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SEM-EDAX and ICP-OES analysis of *Rasa Bhasma* along with its Physico-chemical characterization

Research Article

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Abstract

Abstract- *Rasa Bhasma* is a Herbo-Mineral preparation mentioned by Shri Anantadeva Soori in his textbook *Rasa Chintamani* in the context of *Parada Marana*. Owing to its Hepato-protective indication, it was prepared as per the conventional method and was subjected to various physico-chemical parameters, assessment of inorganic elements, SEM EDAX and ICP-OES for the determination of Mercury. Results revealed that *Rasa Bhasma* was tasteless, grey coloured smooth powder having alkaline nature insoluble in water and alcohol but sparingly soluble in Chloroform with values total ash- 88.10%, acid insoluble ash- 83.03%, loss on drying-0.675%. Amongst inorganic elements, sodium, chlorides and sulphates were found to be present in the *Bhasma*. SEM/EDAX spectra show the results of Mercury, Sulphur, Carbon, Oxygen, in various spectrums. The % of Mercury is maximum i.e., between 65-75% each. Sulphur varies from 25-30%. Carbon is rich in quantity varying from 25-30%. In all the Spectrums, Mercury is associated with Sulphur, forming a major peak and small Mercurial peaks are also seen along with small peaks of carbon. 98.3% mg/kg Mercury was found to be present in the *Rasa Bhasma* by ICP-OES analysis. Physico-chemical parameters of *Rasa Bhasma* were found to be within normal limits concluding that the prepared *Rasa Bhasma* was of standard quality.

Key Words: *Rasa Bhasma,* SEM-EDAX, ICP-OES, Standardisation.

Introduction

Ayurveda has its own unique parameters for qualitative and quantitative analysis of a drug or formulations. The Metallic & Mineral preparation of Ayurvedic medicines should be analysed for physical & chemical properties to confirm the authenticity & safety, before administrating to the patients. Hence it is essential to adopt Ancient & Modern analytical methods for better understanding & interpretation of Physio-chemical changes occurred during the process. So, an attempt has been made to carry out the analysis of *Rasa Bhasma* including its SEM-EDAX and ICP-OES analysis.

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Materials and Methods

3 samples of *Rasa Bhasma* were prepared as per the reference of *Rasa Chintamani* and the one which was found appropriate as per the conventional methods of *Bhasma pariksha* was taken for the analysis in the present study. (1) Physio-chemical analysis of *Rasa Bhasma* was carried out at Soniya College of Pharmacy, Dharwad. SEM - EDX analysis was carried out at Diya Labs Mumbai and ICP-OES was carried out at Cytxon Biosolutions Pvt. Ltd. Hubbali, Karnataka.

Physico-chemical Characterization

- **Determination of total ash:** Take about accurate 2gm ground drug in a previously tared silica dish, previously ignited and weighed. Scatter the ground dry in fine even layer on the bottom of the dish. Incinerate by gradually increasing the heat not exceeding dull red heat (450^{0}C) until free from carbon. Cooled and weighed. Calculate the % of ash with reference to the air-dried drug.(2)

- **Acid insoluble ash:** The ash obtained was taken with dilute HCL filtered through Whitman no. 42 filter paper. The residue was washed with hot water till it was free from chloride. The residue was taken in a crucible, dried & ignited at a low temperature.

Calculate the percentage of acid insoluble ash with reference to the moisture free drug.(3)

- **Loss on drying 110^{0}c:** 1gram of accurately weighed and heated on electric oven up to 110^{0}c and again weighed, the difference in weight was calculated by Initial weighed drug after 110^{0}c=- gram.(4)

- **Determination of pH:** The pH value of an aqueous liquid may be defined as the common logarithm of the reciprocal of the hydrogen ion concentration expressed in grams. The pH value of a liquid is determined by potentiometrically by means of a glass electrode and a suitable pH meter.(5)

- **Solubility:** About one gram of the sample was weighed and dissolved in 10 ml of the solvents. When the sample did not dissolve, an excess of solvent by 10 ml quantity up to 100 ml was added and noted that was soluble in water (1 gram of sample in 100 ml of water) and slightly soluble in chloroform (1 gram of sample in 600 ml to 1000 ml of chloroform) and soluble in alcohol (1 gram sample in 600 ml to 1000 ml alcohol).

