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Research Article

ANALYTICAL STUDY OF *PANCHSHARA RASA* THROUGH XRD, SEM, EDX AND ZP

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ABSTRACT

Rasa Shastra is a branch of medicine, which deals with preparation of the drugs with metals and minerals having wide range of therapeutic efficacy, possessing innate qualities like quick action, low dose, tastelessness, prolonged shelf life and better palatability. *Panchshara Rasa* (PSR) is one such *Rasoushadhi* mentioned in *Bhaishajya Ratnavali,* indicated in *Shukrakshaya*. *Parada*, *Gandhaka* and *Shalmali moola* (*swarasa*) are the main ingredients of PSR. *Shodhana*, *Mardana*, *Bhavana* and *Parpati nirmana* are the important steps involved in preparation of *Panchshara Rasa*. PSR is the blend of *Kharaliya* and *Parpati Rasayana*. In the current study of PSR was analyzed through X-ray diffraction (XRD), Scanning electron microscope (SEM), Energy dispersive X-ray analysis (EDX) and Zeta potential (ZP). XRD of PSR reveals that major peaks are of HgS (Meta cinnabar) compound. SEM study found the smallest grain size in ranging between 467.8 nm to 547.2nm at 7K magnification. EDX study reveals that PSR contains Hg-48.06%, S-38.42%, O-13.52% w/w. PSR shows ZP mean value is -37.4 mV which indicates moderate colloidal stability. The detailed Analytical study (i.e. XRD, SEM, EDX and ZP) of *Panchshara Rasa* will be discussed in the full paper.

KEYWORDS: *Panchshara Rasa,* X-ray diffraction, Scanning electron microscope, Energy dispersive X-ray analysis, Zeta potential.

INTRODUCTION

Ayurvedic drugs are time tested for their efficacy and need no validation for their administration to patients. But, in the present scientific era there is change in the mind set of patients. Safety of the drug to be administered is at par with its efficacy. Analytical study is mandatory to check the raw samples, intermediary products and final product. The presence of free metal or particles of large size in any formulation can lead to damage of vital organs of the body. 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 *Panchshara Rasa* an important *Rasoushadhi* through X-ray diffraction, Scanning electron microscopy, Energy dispersive X-ray analysis and Zeta potential.

Pharmaceutical Process

The pharmaceutical processes involved in the preparation of *Panchshara Rasa* are *Shodhana, Mardana, Bhavana* and *Parpati nirmana*. *Shodhana* is done for *Parada* (Mercury) and *Gandhaka* (Sulphur).[1,2] *Shalmali moola swarasa* was prepared.[3] *Mardana* with *Shalmali moola swarasa* of *Parada* was done for 21 days. *Bhavana* of *Gandhaka* with *Shalmali moola swarasa* was done for 21 days. *Kajjali* was prepared with *Mardita Parada* and *Bhavita Gandhaka. Parpati* was prepared with *Kajjali. Parpati churna* was subjected to *Bhavana* for 21 days with *Shalmali moola swarasa* to obtain *Panchshara Rasa*.[4]

After completion of the pharmaceutical process, the final drug (PSR) was subjected to analysis through X-Ray Diffraction studies (XRD), Scanning Electron Microscope (SEM), Energy Dispersive X-Ray Analysis (EDX) and Zeta Potential (ZP).

MATERIALS AND METHODS

Double distilled Mercury and Crystals of Sulphur were obtained from local market of Tirupati, *Shalmali moola* was procured from TTD's S. S. Ayurvedic Pharmacy, Tirupati and were authenticated by studying its characters as explained in classical texts.[5-7] PSR was prepared in Department of Rasa Shastra, S.V. Ayurvedic College, Tirupati and TTD's S.S. Ayurvedic Pharmacy, Tirupati. Requirement for XRD: Model- Diffractometer system XRD-3003 TT, Manufacturer- General Electronics US. SEM and EDX: Model- EVO MA 15, Manufacturer- Carl Zeiss, Germany. ZP: Model- Malvern Zetasizer Nano, Manufacturer- Malvern Instruments, UK.

XRD

The final product (PSR) was subjected to XRD at Department of Nuclear Physics, University of Madras, Guindy Campus, Chennai.

