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Comparative Pharmaceutico-Analytical Study of Classical, Yashada Bhasma and Potentiated Yashada Bhasma

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Abstract:
Bhasma in Ayurveda are nano-sized particles prepared using unique methods of incineration that are reported for increased bio-availability and therapeutic outcome, when such methods are aided with techniques to further initiate size-reduction it may give rise to therapeutically modified and potent *bhasma* than the ones prepared as per classical lines. Hence the present study attempts to curate *Potentiated Yashada Bhasma* (PYB) by altering few processing methods to the existing incineration methods of *Yashada Bhasma* (CYB) mentioned in classics. Following general and specific purification techniques, PYB was prepared by subjection of ball milling and wet grinding for specified duration as additional steps to the existing method of preparation of CYB. Both the samples prepared passed all classical tests of *bhasma*. The physico-chemical changes revealed changes in total ash, acid insoluble ash and loss on drying. Instrumental techniques using DLS detected particle size of PYB (261.1nm) much lower than CYB (696.3nm). The stability of PYB in Zeta Potential (-28mV) was found satisfactory, thus indicating proper preparation of PYB. The study suggests the effectiveness of potentiation techniques that could be adopted logically to the existing classical methods to considerably reduce operational costs and achieve better therapeutic outcomes in lower doses than required.

Key words: *Yashada, Bhasmeekarana,* Nano-particles, Zinc oxide, Potentiation**,** DLS

INTRODUCTION:

Ayurvedic Pharmaceutics deals with conversion of various raw materials like minerals, metals into bioassimiable therapeutically active compounds called as *bhasmas*.[1] These have been applied as stable formulations ranging in submicronic to nano dimension, exhibiting high bioavailability at lower dose for therapeutic treatments of various diseases.[2] The pharmaceutical processes involved in synthesis of *bhasma* are different for different metals or minerals. Variability of these procedures depends completely upon physical properties, chemical properties of metals and minerals and their targeted therapeutic action.

Nanotechnology is one among the novel area of research in modern material science.[3] Nanoparticles are frequently synthesized by either top–down or bottom–up approaches. Top– down approach is based on the mechanical methods of size reduction by breaking down the bulk materials gradually to nanosized structures. Bottom–up approaches are based on the assembly of atoms or molecules to molecular structure in nanoscale range.[4] Using these approaches a number of processes are available for the biosynthesis of nanoparticles for, e.g., reduction in solution, radiation aspired,

electrochemical, and microwave-assisted process and recently via green chemistry route.[5] Though the similarity in approach of synthesis, all the engineered nanoparticles are not meant for human use the way *bhasma* preparations were meant due to their safety and stability issues.[6]

Yashada bhasma (zinc calyx) is one such preparation consists of various elements which are essential micronutrients responsible for various bodily functions. *Yashada bhasma* (Zinc calyx) is prepared by a series of pharmaceutical processing [7] of metallic *yashada* into submicronic to nano size range zinc oxide, suitable for therapeutic administration.[8] This emphasizes the classical concept of size reduction and potentiation. Based on this *yashada bhasma* can be further potentiated by additional size reduction methods in existing pharmaceutical processing steps in presence of herbal extracts by grinding and milling for its improved therapeutic effect. A classical method of *bhasma* preparation by *bhasmikarana* (incineration) process, which is in practice, is proved safer and efficacious. In *puti lohas* (low melting point metals) like *yashada*, *jarana* (roasting) is an additional procedure between *shodhana* (purification) and *marana* (incineration), for easy *bhasmikarana*. This can be further fine-tuned with additional dry milling to attain lesser particle size and that sample can be further taken to classical method of *bhasmikarana* by grinding and incineration. These additional size reduction methods based on classical concept are better methods to potentiate them further by characterizing them in nano range. It is well known that size reduction of particles increases solubility and hence bioavailability. Therefore, thorough *bhasmikarana* with added size reduction procedures are expected to reduce the size of metal oxide particles enhancing their bioavailability and bioactivity. Interestingly, in modern science, several researchers have demonstrated enhanced bioavailability of nanoparticles as compared to their bulk form. For example, Ishihara et al. reported higher bioavailability of micronized zinc oxide as compared to standard zinc oxide.[9] In another report, poor water-soluble iron compounds when formulated as nanoparticles displayed oral bioavailability similar to soluble salts.[10]

