



## Research Article

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### SOME OBSERVATIONS OF THE ZINC-METALS BASED PREPARATIONS AND ITS PROPERTIES IN AYURVEDA WITH REGARD TO STABILITY OF MEDICINES IN THE NANO STATE

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#### ABSTRACT

In Indian system of medicine, herbo-mineral/ metallic formulations are used since 2<sup>nd</sup> Century. However, the scarcity of complete scientific evaluation of their physico-chemical characterization, therapeutic efficacy and stability study is the major cause for creating hindrance in their acceptance at global level. It is a thinking of modern pharmaceutical scientist, that expiry date express on Ayurvedic medicine is arbitrary. This paper will briefly explore the physico-chemical properties of two different samples of Yashada Bhasma prepared at a time interval of 12 years. During different pharmaceutical process temperature was recorded with non-contact pyrometer. The presence of different elements was determined by Energy dispersive X-ray analysis (EDAX). The surface morphology as well as the nanoparticles nature was observed by scanning electron microscopy (SEM). The crystalline nature of Bhasma was assessed by powdered X-ray diffractometry. This study revealed that both the formulations have nearly same findings in different parameters considered for study and even after the passage of 12 years the Bhasma didn't show any significant changes.

**Keywords:** Indian system of Medicine, Bhasma, EDAX, XRD, SEM, Nanoparticle.

#### INTRODUCTION

Ayurveda is an intricate system of healing, which is the result of thousand years of observation and extensive research of clinical practitioners. Ayurvedic medicine are derived from all source of nature<sup>1</sup>, viz. plants (e.g. flower, fruit, bark, root and latex etc.) animals (e.g. Bone, Horn, Milk, Blood and hairs etc) and minerals (e.g. pure metals and their ores). Metals and minerals are generally converted into easily assailable form called Bhasma. Further, two interesting and wonderful features related to Bhasma are firstly, these are tasteless, convenient in fabrication, more patient's compliance and more efficacious in lower dose<sup>2</sup> with immediate action and other is that, this formulation is free from expiry value<sup>3</sup>. This fact is mentioned for the first time in 12<sup>th</sup> century by Acharya Sharangadhara in his famous book Sharangadhara Samhita in verse 54-56 of first chapter in the context of general principle of expiry of some commonly used dosage form. According to him, potency and efficacy of Asava (Alcoholic preparation), Dhatu (metallic) Bhasma, and Rasa (Mercurial preparation) will increase with the passage of time. Bhasmas are the formulation which is complex compounds forms of metals or minerals obtained by repeated incineration with liquid extracts. Yashada Bhasma is one such Bhasma which is indicated in diabetes, anemia, tuberculosis, cough, gynecological disorders and especially in night sweating<sup>4</sup>. Nanotechnology is the study of materials that measure from one to 100 nanometers (nm), with one

nanometer equal to one-billionth (1/1,000,000,000) of a meter i.e. 1 nm = 10<sup>-9</sup> m. When materials are measured in nanometers, they display extraordinary physical, chemical and electronic properties, opening up new research areas and leading to novel applications. When substances are transformed in nano size they lose their original properties e.g. lead and Mercury nano particles no longer remain toxic in nano state. Numerous studies on nano particles of gold, silver and copper has been reported according to their uses in diverse fields. The research in nanotechnology has shown signs of great potential in medicine and pharmaceutical industries. In life sciences also several nano techniques are in developmental stage wherein the model structure can be easily monitored on computer system to explain the development of novel drug and new drug delivery system. In the present paper stability of drugs in nano state was studied by the researcher. For the above studies the method of grinding and milling was performed for generation of nano particles. The Ayurvedic medicines are also prepared by milling and grinding for the last hundreds of years. Generally it is considered that in Ayurveda, herbo-mineral medicines have not known expiry dates. But the medicines, which are prepared in molecular stage (not in nano), are normally affected by higher atmospheric temperature and also by moisture in the air which causes binding or aggregation of molecules resulting into change of the properties of the medicines and hence there is an 'expiry' to such medicines. As part of the next stage of this research, investigator assessed the expired medicines

to see how they react once broken down to nano scale. The medicines thus transformed in to nano state, would be subjected to various characterization techniques.