Test for Inorganic Elements (6)

- **Test for calcium:** To 10 ml filtrate, add 1 drop dilute NH_4 OH and saturated ammonium oxalate solution. White ppt of calcium oxalate forms.Ppt is soluble in HCL but insoluble in acetic acid.

- **Test for Magnesium:** Filter and separate the white calcium oxalate ppt obtained above, heat and cool the filtrate which with solution of sodium phosphate in dilute ammonia solution gives white crystalline ppt.

- **Test for sodium:** To 2ml of test solution add little uranyl magnesium acetate reagent. Shake well and Keep for few minutes. Yellow crystalline ppt of sodium magnesium uranyl acetate observed.

- **Test for potassium:** To 2- 3 ml test solution, add few drops of sodium cobalt nitrite solution. Yellow ppt of Potassium cobalt nitrite observed.

- **Test for Iron:** To 5 ml test solution, add few drops of 2%potassium ferrocyanide. Dark blue colouration is Observed.

- **Test for sulphate:** To 5ml filtrate, add few drops of 5%$Bacl_2$solution,white crystalline $BaSO_4$ ppt appears. Insoluble in HCL, with lead acetate reagent gives white ppt, soluble in NaOH.

- **Test for phosphate:** To 5 ml test solution prepared in HNO_3, add few drops of ammonium molybdate solution. Heat for 10 min and cool it, yellow crystalline ppt of ammonium phosphor molybdate is observed.

- **Test for chloride:** To 3ml test solution prepared in HNO_3, add few drops of 10% $AgNO_3$ solution. White ppt of $Agcl_2$ is observed.ppt soluble in dil. Ammonia solution.

- **Test for carbonate:** With solution of Magnesium sulphate white ppt is formed.

SEM (Scannin Electron Microscopy)

SEM study is the highly advanced technique of microscopy where high resolution image can be captured. This gives important information on surface topography, elemental composition and particle size. SEM study was carried out by using a cold field emission scanning electron microscope with an intention to quantitatively assess the surface morphology of the sample. Images were captured of sample at different areas of surface. The images were recorded at different magnifications like 2000X, 5000X and 10000X. However the magnification beyond 10000X could not be achieved due to the limitation of the instrument and lack of clarity of image at higher magnification. However particles lesser than one micron size were visible confirming the fineness of particles in sample. Surface topography captured by scanning electron microscope is depicted as image.(7)

EDAX/EDS(Energy-Dispersive Spectrometer)

- Energy Dispersive X-ray Spectroscopy (abbreviated EDS, EDX, or EDAX) is an analysis tool used to determine the elemental composition of a sample.
- EDAX works by analysing the spectrum of emitted X-rays from a sample as a beam of high energy electrons is incident upon its surface.
- By comparing the emitted X-ray photon energies to expected values from elements one may determine which elements are present in a particular sample, and in what ratio.
- Its characterization capabilities are due in large part to the fundamental principle that each element has a unique atomic structure allowing x-rays that are characteristic of an element's atomic structure to be identified uniquely from each other.
- To stimulate the emission of characteristic X-rays from a specimen, a high energy beam of charged particles such as electrons or protons (as in PIXE), or a beam of X-rays, is focused into the sample being studied.
- At rest, an atom within the sample contains ground state (or unexcited) electrons in discrete energy levels or electron shells bound to the nucleus.
- The incident beam may excite an electron in an inner shell, ejecting it from the shell while creating an electron hole where the electron was.
- An electron from an outer, higher-energy shell then fills the hole, and the difference in energy between the higher-energy shell and the lower energy shell may be released in the form of an X-ray.
- The number and energy of the X-rays emitted from a specimen can be measured by an energy dispersive spectrometer.