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.[8]

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 1.540598 A° from copper source is passed on the sample. Detector was set to identify diffracted beams between 10-70 degrees of 2θ range. Obtained values are plotted on graph with the help of inbuilt "Reyflex Software" for further analysis.

SEM and EDX

The final product (PSR) 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.

Procedure

The dried powder was placed over the specimen holder and observed under the microscope at 1,000X 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).[9]

Procedure

Electron beam excitation is used in electron microscopes, 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.

ZP

The final product (PSR) was subjected to ZP at Department of Soil Science, Agriculture 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 toward 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 performing an electrophoresis experiment on the sample and measuring the velocity of the particles using Laser Doppler Velocimetry (LDV).[10]

Sample preparation

A 1% concentration of PSR 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.

OBSERVATION AND RESULTS

X-ray diffraction

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

S. No.	Element/Molecule	JCPDS Ref. No.	2θ	Intensity	FWHM
1.	HgS (Metacinnabar)	96-900-8849	26.43	1000.0	0.3200
		96-900-0005	31.07	68.7	0.3200
2.	S_8 (Sulphur)	96-900-8578	23.13	190.6	0.3200

Table 2: Showing Crystal details of JCPDS entries

Phase classification		
Name		
Mineral Name		Metacinnabar
Formula		HgS
I/Icor		26.980000
Sample Name		9008848
Quality		C (calculated)

Crystal structure		
Crystallographic data		
Space group		F -4 3 m (216)
Crystal system		Cubic
Cell parameters		a= 5.8517 Å

Phase classification		
Name		Sulphur
Mineral Name		Sulphur
Formula		S_8
I/Icor		2.200000
Sample Name		9008577
Quality		C (calculated)

Crystal structure		
Crystallographic data		
Space group		F d d d (70)
Crystal system		Orthorhombic
Cell parameters		a= 10.4670 Å b= 12.8700 Å c= 24.4930 Å

