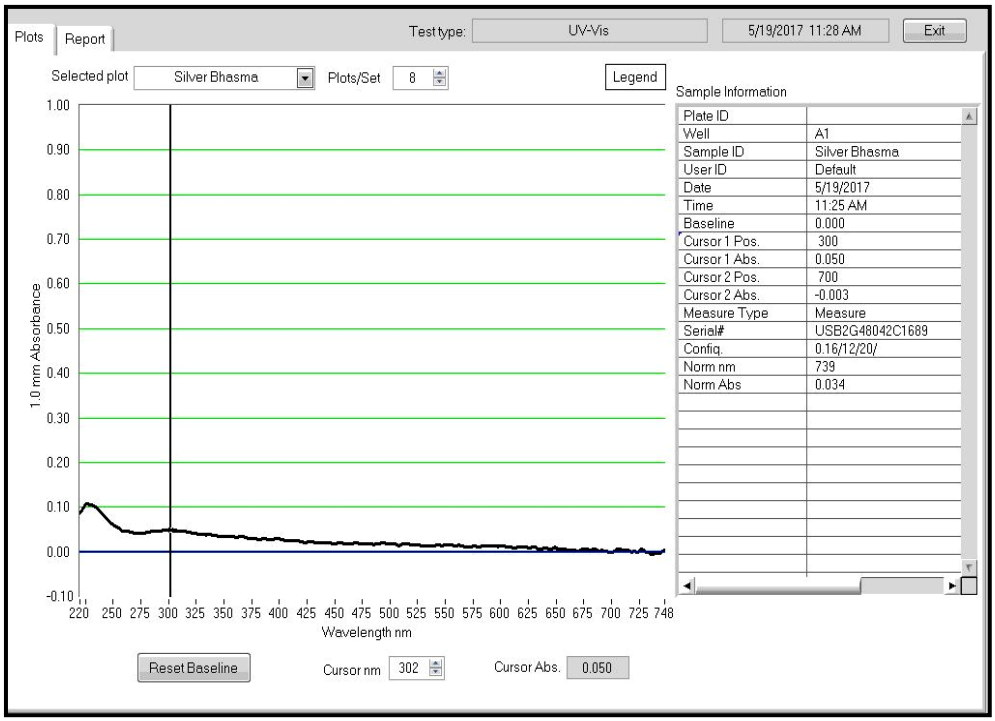
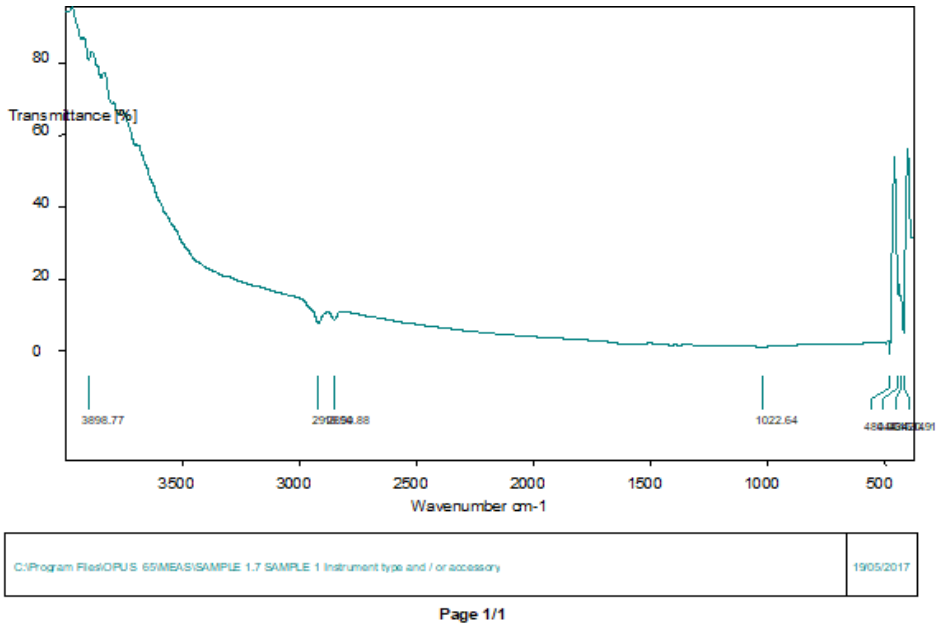