Thus, it is safe to assume that *bhasma* (calyx) contain nanoparticles that lead to enhanced bioactivity. Thus, the study is aimed to potentiate *yashada bhasma* (zinc calyx) by adopting further size reduction techniques addition to classical methods and comparatively evaluate their particle size and various physico chemical properties using advanced instrumental techniques.

MATERIALS AND METHODS:

Materials:

The raw material *Yashada* (raw zinc) has been sourced from Dhootapapeshwar Pvt. Ltd (batch no. 1PRML00278/Jun/2021) with certificate of analysis having purity of 99.62% w/w. Additional ingredients, including *Parada* (Mercury), *Gandhaka* (Sulphur), *Tilataila* (Gingelly oil), *Kanji* (sour gruel), *Kulattha* (Horse gram) seeds, *Sudha Churna* (Limestone), and *Apamarga* (*Achyranthes aspera* Linn.) Panchanga *Churna*, were obtained from the GMP-certified KLE Ayurveda Pharmacy in Khasbag. *Takra* (Buttermilk) was prepared using milk purchased from a local cowshed and *gomutra* (cow's urine) was also collected from a local cowshed. *Kumari* (Aloe vera) was procured from the natural habitat in Belagavi. All the drugs were authentified by the experts and physico chemical analysis of raw drugs was done in the AYUSH Approved Drug Testing Laboratory at Shri BMK Ayurveda Mahavidyalaya. The instrumental characterizations were done in KLE college of Pharmacy, Nanowatts Bangalore and IIT Dharwad and IIT Bombay.

Methods:

Pharmaceutical part: This has been initiated with *samanya shodhana* (general purification) of 1kg of raw *yashada* (zinc). The raw *yashada* was placed in a *loha darvi* (iron ladle) and melted completely. The molten zinc was poured into *kanji* (sour gruel) using *pithara yantra* (Special instrument). After this, the zinc was removed, washed in warm water, dried, weighed, and pH of media was checked. This process was repeated two more times according to the traditional texts. The same purification method was followed with other media, such as, *takra* (buttermilk), *kulattha kwatha* (horse gram decoction) *gomutra* (cow urine) and *tilataila* (sesame oil) in each three times. Following this, *visesha shodhana* (special purification) was carried out using *churnodaka* (limewater) as the medium, repeating the process seven times in the same manner as for *samanya shodhana*.[7]

Jarana (Roasting) was then performed on the purified *yashada*. This was done by adding one-fourth the weight of *apamarga panchanga churna* (*Achyranthes aspera* Linn) to the purified *yashada* in a wide-mouthed iron vessel. The *churna* was gradually added to molten state of zinc and the mixture was triturated using an iron ladle. The mixture when

turns to complete powder form, it was then made a heap in the center of iron vessel and covered with a *sharava* (earthen plate) and intense heat was given until at the edges of *sharava* look *jwalaangara nibham* (red hot). After this stage was reached, the fire was extinguished, and the vessel was allowed to cool naturally. The next day, after self-cooling, the contents were removed from the iron vessel and sieved to obtain a fine powder of *jarita yashada*. This *jarita yashada* was mixed with sufficient quantity of water and washed repeatedly to remove excess alkalinity by checking pH each time and it was dried to powder and used for further processing.[7] After this *jarana,* the procedure was divided into two steps as: preparation of classical *yashada bhasma* and preparation of potentiated *yashada Bhasma*

Preparation of Classical *yashada bhasma* (CYB):

