

SEM AND EDX ANALYSIS OF KASEESA BHASMA

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ABSTRACT

Rasashastra is a specialized branch of Ayurveda which deals with the pharmaceutics of its unique and potent preparations. *Bhasmas* (calx) are one among such preparations which are prepared after various *Samskaras* (processings) like *Shodhana* (purification), *Jarana* (roasting), *Marana* (incineration), *Amrutikarana* (nectarization) etc. They are said to be properly prepared if they pass certain *bhasma parikshas* (tests) enlisted in classical *Rasashastra* texts. Modern tools of analysis help to understand the *bhasmas* in a better way that we could know not only by naked eyes. It gives us a microscopic vision to understand its structure and components rather than only satisfying the modern scientific world. Hence the present study was carried out to evaluate the analysis of *Kaseesa bhasma* by carrying out SEM (Scanning Electron Microscopy) and EDX (Energy Dispersive Spectroscopy). SEM analysis showed the particle size ranging between 409.5 nm at 4Kx magnification and 327.5 nm at 10Kx magnification. EDX showed the presence of elements Fe-59.66%, O-33.89%, S-3.02%, K-1.80%, Ca- 1.63% w/w.

Keywords: *Kaseesa bhasma*, SEM (Scanning Electron Microscopy), EDX (Energy Dispersive Spectroscopy)

INTRODUCTION

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. *Kaseesa* (Ferrous Sulphate- $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$) is mentioned as one of the *Uparasa* by most of the *Ayurveda Rasa shastra* texts. It is available both naturally and prepared artificially. It is a green coloured crystalline compound also called as Green Vitriol. *Kaseesa* crystals of good quality were ob-

tained from laboratory supplies. It was subjected to *shodhana* by soaking it in *Bhrungaraja swarasa*¹ (*Eclipta alba*), after the *shodhana* purified *kaseesa* was subjected to *marana* (incineration) by triturating it with *Snuhipatra swarasa*² (juice of leaves of *Euphorbia nerifolia*). The fine red coloured *bhasma* obtained so was subjected to SEM and EDX analysis.

MATERIAL AND METHODS:

The process was carried out in two steps:

1. Pharmaceutical study
 2. Analytical study
- 1. Pharmaceutical process:** pharmaceutical process was also carried out in two steps:
- **Shodhana (purification)**
 - **Marana (Incineration)**

Pharmaceutical study:

Shodhana:

It was carried out by soaking *Kaseesa* with *Bhrungaraja swarasa (Eclipta Alba)* for one day until the juice was completely absorbed in *kaseesa*. After *shodhana* it was kept for drying and dried *kaseesa* powder was kept in an air tight container because of its hygroscopic nature.

Marana:

Marana was carried out by preparing fresh juice of *Snuhipatra* and triturating purified *kaseesa* by it, after the liquid was well absorbed in *kaseesa* it was subjected to *chakrikanirman* (pellets of coin shape). The *chakrikas* were kept in drier for drying after drying they were kept in between two *sarava* (earthen pots) and the joined was sealed with fuller's earth and kept for drying. After the seal was dried the *sarava* was kept in a pit. 40 cow dung cakes were arranged in two layers of 20 cakes each amidst these two layers *sarava* was kept. Later it was subjected to *puta* (incineration by providing heat through cow dung cakes). Whole process was repeated for 3 times until the *Nirmalatva* (absence of sour taste which is special test for *Kaseesa bhasma*) was obtained. On opening the

sarava fine red coloured *bhasma* of *kaseesa* was obtained.

Analytical study:

SEM and EDX

Kaseesabhasma 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 aluminium powder. 5) Spreading and vapour sputtering unit.

Procedure

The dried powder was placed over the specimen holder and observed under the microscope at 4Kx to 10Kx. Microphotographs were taken with the in built 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).³

Procedure

EVO MA 15 Carl- Zeiss, Germany model was used for SEM-EDX analysis. 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.

RESULTS:

- Immediately after adding *Bhrungaraja swarasa* in *kaseesa* dark green colour was obtained. But as the process got progressed the colour of *kaseesa* became light in colour and also it got light in weight.
- *Shodhita kaseesa* was subjected to *puta* for 3 times until the desired qualities of *bhasma* were not obtained.