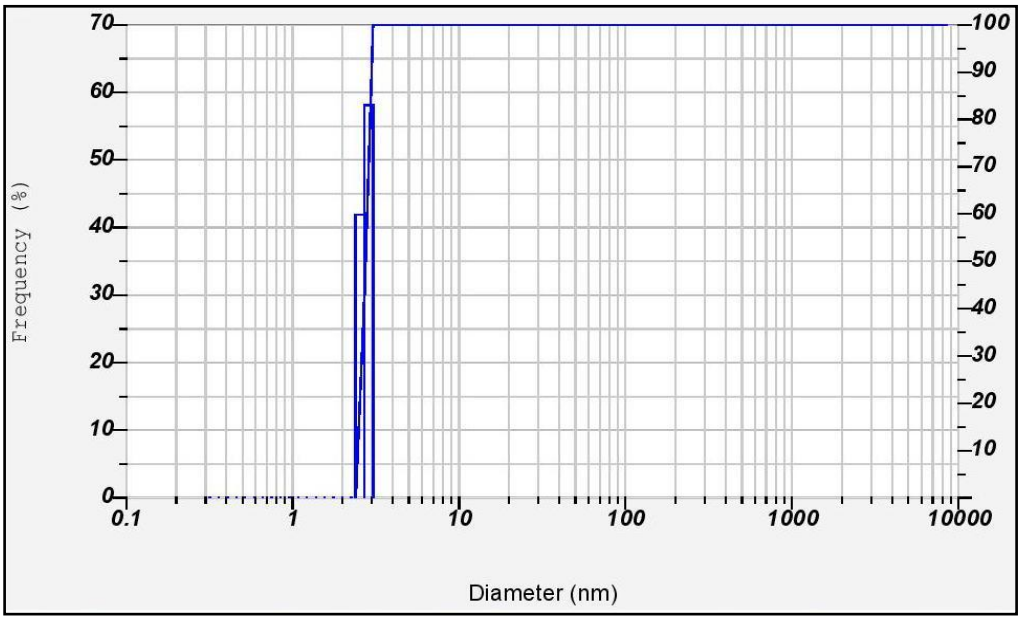
Table No.1Various peaks in FTIR analysis of *Rajata bhasma* and their correlation with compounds

S.No.	Actual peak	Bond	Type of bond	Specific type of bond	Appearance
1.	3898.77	-	-	-	Not found
2.	2918.94	C - H	Alkyl	Methylene	Medium to strong
3.	2850.88	C - H	Alkyl	Methyl	Medium to strong
4.	1022.64	C – N	Aliphatic Amine	Any	Weak
5.	480.05	-	-	-	Not found
6.	444.15	-	-	-	Not found
7.	434.14	-	-	-	Not found
8.	42091	-	-	-	Not found

Image No. 2 Showing various peaks obtained in FTIR analysis of *Rajata bhasma*



Graph No. 1: Showing the result of Particle size analysis of *Rajata Bhasma*



- The mean particle size of *Rajata Bhasma* is 2.7 nm.
- *Rajata bhasma* sample showed a Zeta potential value of -19.3 **mV,** which indicates colloidal stability.