To prepare *Classical yashada bhasma*, the *jarita yashada* was then weighed and placed in the center of the *khalva yantra* (grinding apparatus), where it was triturated with 1/4th quantity of *shuddha parada* (purified mercury). *shuddha gandhaka* (purified sulfur) to prepare *kajjali* (black sulfide mixture). After obtaining *kajjali siddha laxanas*, *bhavana* (wet grinding) was carried out with *kumari swarasa* (Aloe vera juice) and *nimbu swarasa* (lemon juice) until the mixture reached the proper consistency for forming *chakrikas* (small discs). These *chakrikas* approximately resembling the size of *kuppilu beeja* (3 to 5 grams), were shaped and left to dry. Once, completely dried, the *chakrikas* were arranged between two *sharavas* (earthen plates), sealed with seven layers of *kapad mitti* (cloth coated with mud), and dried in sunlight. After thorough drying, the *samputa* was subjected to *puta* (incineration) in an electric muffle furnace at 950°C by maintaining peak temperature for 15 mins. After this furnace was made off and left for self-cooling. This procedure was repeated one more times, adhering to the guidelines of *bhasma siddhi lakshanas*[11] Observations done during the preparation are tabulated in Table 1-3.

Preparation of Potentiated *Yashada bhasma* (PYB):

The same method was followed in the preparation of Potentiated *yashada bhasma*, with additional size reduction in processing with modern instruments (Ball mill and Wet grinder). The potentiated *yashada bhasma* was prepared by additional size reduction at three different steps of classical *bhasmikarana* and increased number of *puta* (Total 4) even after attainment of *bhasma siddha laxanas* after second *puta*. Observations are tabulated in Table 4-6.The interventional steps are: dry milling of *jarita yashada* using ball mill for 24 hours, dry milling of PYB *kajjali* for additional 48 hours and thorough and continuous wet grinding in wet grinder for 24 hours in each *puta* before palettization.

Analytical Part: Both the samples were subjected to classical and modern instrumental analysis.

***Bhasma pareeksha* (Classical parameters):** The classical evaluation parameters like *varna* (color), *rekhapurna* (fineness), *varitara/unama* (lightness), *nischandra* (lusterless), *niruttha* and *apunarbhava* (absence of free metal) helps to judge the *bhasma siddha laxana* (completion of calcination process).

Physico chemical analysis: To ascertain the quality of *bhasma*, based on performed procedure of incineration and check any contamination including moisture during and after the preparation of *bhasma*, basic physical tests like loss on drying, ash value, acid insoluble ash were carried out.

Instrumental Characterization: To evaluate the end products (CYB, PYB) with special reference to particle size, distribution, stability, morphology, compound formed, shape and elemental composition various instrumental analysis were done like ICPAES, DLS- Zeta potential, XRD and SEM.

OBSERVATIONS:

Table 01. Ingredients and their quantity in each *puta* of CYB

Sl. No.	*Puta*	Ingredients	Quantity
1.	1st *Puta*	*Jarita yashada*	200 gm
		Shuddha Parada	50 gm

		Shuddha Gandhaka	50 gm
2.	2nd *Puta*	1st *Puta Bhasma*	150 gm
		Shuddha Parada	37.5 gm
		Shuddha Gandhaka	37.5 gm

Table 02. *Bhavana dravya* quantity and weight of *chakrika* at each incineration of CYB

Sl. No	Date	Quantity of *bhavana dravya*		Hours of *bhavana*			Weight of *chakrika* in *bhasmikarana*	
		Kumari	***Nimbu***	***Kumari***	***Nimbu***	**Total**	**Before**	**After**
1.	1st *puta*	150 ml	150 ml	8 hrs	6 hrs	15 hrs	330 gm	150 gm
2.	2nd *puta*	100 ml	70 ml	3 hrs	3 hrs	6 hrs	270 gm	160 gm

