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Research Article

STANDARDIZATION OF *YOGAAMRUTO RASA* BY USING MODERN ANALYSIS TECHNIQUES

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ABSTRACT

Rasa Shastra is a partially independent branch of Ayurvedic medicine, which deals with preparation of the drugs with metals and minerals to produce the drugs with higher efficacy in lower dose with good palatability. *Yogaamruto Rasa* (YMR) is one such *Rasoushadhi* mentioned in *Rasa Kamdhenu* indicated for all types of *Kushta*. *Parada, Gandhaka, Tamra churna, Vatsanabha, Vacha, Trikatu, Musta* and *Vidanga* are the main ingredients of YMR. *Shodhana, Mardana, Murchchana, Pishti nirmana, Aagni paaka* are the important steps involved in preparation of YMR. Till date no standards are available for the above drug. Need of the hour is to revalidate the safety and efficacy of the above said formulation. In the current study of YMR it was subjected to analysis through X-ray diffraction (XRD), Scanning electron microscope (SEM), Energy dispersive X-ray spectroscopy (EDX) and Zeta potential (ZP). XRD of YMR reveals that major peaks are of Cu_20 (Cuprite) and minor peaks of HgS (Meta Cinnabar), Cu_2S (Cuprous Sulphide). SEM study found the smallest grain size ranging between115nm at 5Kx magnification to 82.11 nm at 7Kx magnification. EDX study reveals that YMR contains significant percentage of O-25.96%, Cu-22.82%, C-20.41%, Hg- 12.84 %, S-9.8%. w/w. ZP mean value for YMR is 51.4 mV which indicates moderate colloidal stability.

KEYWORDS: *Yogaamruto rasa, Rasa Kamdhenu*, X-ray Diffraction, Scanning Electron Microscopy, Energy Dispersive X-ray Spectroscopy, 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 essential part of any thesis scientific work. 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 contra-indications of any formulation. 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 *Yogaamruto rasa* an important *Rasoushadhi* through X-ray diffraction, Scanning electron microscopy, Energy dispersive X-ray analysis, and Zeta potential.

Pharmaceutical process:

The pharmaceutical procedures adopted in this study are *Shodhana, Pishti nirmana (Murchchana)*[1], *Mardana and Agnipaka*. *Shodhana* is done for *Parada, Gandhaka, Tamra and Vatsanabha*[2,3,4,5]. *Pishti nirmana* was carried out by doing rigorous *Mardana* of *Shuddha Parada* and *Shuddha Tamra churna* with *Nimbu swarasa* till the accepted properties of *Pishti* were obtained. *Tamra dhatu pishti* obtained so was arranged amidst layers of *Shuddha Gandhaka* in an Iron vessel, *Katu taila* was poured over *Shuddha Gandhaka* and kept undisturbed until *Taila* was completely absorbed in *Gandhaka* layers. After that it was subjected to *Agni paaka* at low flame (*Alpa agni)* until a dome like structure over the *Pishti* was formed and gradually which got completely dried. *Patra* was taken out of the gas stove and kept for self cooling, after self cooling *Katu taila Gandhaka* layer formed like dome over and around the *Pishti* was removed and it was lifted up carefully as a whole from the bed of *Patra* and taken in *Khalwa yantra* for grinding. After making fine *Churna* of *Pishti* the remaining herbal drug Churnas of *Vatsanabha, Vacha, Trikatu, Musta and Vidanga* were added in appropriate amount as mentioned in reference of the drug to obtain *Yogaamruto rasa*[6].

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

MATERIALS AND METHODS

Double distilled Mercury and Sulphur were obtained from laboratory shop Tirupati. *Vatsanabha, Vacha, Trikatu, Musta, and Vidagna were* procured from TTD's S. S. Ayurvedic Pharmacy, Tirupati. YMR was prepared in Department of Rasa Shastra, S.V. Ayurvedic College, Tirupati. Requirement for XRD: Model- Power X-ray Diffractometer D8 advance, Manufacturer- Bruker Germany. SEM and EDX: Model- EVO MA 15, Manufacturer- Carl Zeiss, Germany. ZP: Model- Malvern Zeta sizer Nano, Manufacturer- Malvern Instruments, UK.

XRD

The final product (YMR) was subjected to XRD at Department of Nuclear Physics, Vellore Institute of Technology, Vellore.