## MATERIAL AND METHODS

Zinc metals used for the preparation of Bhasma was procured from local market. Bhasma formulation was prepared according to the procedures mentioned in Ayurvedic texts and the materials required in these preparations were also procured from local market.

### Yashada Bhasma preparation

The traditional method for Yashada Bhasma preparation is long and involves several steps. Yashada Bhasma used in our study was prepared by the following method. Traditional names of each step have been mentioned in parentheses. In the first step, about 3 kg of raw zinc was melted and quenched into four time of its quantity into banana rhizome juice. The procedure (Samanya Shodhana) was repeated seven times. Again partially coarse material obtained from this step was subjected to quench into quick lime (0.5 % w/v) solution. This course of action (Vishesh Shodhana) was performed seven times. In the second step, the product obtained from previous step was melted in iron pan at 450° C, while adding one fourth quantity of coarse powder of *Achyranthus aspera* slowly. This process known as, Jarana was continued for 12 h at 450° C and the whole material was allowed to self-cooling (Swangsita). In the third process washing (Dhavana) was done with water to remove the excess of alkalinity, which was added by the ash of *Achyranthus aspera*. In the last step, material was triturated (Bhavana) with desired amount i.e., 160 ml of fresh pulp of *Aloe vera* in mortar and pestle for 8 h, until the sufficient amount of liquid was evaporated to become semisolid mass. This dough like material was used to make the pellets of about 3.5 - 5.0 g and dried in direct sun. Completely dried pellets was placed inside an earthen pot and covered with equal size of another pot. These two pots were completely sealed with mud smear cloth pack and the entire assembly was called sarawasamputa. The sealed pots were kept in dry cowdung (uppala) in incineration chamber (Gaja puta) of dimension about 57 cm x 57 cm x 57 cm and allowed to burn for 24 h and during that operation the peak temperature reached upto 800 ± 40°C for about 1 h thereafter this temperature was decreased in steady pattern. Due to incineration the color of pellets was changed from metallic grey to pale yellow. These pellets were crushed to form fine powder. This whole process of trituration, pelletization, drying, incineration and powdering was performed for seven times and the obtained final powder was considered as Bhasma. The Bhasma preparation was coded as (YJK-7). Another

sample of the Yashada Bhasma prepared by same reference method (YMK-7) dated 12 years back was procured from renowned Ayurvedic pharmacy for comparison.

### Elemental Analysis with Energy Dispersive X-Ray Analysis (EDAX)

Qualitative determinations of bulk elemental composition in Bhasma samples were carried out by EDAX (Carl Zeiss AG supra 40 WDS) which was attached with Field emission Scanning Electron Microscope (FESEM). For FESEM analysis the sample was sprinkled over the carbon coated black tape and Gold solution was used for coating over it. The operating parameter were; 30 KV, count-rate 1,500 ± 500 counts/s, working distance 10 mm, chamber pressure set to < 2.2 x 10<sup>-4</sup> torr, tilt angle 0° and accumulation time 50 sec. A gaseous secondary electron detector (GSED) was used for the image formation. The relative elemental compositions of the Bhasma particles were computed directly with Norton system sin software.

### Powder X-Ray Diffraction (PXRD)

To determine the different crystalline phase present in the Bhasma preparation XRD studies was done. XRD pattern were obtained using an X'pert high Score Plus. Powdered samples were studied by a thin layer in conventional cavity mounts. The samples were scanned from (10-90°) 2θ. The Cu anode X-ray was operated at 40 kV and 30 mA to give monochromatic Cu Kα X-rays (λ = 1.54056 Å). The average crystalline size (L) of Bhasmas was calculated from XRD graph using the Debye-Scherrer formula-

$$L = \frac{k\lambda}{\beta \cdot \cos\theta}$$

Where λ is the X-ray wavelength in nanometer (nm), β is the peak width of the diffraction peak profile at half maximum height resulting from small crystallite size in radians and k is a constant related to crystallite shape, normally taken as 0.9

The Scherrer's equation predicts crystallite thickness if crystals are smaller than 1000 Å to 100 nm. The mean crystalline size was calculated after averaging the crystal size value from most intense peak of the XRD.