Table 03. Observations during each incineration of CYB

Sl. No	Puta	Observations & Results
1.	**1st** *puta*	All the *chakrikas* completely baked and turned off white colour. The *chakrikas* were soft to touch and were breaking with slight difficulty. After powedring of *chakrikas*, the *bhasma* was smooth in texture. *Varitara* was not passed completely but partially passed. *Rekhapurnata* was appreciated.
2.	2nd *puta*	All the *chakrikas* were completely baked and turned into light yellow colour. The *chakrikas* softer than the 1st *puta* and can be easily broken. The texture of the *chakrikas* was soft like butter while touching. *Varitara was* completely passed. *Niruttha* and *apunarbhava* test were also passed.

Table 04. Ingredients and their quantity in each incineration of PYB

Sl. No.	***Puta***	Ingredients	Quantity
1.	1st *Puta*	*Jarita yashada*	200gm
		Shuddha Parada	50gm
		Shuddha Gandhaka	50gm
2.	2nd *Puta*	1st *Puta Bhasma*	140 gm
		Shuddha Parada	35 gm
		Shuddha Gandhaka	35 gm
3.	3rd *Puta*	2nd *Puta Bhasma*	60 gm
		Shuddha Parada	15 gm
		Shuddha Gandhaka	15 gm
4.	4th *Puta*	3rd *Puta Bhasma*	37 gm
		Shuddha Parada	9.3 gm
		Shuddha Gandhaka	9.3 gm

***Note:** Quantity of *bhasma* obtained at each *puta* was not fully taken for next *puta*, decided as per the quantity of *Sh. Parada*.

Table 05. *Bhavana dravya* quantity and weight of *chakrika* at each PYB *puta*

Sl. No	Date	Quantity of *Bhavana dravya*		Hours of *bhavana*			Weight of *Chakrika* in *Bhasmikarana*	
		Kumari	***Nimbu***	***Kumari***	***Nimbu***	**Total**	**Before**	**After**

3.	1st *puta*	800ml	250ml	15hrs	9hrs	24hrs	323gm	196gm
4.	2nd *puta*	600 ml	200 ml	15hrs	9hrs	24hrs	208 gm	116 gm
5.	3rd *puta*	500 ml	170 ml	15hrs	9hrs	24hrs	87 gm	43 gm
6.	4th *puta*	200 ml	80 ml	15hrs	9hrs	24hrs	53 gm	25 gm

Table 06. Observations during each incineration

Sl. No.	*Puta*	Observations & Results
1.	**1st *Puta***	All the *chakrikas* completely baked and turned off white colour. The *chakrikas* were soft to touch and were breaking with slight difficulty. After powedring of *chakrikas*, the *bhasma* was smooth in texture. *Varitara* was not passed completely but partially passed. *Rekhapurnata* was appreciated.
2.	**2nd** *puta*	All the *chakrikas* completely baked and turned into yellow colour. The *chakrikas* were soft to touch and were breaking with slight difficulty. After powedring of *chakrikas*, the *bhasma* was smooth in texture. The colour of the *bhasma* faded when compared to the 1st *puta.* *Varitara was* passed. *Rekhapurnata* was appreciated.
3.	**3rd** *puta*	All the *chakrikas* were completely baked and turned into yellow colour. The *chakrikas* softer than the 2nd puta and can be easily broken. The texture of the *chakrikas* was very soft like butter while touching. The colour of the *bhasma* still more faded when compared to the 2nd *puta* *Varitara* completely passed. Except *niruttha* and *apunarbhava* rest all *bhasma pariksha* were done.
4.	**4th** puta	All the *chakrikas* completely baked and turned into yellow colour. The *chakrikas* softer than the 3rd *puta*. The texture of the chakrikas was softer like butter while touching when compared to 3rd *puta*. The colour of the *bhasma* still more faded when compared to the 3rd *puta* *apunarbhava* and *niruttha* were also passed.