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. [7]

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 2θrange. Obtained values are plotted on graph with the help of inbuilt "Reyflex Software" for further analysis.

SEM and EDX

The final product (YMR) 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 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). [8]

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.

Zeta Potential

The final product (TMR) 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 Zeta sizer 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). [9]

Sample preparation

A 1% concentration of YMR 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

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

S.No	Element/Molecule	JCPDS Ref.No	2θ	Intensity	FWHM
1.	Cu_2O (Cuprite)	96-101-0927	29.48	1000	.2297
2.	HgS (Metacinnabar)	96-101-1369	30.53	138.9	.2997
3.	Cu_2S (Cuprous Sulphide)	96-152-9747	29.05	222.3	.2297

Table 2: Showing Crystal details of JCPDS series

Phase classification		
Name		Copper(I) oxide
Mineral Name		Cuprite
Formula		Cu_2O
I/Ic		11.010000
Sample Name		1010926
Quality		C (calculated)
Crystal structure		
Crystallographic data		
Space group		P n -3 m (224)
Crystal system		Cubic
Cell parameters		a= 4.2520 Å
Z		2

Phase classification		
Name		Mercury sulfide
Mineral Name		Metacinnabar
Formula		HgS
I/Ic		26.969999
Sample Name		1011368
Quality		C (calculated)

Crystal structure		
Crystallographic data		
Space group		F -4 3 m (216)
Crystal system		Cubic
Cell parameters		a= 5.8580 Å
Z		4

Phase Classification		
Name		Cu2 S
Formula		Cu_2S
I/Ic		2.240000
Sample Name		1529746
Quality		C (calculated)
Crystal structure		

Crystallographic data		
Space group		P 63/m m c (194)
Crystal system		hexagonal
Cell parameters		a= 3.9590 Å c= 6.7840 Å
Z		2