### Fourier Transmission Infrared Spectroscopy

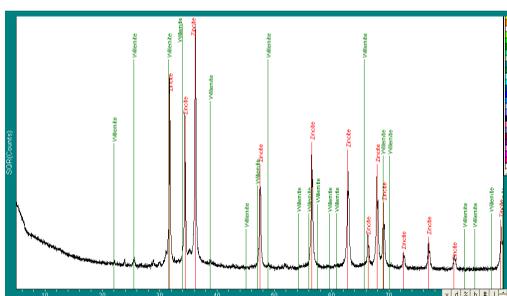
The FTIR Spectra were recorded on a Shimadzu FTIR spectroscopy (IR prestige 21, 200 VCE) in absorption mode with 50 scan range was fixed in the region between 400 to 4500 cm<sup>-1</sup>.

Table 1: Comparison of elemental composition of Bhasma detected by EDAX method

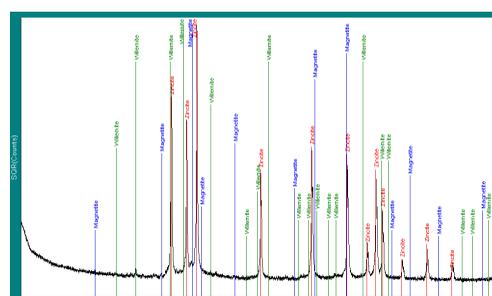
Elements	YJK7		YMK7	
	wt (%)	at (%)	wt (%)	at (%)
O	20.23	44.22	30	35.54
Al	0.24	0.31	0.15	0.61
Si	1.0	1.24	1.99	1.07
S	0.07	0.07	0.06	0.15
K	- 0.15	- 0.13	- 0.02	- 0.11
Sn	0.01	0.0	0.01	0.20
Ca	1.23	1.07	0.63	0.56
Fe	0.81	0.51	3.26	4.56
Cu	2.29	1.26	1.97	1.06
Zn	61.83	33.07	67.09	38.06

Table 2: Phases and crystal size of Bhasma

Formulation code	Compound identified		Mean crystal size (nm)	Crystallites size by modified Scherrer's equation	
	Major phase	Minor Phase		L (nm)	L (nm)
YJK7	ZnO	Zn <sub>2</sub> SiO <sub>4</sub>	46.46	48.92	70.08
YMK7	ZnO	Zn <sub>2</sub> SiO <sub>4</sub> + Fe <sub>3</sub> O <sub>4</sub>	52.61	52.12	79.79

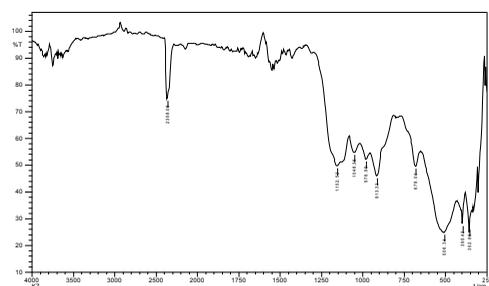


YJK-7: ZnO + Zn<sub>2</sub>SiO<sub>4</sub>-slight (Willemitite)

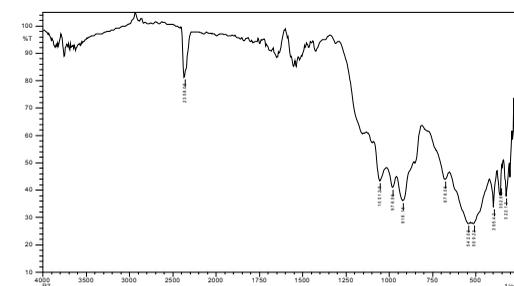


YJM-7: ZnO + Zn<sub>2</sub>SiO<sub>4</sub>(Wiemite)-Slight + Fe<sub>3</sub>O<sub>4</sub> (magnetite)- Slight