As the energy of the X-rays is characteristic of the difference in energy between the two shells, and of the atomic structure of the element from which they

were emitted, this allows the elemental composition of the specimen to be measured.(8)

ICP-OES Analysis

Inductively coupled plasma/optical emission spectroscopy (ICP/OES) is a powerful tool for the determination of metals in a variety of different sample materials.(9)

Results

Table 1: Showing Organoleptic Parameters For *Rasa Bhasma*

Taste	Tasteless
Colour	Grey
Touch	Smooth
Smell	No smell
Appearance	Amorphous

Table 2: Showing Solubility Results

	Rasa Bhasma
Water	Insoluble
Alcohol	Insoluble
Chloroform	Sparingly soluble

Table 3: Showing the Results of Inorganic Elements

	Rasa Bhasma
Calcium	Absent
Magnesium	Absent
Sodium	Present
Potassium	Absent
Chloride	Present
Sulphates	Present
Carbonates	Absent
Phosphates	Absent

Table 4: Physico-chemical analysis

S.No.	Parameter	***Rasa Bhasma***
1	Total Ash	88.10%
2	Acid insoluble ash:	83.03%
3	Loss on Drying at 110 C	0.675%
4	pH	8.74%

% of Mercury by ICP-OES Analysis was found to be 98.3% mg/kg.

Discussion

In the present study, *Rasa Bhasma* was evaluated with the aid of classical and modern analytical parameters. *Rasa Bhasma* is very well known for its Hepato-protective activity**.(10)** It is a Grey coloured fine powder possessing *Rekhapoornatwa* which indicates the fineness, capable of reaching minute pores of cells of the body when administered internally and *Nischandratwa gunas* indicating the absence of free metallic portion in it. If it is not free from metallic portion, it may become harmful to the body. Hence this test plays a major role in analysing the quality of *Bhasma*.

Physico-chemical analysis

- **pH-** The alkaline nature of *Rasa Bhasma* may be attributed to the *Teekshnata* and *Ushnata* of *Apamarga Kshara* used in the preparation of the same. The pH of *Rasa Bhasma* is 8.74, which is slightly alkaline & it is not irritant to gastric mucosa and was under limits.

- **Loss on Drying at 110⁰ C-** This Procedure determines the amount of volatile matter (i.e., water drying off from the drug).The least the moisture content better the standard of drug i.e., free from microbial contamination. *Rasa Bhasma* was having least value on loss on drying which indicates that it is not prone for microbial contamination.

- **Total Ash:** Ash value denotes the amount of inorganic substances present in the product. *Rasa Bhasma* has less ash value indicating good quality of drug.

- **Acid Insoluble Ash:** It is intended to provide a step towards the evaluation of physiological availability of drug. *Rasa Bhasma* having value 83.03 indicates better quantity of drug is absorbed in the acidic media of stomach.

- **Inorganic elements:** In the present study the drugs used are both herbal and mineral and *Shodhana Dravya* and *Kshara* contain many inorganic elements in it. To know the fate of these inorganic elements after going through many therapeutic procedures like *Mardana, Prakshalana, Bhasmikarana* and test for inorganic elements was conducted. *Rasa Bhasma* was found to contain Sodium, Iron, Chloride, Sulphates commonly in it. These 4 elements are also present in the body in large quantity indicates its vital role in human body.

- **Sodium:** Strongly affects distribution of water through osmosis and is a part of bicarbonate buffer system. Functions in nerve and muscle action potential conduction. This may help in maintaining disturbed fluid balance in renal failure.

- **Iron:** Component of haemoglobin reversibly binds O_2. Component of cytochromes involved in electron transport chain. It may help in relieving anaemia due to renal failure.