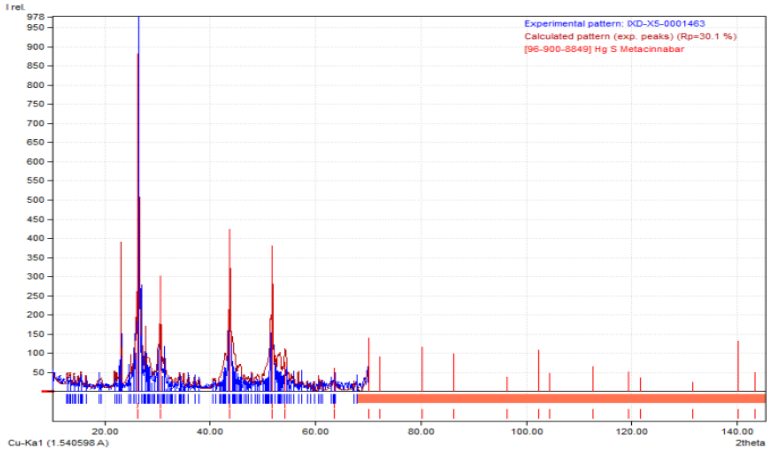

Fig. 1: Showing XRD report of PSR

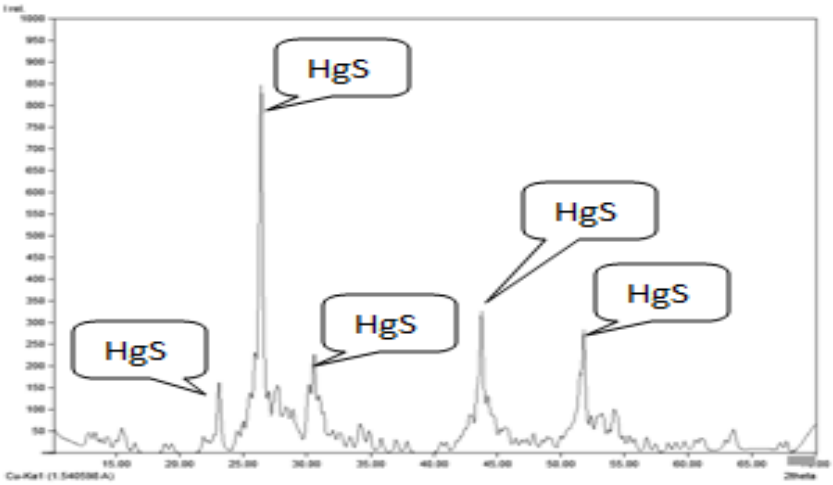

Fig. 2: Showing peaks of HgS

XRD of PSR shows that major peaks are of HgS (Meta Cinnabar) compound with cubic structure and minor peak is S_8 (Sulphur) with orthorhombic structure. The HgS peaks are detected at diffraction angle of 26.43 and 31.07. The JCPDS reference numbers are 96-900-8849 and 96-900-0005 respectively. S_8 (Sulphur) peak is detected at diffraction angle of 23.13 the JCPDS reference number is 96-900-8578.

Scanning electron microscope

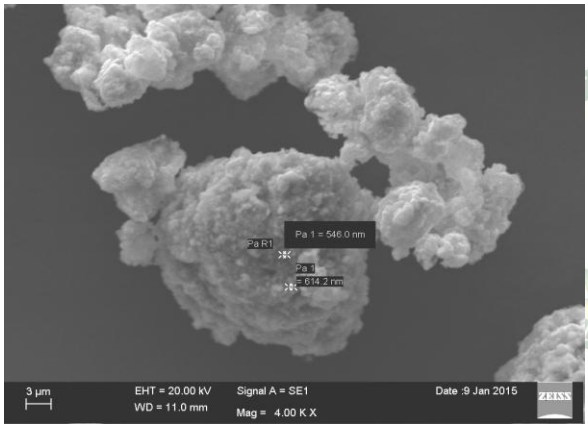

Fig. 3: Showing SEM report of PSR (4KX magnification)

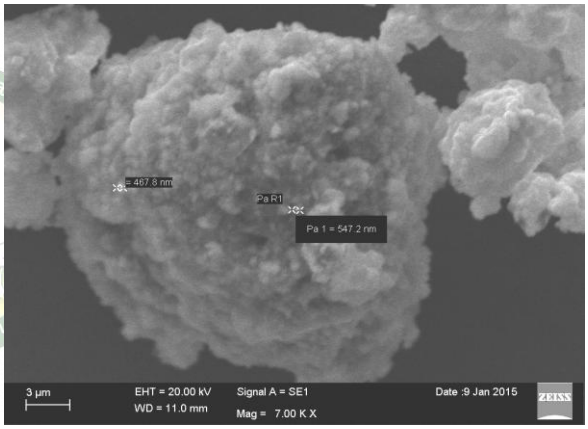

Fig. 4: Showing SEM report of PSR (7KX magnification)

The SEM images that were obtained from various regions of sample clearly depicts that at different magnifications the grain size was commonly found to be ranging between 546.0 to 614.2 nm at 4K magnification and 467.8 to 547.2 nm at 7K magnification.

Energy dispersive X-ray analysis

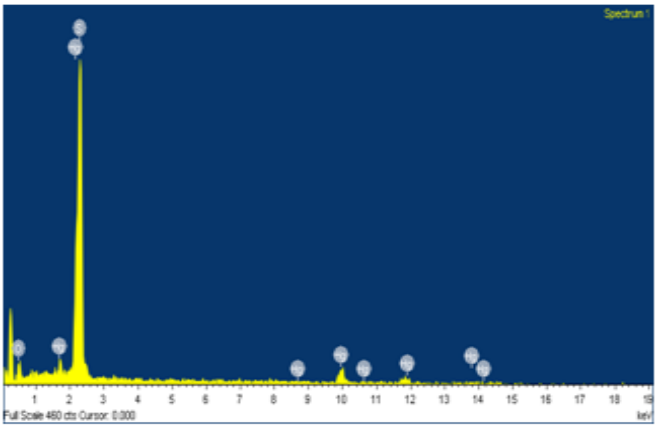

Fig 5: Showing EDX report of PSR

Element	Weight %	Atomic %
O K	13.52	37.02
S K	38.42	52.49
Hg K	48.06	10.50

EDX study reveals that PSR contains Hg-48.06%, S-38.42%, O-13.52% w/w.