Figure 1: XRD spectra of Yashada Bhasma

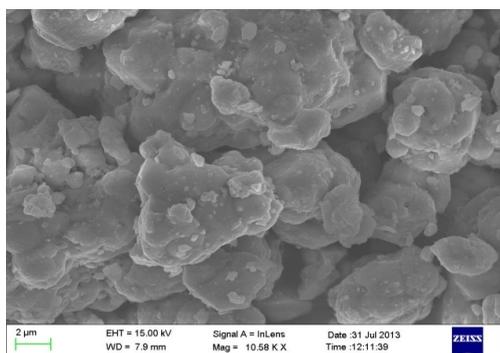


YJK7

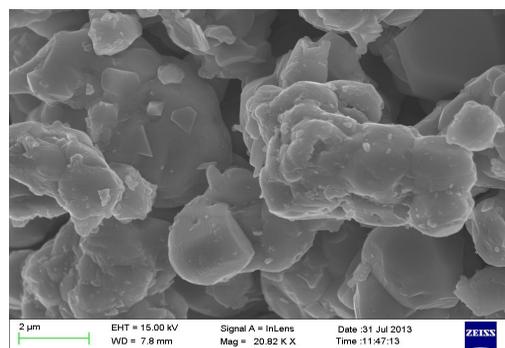


YMK7

Figure 2: FTIR study, whole FTIR analysis of both Yashada Bhasma samples



YJK7



YMK7

Figure 3: SEM micropicture of Yashada Bhasma Samples

## RESULTS AND DISCUSSION

### Chemical Composition: Analysis by EDAX

EDAX analysis shows the presence of following elements viz. O, Mg, Al, Si, S, K, Ca, Mn, Fe, Cu, Zn and Sn in the formulation, where Zinc (61-68 %) was present as major element followed by oxygen (20-30 wt %). As SEM coupled EDAX, microprobe analysis is semi quantitative, analysis of several spots was carried out, and finally an average value was considered. The average of different element analyzed in two samples of the formulation is shown in Table 1. The ratio of atomic weight percentage of Zinc and oxygen was found to be 1.86:1 and 1.76: 1 in sample YJK7 and YMK7 respectively. As the formulation showed number of trace elements, it can be inferred that if a definite amount of oxygen would bound to these trace element, the net oxygen available for Zinc would be even less than the recorded values. This strongly suggests that every atom of Zn was not bound to oxygen atom in ZnO molecule. Moreover, Zinc oxide is thermochromic<sup>5</sup>, as it changes its colour from white to yellow on heating. When the temperature is increased above 300°C, this feature would be seen in case of ZnO. It was due to the formation of crystal lattice defects due to loss of oxygen and the formation of the non-stoichiometric  $Zn_{1+x}$  with  $x$  increasing with the temperature<sup>6</sup>. Yashada Bhasmas present in market supplied by different pharmacies, shows different ranges of colours from off-white to dark yellow. So it may be concluded that material obtained after incineration of Zn was oxygen deficient. This highly oxygen deficient ZnO in medicinal preparation like Yashada Bhasma raises the possibility that oxygen deficient property may be one of the attributing factors impacting the medicinal value in it. Very little attention has been paid to the therapeutic application of oxygen deficient materials; recent findings have shown importance of the oxygen deficiency in the different diseases by lethal oxidative stress<sup>7</sup>.

### Phase Identification

Figure 1 shows the whole XRD plots of both Bhasma. The sharp intense peaks of ZnO confirm the good crystalline nature of ZnO and the peak originates from plane which reflects the formation of hexagonal ZnO. No peaks of Zn or other impurities were detected in the spectrum, revealing the phase purity of the products. The  $d$  values were computed and compared with standard  $d$  values of ZnO (JCPDS No. 036-1451) resulting in excellent matching for embedded samples. Formation of  $Zn_2SiO_4$  may be due to presence of Silicon found in pulp of *Aloe vera* juice.  $Fe_3O_4$  may be formed due to traces of iron that comes from environmental conditions, like processing vessels or storage container. But still presence of magnetite and willemite was very minute.