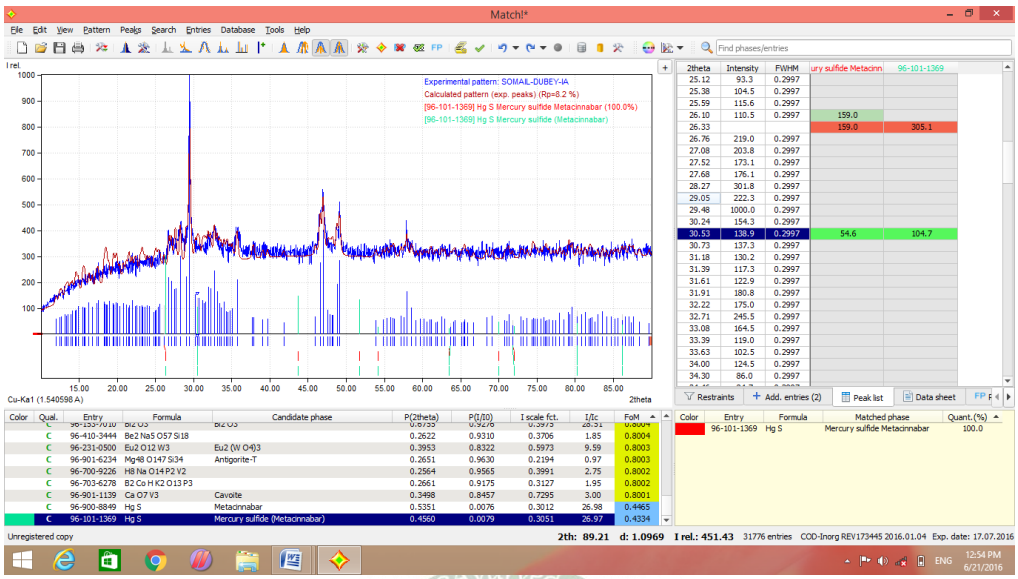

Fig. No. 1: Showing XRD report of YMR

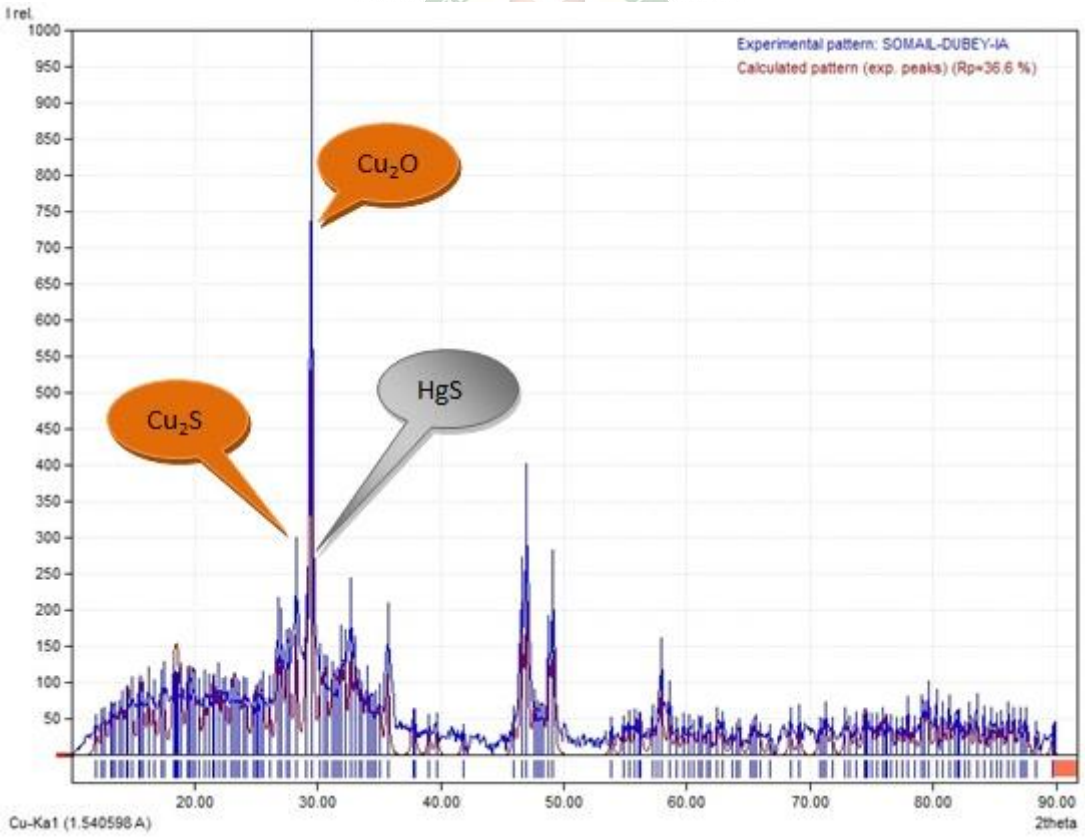

Fig. No. 2: Showing XRD peaks of YMR

XRD of *Yogaamruto rasa* shows major peaks of Cu_2O (Cuprite) with cubic structure and minor peaks of HgS (Meta Cinnabar), Cu_2S (Cuprous Sulphide) compound with cubic and hexagonal structures respectively. The Cu_2O peak was detected at diffraction angle of 29.48 the JCPDS reference numbers is 96-101-0927, HgS peak was detected at diffraction angle of 30.53 the JCPDS reference numbers is 96-901-1369, Cu_2S peak was detected at diffraction angle of 29.05 the JCPDS reference numbers is 96-152-9747.