- **Chloride:** Plays vital role in acid base balance of blood, water balance, and formation of HCl in stomach. It may help in maintaining blood volume which is disturbed in renal failure.

- **Sulphates:** It is component of hormones and vitamins and many proteins and regulates many body activities. It may help in nutrition of body. It means that the elements needed for therapeutic or health aspect was preserved in desired quantity, in the end product after undergoing through different therapeutic procedures.

SEM/EDAX Analysis:

Fig.1 Image and Spectrum at 2000 resolution

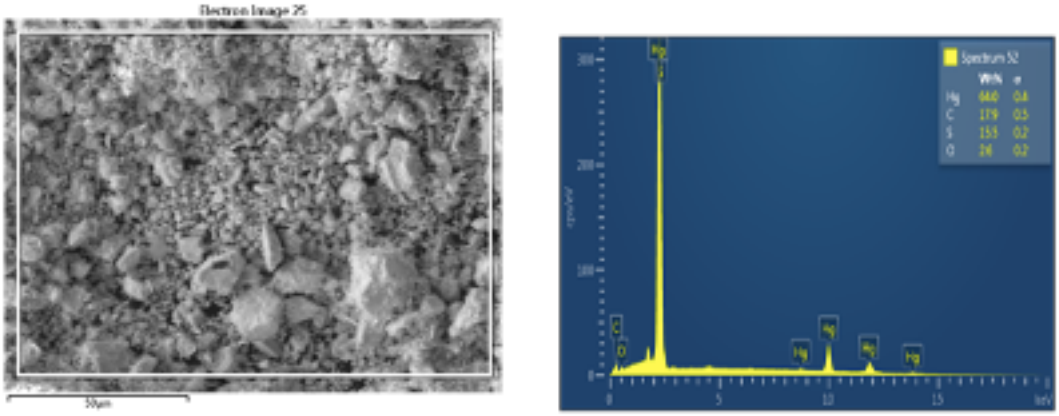

Fig. 2 Image and Resolution at 5000 resolution

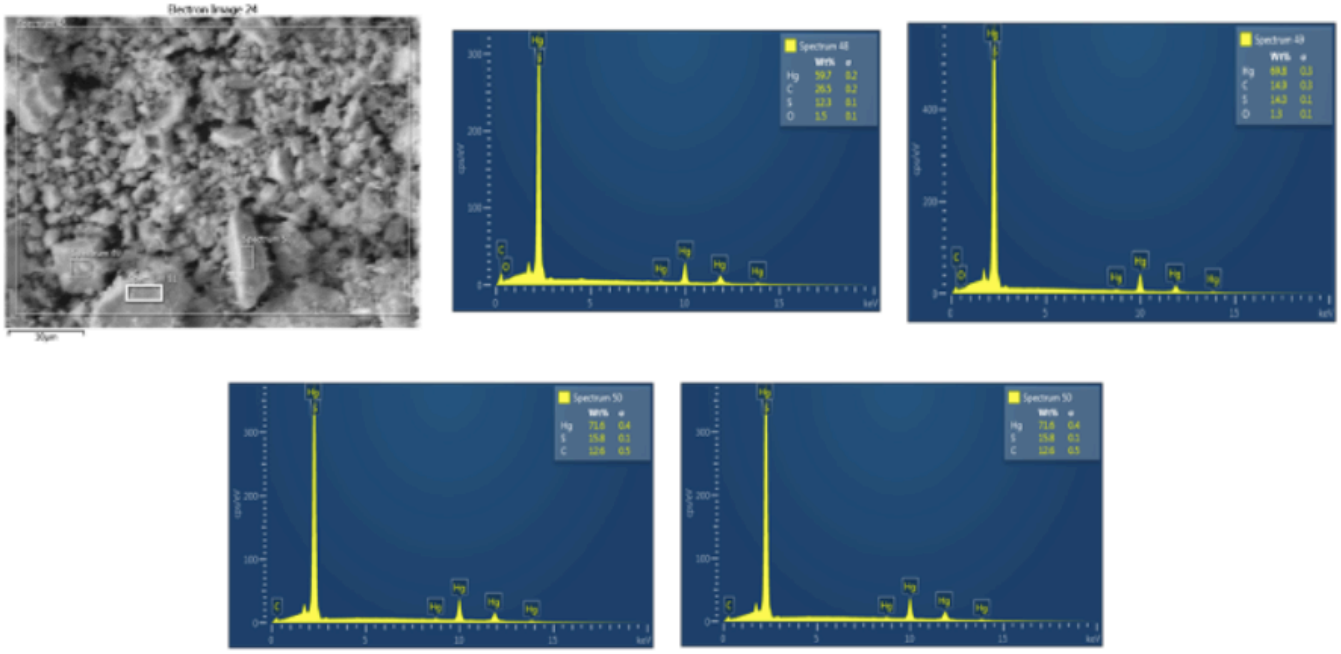

Fig. 3 Image and Spectrum at 10,000 resolutions

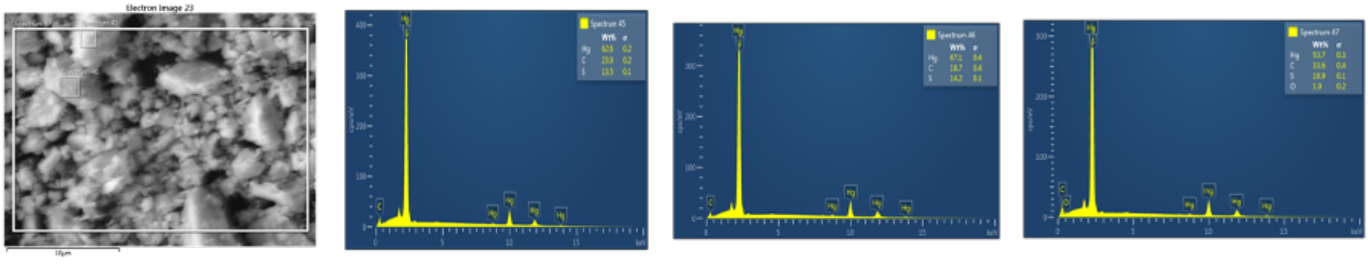

Fig. 1, 2 and 3 show the results of Mercury, Sulphur, Carbon, Oxygen, in various spectrums. The % of Mercury is maximum i.e., between 65-75% each. Sulphur varies from 25-30%. Carbon is rich in quantity varying from 25-30%. In all the Spectrums, Mercury is associated with Sulphur, forming a major peak and small Mercurial peaks are also seen along with small peaks of carbon. Mercury being associated with Sulphur is suggestive of combination of organic sulphide present in Mercury which could have been made its presence during *Parada shodhana* with *Lashuna*. Organic Sulphur present in *Lashuna*, binds to Mercury with the formation Mercuric Sulphide HgS. Hence the major peak of Mercury is associated with Sulphur demitting Mercuric Sulphide HgS. Carbon is the other element next to Mercury was found in the product, could be the result of combined organic compound during *Puta* procedure as *Bhringaraja Swarasa* was added. Oxygen is the other element found which could be the result of oxidative reaction during *Puta* process. It is also evident

by the elemental analysis of *Rasa Bhasma* where Sulphur is present in the form of sulphate.

Conclusion

Physico-chemical parameters of *Rasa Bhasma* were found to be within normal limits. SEM/EDAX spectra show the results of Mercury, Sulphur, Carbon, Oxygen, in various spectrums. In all the Spectrums, Mercury is associated with Sulphur, forming a major peak and small Mercurial peaks are also seen along with small peaks of carbon. 98.3% mg/kg Mercury was found to be present in the *Rasa Bhasma* by ICP-OES analysis. Thus we can conclude that prepared *Rasa Bhasma* is of standard quality.

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