RESULTS:

Bhasma Pareeksha

Classical *yashada bhasma* took 2 *puta* whereas Potentiated *yashada bhasma* was given 4 *puta* even after attaining *bhasma siddha laxanas* after 2nd *puta* to get additional size reduction. The observational results of classical *bhasma* parameters and organoleptic characters after each successive *puta* of both the samples are enlisted in Table 7.

Table 07. Organoleptic characters and *bhasma pareeksha*

Parameters	CYB		PYB			
	1st *puta*	2nd *puta*	1st *puta*	2nd *puta*	3rd *puta*	4th *puta*
Colour	Off white	Light yellow	Off white	Light yellow	Light yellow	Pale yellow
Taste	Tasteless	Tasteless	Tasteless	Tasteless	Tasteless	Tasteless
Touch	Soft	Softer	Soft	Softer	Very soft	Very soft
Appearance	Powder	Fine powder	Powder	Fine powder	Amorphous	Amorphous
Rekhapurnatva	Positive	Positive	Positive	Positive	Positive	Positive
Varitara	Partially positive	Positive	Partially positive	Partially positive	Positive	Positive
Unama	Partially positive	Positive	Partially positive	Positive	Positive	Positive
Nishchandrata	Positive	Positive	Positive	Positive	Positive	Positive
Niruttha	Not done	Positive	Not done	Not done	Not done	Positive

Apunarbhava	Not done	Positive	Not done	Not done	Not done	Positive

Physical Characterization

Physical parameters of both the sample are comparable with slight differences in total ash, acid insoluble ash and loss on drying. Results shows increased organic matter in PYB sample as depicted in Table 8.

Table 08. Physical parameters

Sl.No.	Physicochemical analysis	CYB	PYB
1.	Total ash	98.7%	96.2%
2.	Acid insoluble ash	52.331 %	55.24%
3.	Loss on drying	0.09 %	0.29%

Instrumental characterization

ICPAES: Elemental Analysis of *Yashada* at different steps of processing was done to analyze the percentage of major element zinc and results are depicted in Table 9. Results show that there is subsequent decrease in zinc percentage from raw *yashada* to potentiated *yashada bhasma*.

Table 09. ICPAES Results of raw, intermediate and final samples

Sl. No	***Yashada* samples**	**% of zinc**
1.	Raw *yashada*	99.8%
2.	*Shodita yashada*	92.9%
3.	*Jarita yashada*	82.2%
4.	Classical *yashada bhasma*	77.13%
5.	Potentiated *yashada bhasma*	70.29%

Particle size

Particle size analysis of CYB and PYB samples done using dynamic light scattering (DLS) method by particle size analyser (Malvern Zetasizer) are as shown in Table 10 and Fig 1 & Fig 2. Particle size findings suggest that particle size of PYB was reduced two and half time than CYB. Polydispersibility index of PYB is 0.412 which suggest uniform particle size distribution contributing to more homogeneity compared to CYB with PDI 1 which shows slightly greater heterogeneity.

Zeta potential

In present study both the samples exhibited negative zeta potentials as shown in Table 10 and Fig 3 & Fig 4, indicating that both samples are negatively charged and show good colloidal stability. The difference is very minimal. Both the samples are expected to remain well dispersed and demonstrate strong electrostatic repulsion, preventing aggregation on suspension.

Table 10. Particle size and Zeta potential Analysis

Samples	**Particle Size(nm)**	**PDI**	**Zeta potential (mV)**
CYB	696.3	1	-28.88
PYB	261.1	0.412	-28.20