Scanning Electron Microscope

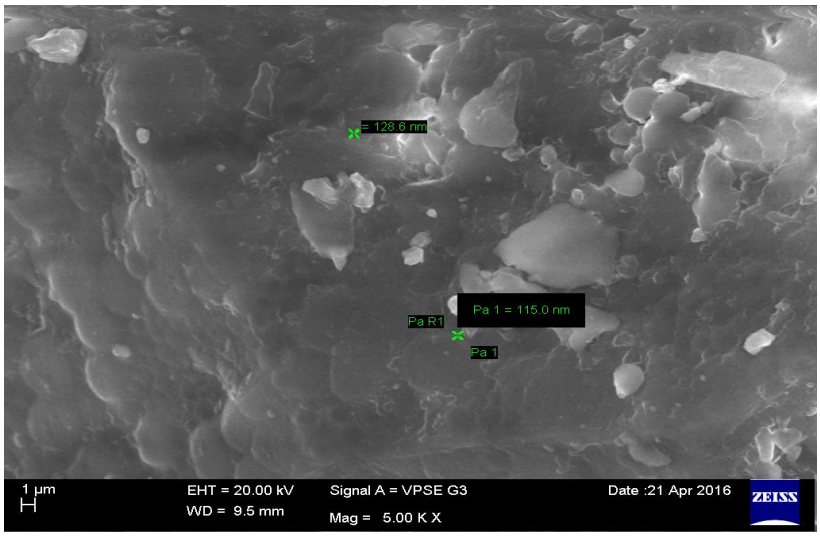

Fig. No. 3: Showing SEM Report of YMR at 5Kx Magnification

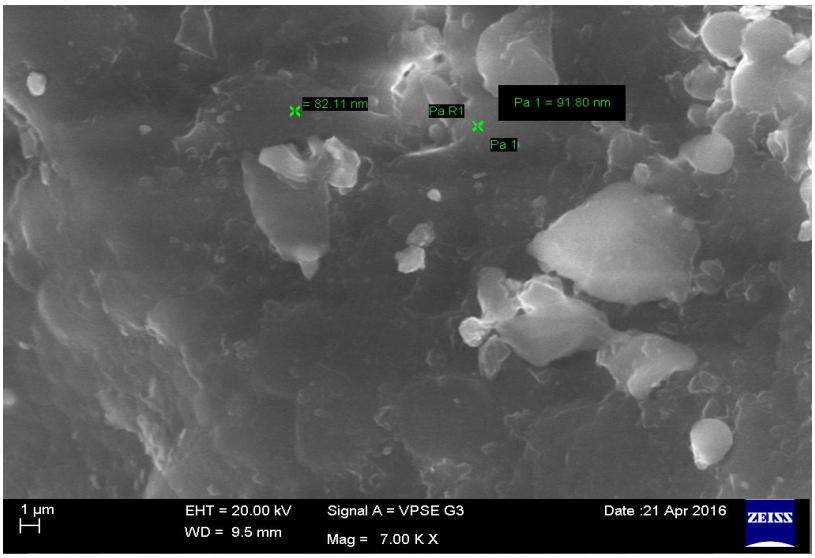

Fig. No. 4: Showing SEM Report of YMR at 7Kx Magnification

Smallest particle size was found to be ranging between **115.0 nm at 5Kx** magnification to **82.11 nm at 7Kx** magnification.

Energy Dispersive X-Ray Spectroscopy Analysis (EDX)

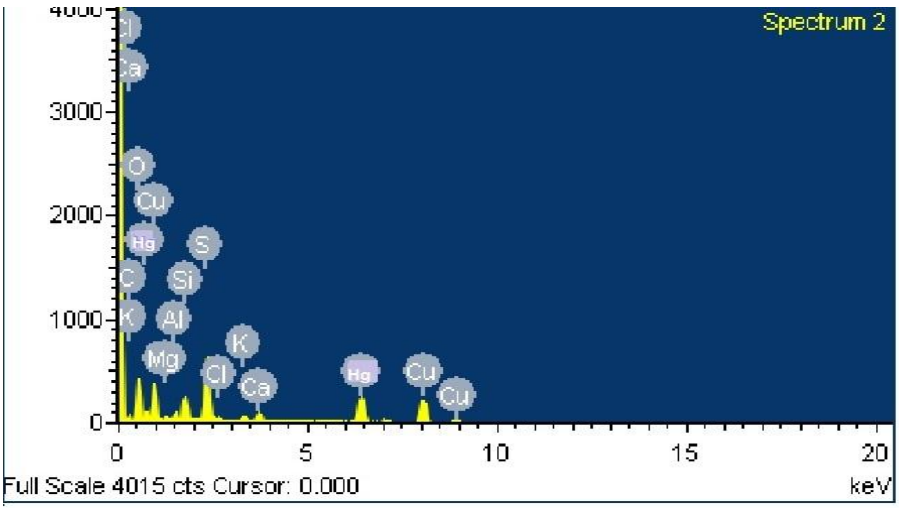

Fig. No. 5: Showing EDX report of YMR

Element	Weight%
C K	20.41
O K	25.96
Mg K	1.27
Si K	3.72
S K	9.80
Cl K	0.58
K K	0.89
Ca K	1.70
Hg K	12.84
Cu K	22.82
Totals	100.00

EDX study reveals that *Yogaamruto rasa* has significant percentage of O-25.96%, Cu-22.82%, C-20.41%, Hg- 12.84 %, S-9.8% w/w.