Graph No. 2: Showing Zeta potential distribution of *Rajata bhasma*

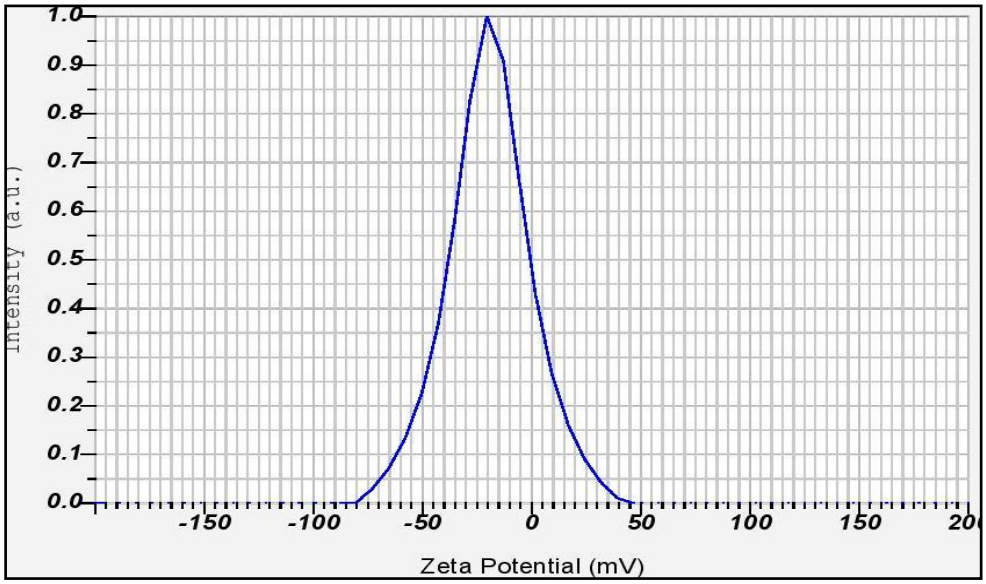


Image No. 3: Showing SEM result of *Rajata bhasma* (Mag. 5Kx)

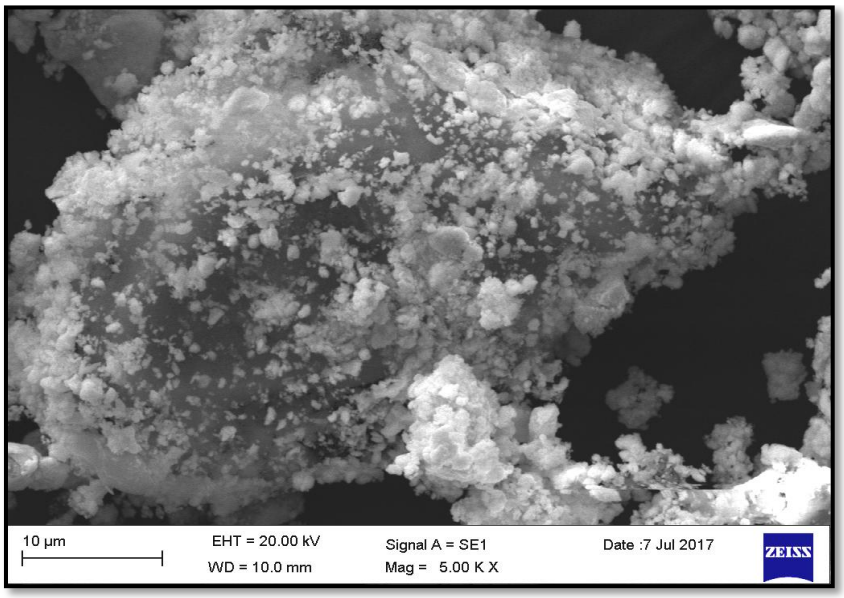


Image No. 4: Showing SEM result of *Rajata bhasma* (Mag. 10Kx)