Table no. 1: Showing the result of *Kaseesa shodhana*:

S. No.	Weight of <i>kaseesa</i> before <i>shodhana</i>	Weight of <i>kaseesa</i> after <i>shodhana</i>	Loss of weight	% age weight loss
1.	100 gm.	95 gm.	5 gm.	5%

Table no. 2: Showing the changes in *Kaseesa* during *marana* process:

S. No.	Colour	<i>Rekhapuranatva</i>	<i>Varitara</i>	<i>Gatarasatvam (Nirmalatva)</i>
1st Puta	Blackish Red	+ve	-ve	-ve
2 nd Puta	Dark Blackish red	+ve	+ve	-ve
3 rd Puta	Dark Red	+ve	+ve	+ve

Table no. 3: Showing the weight change during the *marana* process:

S. No.	Weight before <i>puta</i>	Weight after <i>puta</i>	Weight loss	% age weight loss
1st Puta	95 gm	60 gm	35 gm	37
2 nd Puta	60 gm	52.8 gm	7.2	12
3 rd Puta	52.8 gm	49.6	3.2	6



Fig. 1: *Ashuddha Kaseesa*



Fig.2: *Shuddha Kaseesa*



Fig. 3: *Bhavana of Snuhi patra*



Fig. 4: *Chakrikas of Kaseesa*

Swarasa in Shuddha Kaseesa



Fig. 5: *Puti of Kaseesa after sealing in sarava*



Fig. 6: *Kaseesa bhasma*

Scanning Electron Microscopy (SEM):

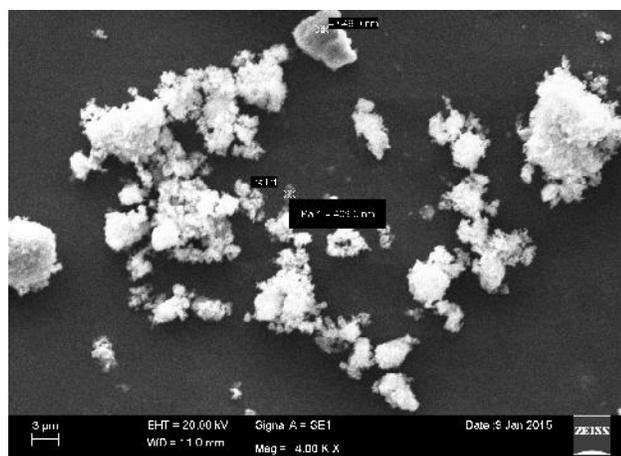


Fig. 7: Showing SEM report of *Kaseesa Bhasma* at 4 Kx magnifications.

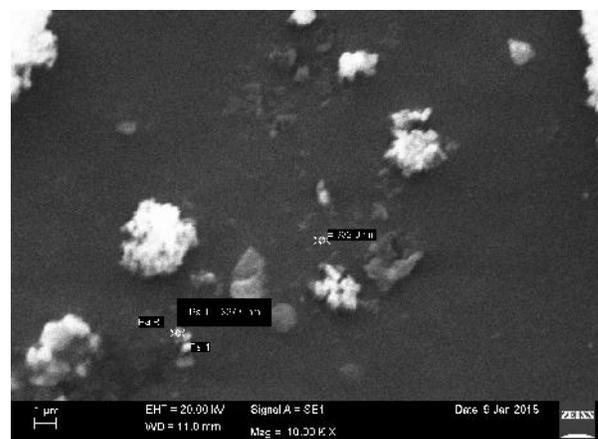


Fig. 8: Showing SEM report of *Kaseesa bhasma* at 10 Kx magnifications.

Energy Dispersive Spectroscopy (EDX):