Zeta Potential Analysis (ZP)

Table 3: Showing Zeta potential measurement result of YMR

Measurement Type	Zeta Potential
Sample Name	*Yogaamruto rasa*
Temperature of the holder	25.0^{0}C
Viscosity of the dispersion medium	0.895 mPa·s
Conductivity	0.135 mS/cm
Electrode Voltage	3.4 V

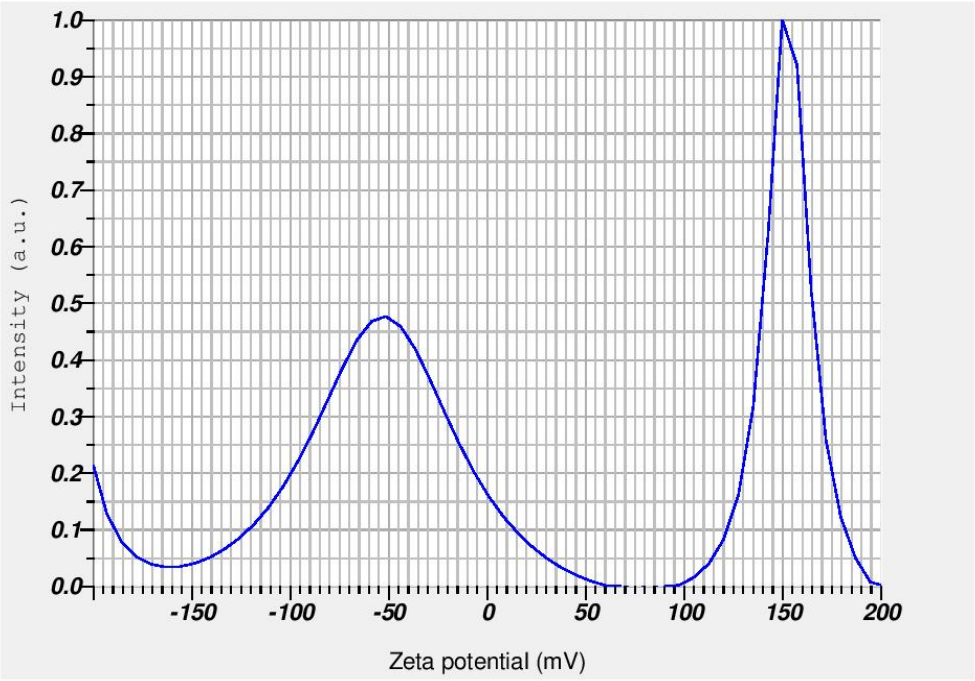

Fig. No. 6: Showing ZP distribution of YMR

Yogaamruto rasa sample showed a Zeta potential value of 51.4 mV and Electrophoretic Mobility mean 0.000398 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 YMR shows that major peaks are of Cu_2O (Cuprite) with cubic structure and minor peaks of HgS (Meta Cinnabar), Cu_2S (Cuprous Sulphide) compound with cubic and hexagonal structures respectively. The major peaks formed were sharp due to crystalline nature of Cu_2O.

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 was found to be ranging between 115.0 nm at 5Kx magnification to 82.11 nm at 7Kx magnification. Small size of particles was again attributed by fine *Pishti* and use of very fine powder of herbal drugs which were prepared by using sophisticated instruments like hammer crusher and swifter machine. Smaller size of particles attribute to better Pharmokinetic action of drug i.e drug absorption, assimilation and distribution in body.

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. EDX study reveals that *Yogaamruto rasa* has significant percentage of O-25.96%, Cu-22.82%, C-20.41%, Hg- 12.84 %, S- 9.8% w/w.

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 YMR found to be 51.4 mV which indicates moderate colloidal stability.

CONCLUSION

YMR 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. XRD analysis was carried out for YMR. It was clear in the report that major peaks are of Cu_2O (Cuprite) and minor peaks of HgS (Meta Cinnabar), Cu_2S (Cuprous Sulphide). In SEM smallest particle size was found to be ranging between 115.0 nm at 5Kx magnification to 82.11 nm at 7Kx magnification. EDX report showed significant percentage of significant percentage of O-25.96%, Cu-22.82%, C-20.41%, Hg- 12.84 %, S- 9.8% w/w. The ZP (mean) value of YMR was found to be 51.4 mV.

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