Zeta Potential

Table 3: Showing ZP measurement report of PSR

Measurement Type	**Zeta Potential**
Sample Name	*Panchshara Rasa*
Temperature of the holder	25.0^{0}C
Viscosity of the dispersion medium	0.895 mPa·s
Conductivity	0.163 mS/cm
Electrode Voltage	3.4 V

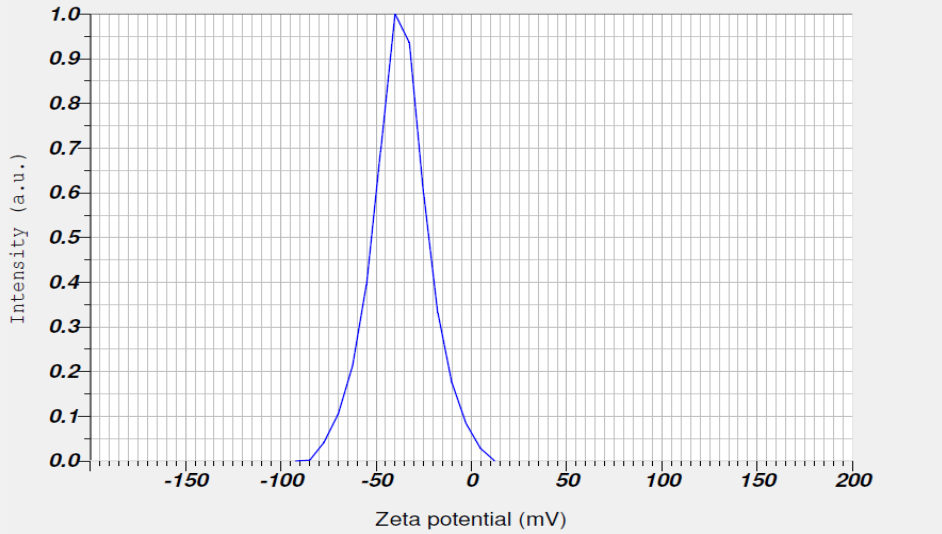

Fig. 6: Showing ZP distribution of PSR

PSR sample showed a Zeta potential (mean) value of -37.4 mV and electrophoretic mobility mean is -0.000290 cm^2/Vs.

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.

XRD has been in use in two main areas, for the fingerprint 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 are etc. 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. XRD of PSR shows that major peaks are of HgS (Meta Cinnabar) compound with cubic structure and minor peak is S_8 (Sulphur) with Orthorhombic structure. The major peaks formed were sharp due to crystalline nature of HgS. The other peaks like Sulphur formed were not sharp as those HgS.

SEM is an analytical technique that 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. It can magnify objects to extreme levels where even structure of nano particles could be clearly visible. Smallest particle size of PSR was found to be ranging between 546.0 nm at 4K magnification to 467.8 nm at 7K magnification. The size of the particle might have reduced because of trituration. Particles are found to be agglomerated due to presence of natural tannins which have partial binding nature. Smallest particle size proves that the drug can be easily absorbed in body and exhibit its therapeutic effect quickly due to its nano particle size.

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. The analysis of PSR confirmed the presence of elements viz. Hg, S and O with percentage as 48.06%, 38.42%, 13.52% respectively showing significant percentage of Mercury and Sulphur in the drug.

ZP 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 PSR found to be -37.4 mV which indicates moderate colloidal stability.

CONCLUSION

PSR was subjected to analysis with highly sensitive analyzers like XRD, SEM, EDS and ZP for checking its identity, crystalline structure, particle size, absorption power and stability. XRD analysis was carried out for PSR. It was clear in the report that major peaks are of Meta cinnabar (HgS) and minor peak is of S_8. In SEM analysis smallest grain size was commonly found in 546.0nm at 4K magnification and 467.8nm at 7K magnification. The size of the particle might have reduced because of trituration. It accounts for its bioavailability and efficacy. EDX report showed significant percentage of Hg, S and O i.e. 48.06%, 38.42%, 13.52%. The ZP (mean) value of PSR was found to be -37.4 mV.

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