***PDI- Polydispersibility index**

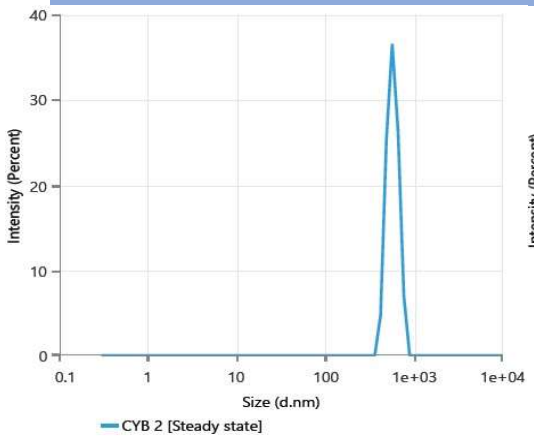

Fig.1 Particle size of CYB

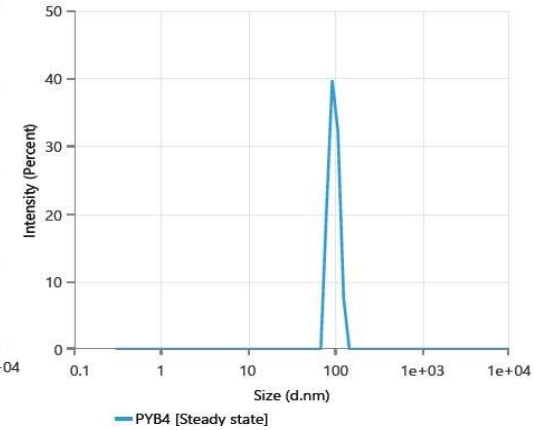

Fig. 2 Particle size of PYB

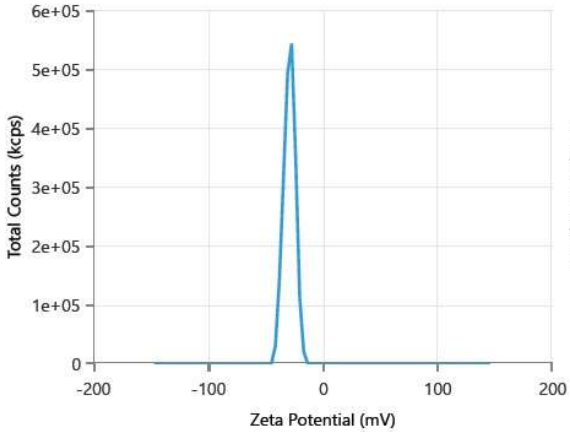

Fig. 3. Zeta potential of CYB

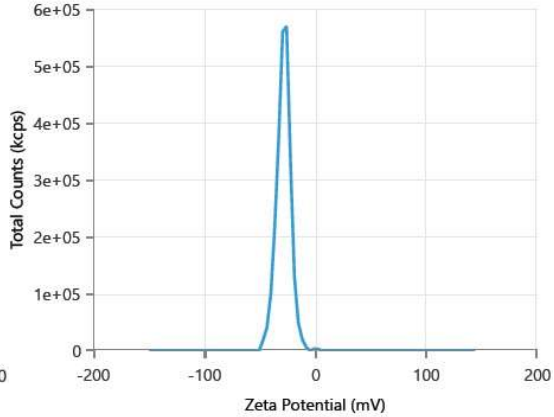

Fig. 4. Zeta potential of PYB

X-Ray Diffraction

X-ray diffraction study (XRD) of both samples done using Rigaku smart Lab S 200 confirms that they contain ZnO with wurtzite phase and the entire diffraction peaks are in agreement with the standard JCPDS data (card No. 36-1451) as shown in Fig. 5 & 6.