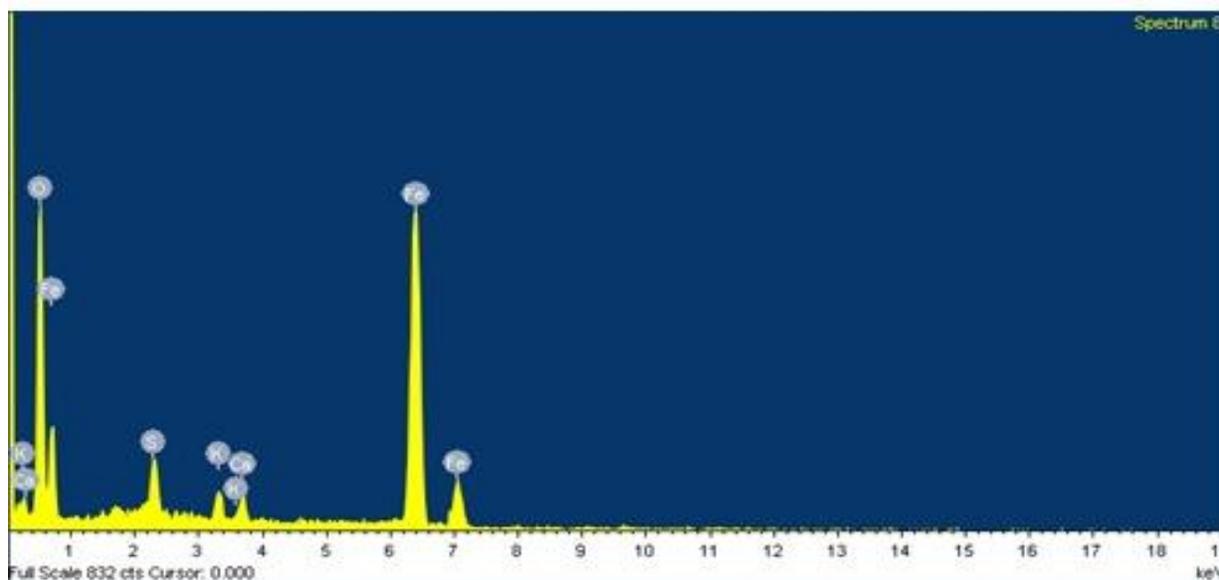


Fig. 9: Showing EDX report of *Kaseesa bhasma*:

Table no. 4: Showing elemental percentage of *Kaseesa bhasma*:

S. No.	Element	Weight%
1.	O K	33.89
2.	S K	3.02
3.	K K	1.80
4.	Ca K	1.63
5.	Fe K	59.66
6.	Totals	100.00

EDX report reveals that *kaseesa bhasma* has significant percentage of Fe-59.66%, O-33.89%, S-3.02%, K-1.80%, Ca- 1.63% w/w.

DISCUSSION

Kaseesa is mentioned as *Uparasa* in *Ayurveda Rasa shastra* text. *Kaseesa* is astringent, hot in potency, cold in quality. It blackens the hair, alleviates leucoderma, eye diseases, poisoning, cures diseases of *Vata- Sleshma*, dysuria, pruritus, anaemia, helminthiasis, fever and splenomegaly. It facilitates menstrual flow, provides strength and acts as astringent on external application⁴. Being having so much of uses in day to day clinical practice an attempt was made to prepare *kaseesa bhasma* by adopting simple method of *shodhana* and *marana* that can be practised even at small level.

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 us from misinterpretations of results obtained. 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. Here *kaseesa bhasma* analysis was done by using SEM and EDX.

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 409.5 nm at 4Kx magnification and 327.5 nm at 10Kx magnification for *kaseesa bhasma*. Small size of particles was again attributed by the triturating and *marana*. SEM is used to know particle size of any drug this gives information about the fineness of drug. Smaller the size of the particle in drug better is its absorption and pharmaceutical action.

EDX is used to know the elemental percentage in any drug. Particularly in case of *bhasma* it shows the authenticity of pharmaceutical process to know the major share of element that contributes to action of the drug. EDX report of *kaseesabhasma* reveals major percentage of Fe-59.66%, O-33.89%, S-3.02%, K-1.80%, Ca- 1.63% w/w. As the major elements present in *kaseesabhasma* is Iron so it can be known that the above pharmaceutical process was appropriate for making *kaseesabhasma*.

CONCLUSION

Kaseesa is an important *dravya* mentioned in *uparasavarga* in *ayurvedicRasa shastra* text finds its utility in most of the diseases. An attempt was made here to study its pharmaceutical process and then subjecting it to SEM and EDX analysis. SEM report of *kaseesabhasma* showed particle size to be ranging between 409.5 nm at 4Kx magnification and 327.5 nm at 10Kx magnification. EDX report showed major percentage of Fe-59.66%, O-33.89%, S-3.02%, K-1.80%, Ca- 1.63% w/w.

ACKNOWLEDGMENT:

Authors would like to acknowledge Dr. Sridhar Reddy, Professor, Department of Physics, S.V. University, Tirupati, and his Ph.D.

scholar Mrs. Ajeetha bonda for their cordial support and cooperation during the analytical study.

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Source of Support: Nil

Conflict Of Interest: None Declared

How to cite this URL: Dubey Somil : Sem And Edx Analysis Of Kaseesa Bhasma. International Ayurvedic Medical Journal {online} 2017 {cited March, 2017} Available from: http://www.iamj.in/posts/images/upload/693_699.pdf