### Crystal Structure Determination with XRD

XRD pattern of Yashada Bhasmas is shown in Figure 1. Sample identification was done by matching  $d$  spacing with the standard JCPDS database. Results showed that ZnO is the major crystalline phase present in Yashada

Bhasma. Peaks at  $d = 2.47 \text{ \AA}$  ( $2\theta = 36.25^\circ$ ),  $2.81 \text{ \AA}$  ( $2\theta = 31.75^\circ$ ) and  $1.62 \text{ \AA}$  ( $2\theta = 56.58^\circ$ ), confirm the presence of ZnO (hexagonal) as the major crystalline phase in the sample. The mean crystal size of the Yashada Bhasma particles was found between 49 nm to 80 nm. To date, a great deal of attention has been focused on nanoparticles. As the particle size of ZnO decreases, its chemical reactivity, transparency, ultraviolet-filtering efficiency and dispersion properties also increases<sup>8</sup>. Nano size particles are attached with the cell surface and can diffuse readily inside the cells<sup>9</sup>. Thus, the size of particle is able to influence the efficacy as shown by others studies. Due to the extreme small size (49-80 nm) these particles possess medicinal properties in Bhasma Preparation. It can be possible that small particles (10-50 nm size range) are able to enter the cells by permeation, whereas larger size particles ( $> 100 \text{ nm}$ ) cannot permeate inside the cells easily<sup>10</sup>. One of the critical factors for toxicity is the size of nanoparticles conversion of nanoparticle into micron particle to decreases its toxicity.<sup>11-13</sup> Some studies also indicated that ZnO nanoparticles show antibacterial activity apparently greater than as shown by microparticles<sup>14</sup>.

### FTIR

The comparative FTIR spectral analysis of both Yashada Bhasma has been shown in Figure 3 Spectra of both the samples showed similar pattern of bending and stretching.  $V_{\max}$  around  $3500-4000 \text{ cm}^{-1}$  showed the presence of water molecule in all spectra. Due to absorption of peak  $V_{\max}$  around  $900-1250 \text{ cm}^{-1}$  showed the presence of  $(SiO_4)_n$ . The bands obtained in the range between  $478 \text{ cm}^{-1}$  to  $685 \text{ cm}^{-1}$  corresponds to the stretching of Zn-O-Zn. An important intrinsic property of ZnO is the presence of abundant, stable surface hydroxyl functional groups. This surface property is reported to result in less agglomeration of the particles<sup>15</sup>. Figure 2 shows the pattern of FTIR spectra of different samples.

### Morphology analysis by SEM

Particle shape and size analysis of Yashada Bhasma samples was performed by SEM. The photomicrograph of bulk particle showed a wide distribution of size in the preparation. It was observed that the particles were smooth, spherical and present in aggregates. Figure 3 shows micro picture of samples.

### CONCLUSION

Although Bhasma are complex materials, we have tried to show that physico-chemical analysis is useful to correlate the possible mode of action on biological model. Zinc is the major element present, next to oxygen. These formulations are highly oxygen deficient in nature. In a crystal, only half of the total zinc atoms bound to oxygen atom. It contains hexagonal ZnO in major phase and  $Zn_2SiO_4$  in minor phase in both preparations. ZnO exist in hexagonal Wurtzite crystalline form. This crystalline form is the most stable form of ZnO among three forms viz. Wurtzite, rock salt and zinc blende. This could be possible

due to different pharmaceutical steps that were performed during Bhasma preparation and this may most likely be the cause of having no expiry of this traditional nanomedicine. The particle size distributed in both preparations was 50-80 nm. A significant number of particles were present in the material within the nanometer range of 10- 25 nm. The hydroxyl functional groups attached to the ZnO nanomaterials has been successfully investigated by FTIR analysis. Sophisticated analysis of the sample procured from pharmacy, which was manufactured nearly 12 years back, showed similar findings that is the clear cut indication for non-expiry of Ayurvedic Bhasma preparation. So it may be strongly concluded that application of different unit operation may be responsible to add up the medicinal value and prolongs the shelf life of nano-particle of ZnO which might be due to smooth and spherical morphology, high stability of molecule, antimicrobial property and oxygen deficient nature.

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