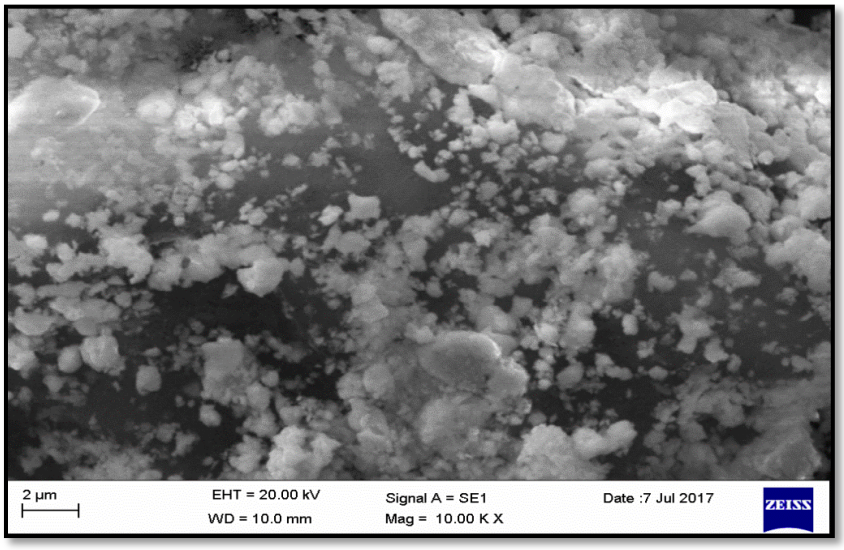
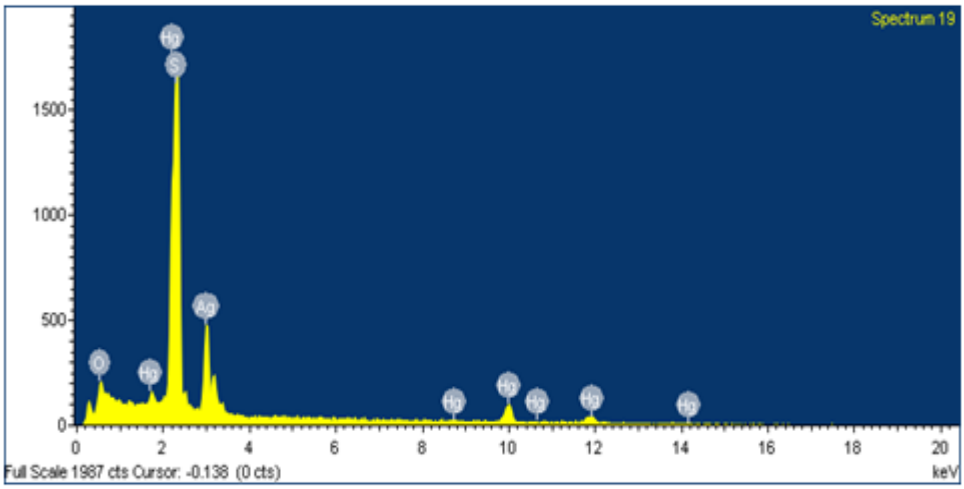


Table No. 2: Showing the quantity of all the elements in *Rajata bhasma*

Element	Weight%
S K	20.34
O K	7.43
Ag L	26.72
Hg M	45.51
Totals	100.00

Graph No. 3: Showing EDS graph of *Rajata bhasma*



Graph No. 4: Showing XRD graph of *Rajata bhasma*

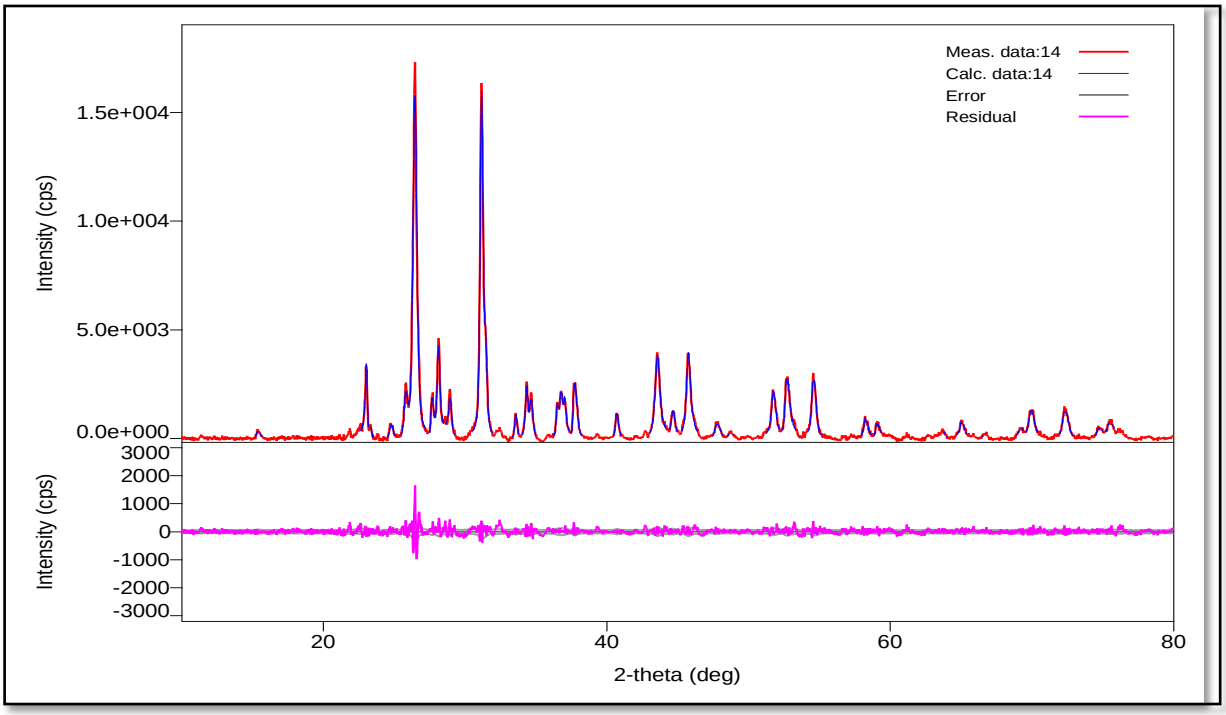


Table No. 3: Showing the details of matching peaks of XRD data for *Rajata Bhasma*:

S.No	Element/Molecule	JCPDS Ref.No	2θ	Intensity	FWHM	h k l
1.	HgS (Cinnabar)	00-042-1408	26.45	100	0.216	1 0 1
2.	HgS (Cinnabar)	00-042-1408	31.14	93	0.24	1 0 2
3.	Ag_2S (Silver Sulfide)	00-002-0998	43.55	23	0.168	- - -
4.	Ag_2S (Silver Sulfide)	00-024-0715	25.82	14.2	0.24	-1 1 2

DISCUSSION

Analytical study is an essential part of any research work. It provides us with experimental data and makes us know about certainty of our assumptions and prevents from miss interpretations. It provides us with knowledge about identity, size, structure of chemical constituents and physical properties. It hints us about toxic properties of drugs, if any.

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 inter-atomic distance and angle. X-ray diffraction is one of the most important characterization tools used in solid state chemistry and materials 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 Ag_2S were seen in XRD. During *Shodhana* and *Marana* process silver reacts with Mercury and Sulphur present in *Kajjali* to form Cinnabar and Silver Sulfide.

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. The distribution of particles in *Rajata bhasma* shows crystalline particles which are smaller in size.

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 *Rajata bhasma* confirmed the presence of elements like Sulphur, Silver and Mercury because of the *Kajjali* used in the preparation of *Rajata 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 the particles of *Rajata bhasma* is 2.7nm. The nano size of drug is indicative of its quick absorption and faster dispersion into body resulting in 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 *Rajata bhasma* was found to be -19.3 mV which indicates colloidal stability.

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 *Rajata bhasma* showed maximum absorption at 302 nm which indicates the presence of Silver in the sample.

FTIR was performed to detect the presence of functional groups or organic legends in *Rajata 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. *Rajata bhasma* showed 8 peaks between the wave length 3351.01cm^{-1} to 575.64 cm^{-1}. **FT- IR** analysis of Rajata *bhasma* reveals the presence of functional groups C-H, C-N, i.e. alkyl and aliphatic amine groups.

CONCLUSION

The *Rajata bhasma* passed all *Bhasma pariksha* viz. *Varitaratwa, Rekhapurnata, Nischandrata* and *Slakshanatwa* which proves that drug has attained its *Bhasma* form properly which proves that neither less nor more heat is desirable and *Supaka* is essential for making a drug safe and efficacious. The analytical study depicts the vision of the ancient seers regarding the pharmaceutical procedure adopted in the preparation of *Bhasma* in making them completely safe for therapeutic usage which was reflected in all the sophisticated analytical tests employed in this research work.

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