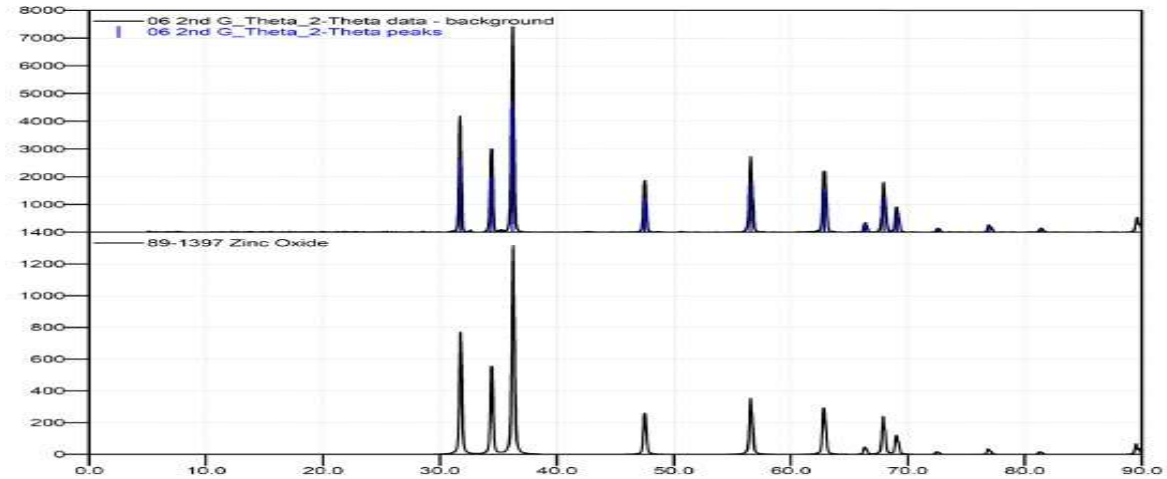

Fig 5. XRD of CYB

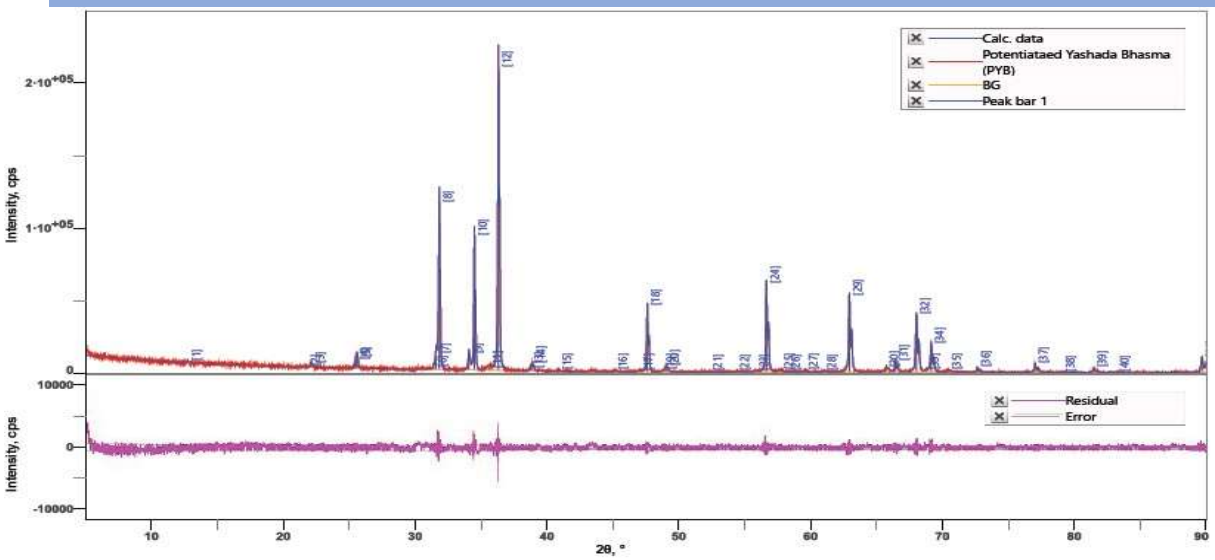

Fig 6. XRD of PYB

SEM analysis:

SEM analysis revealed that the shapes of particles of *bhasma* of both samples were majorly rectangle, spherical and hexagonal shapes. Compared to CYB in PYB the hexagonal wuzurite structure can be well appreciated. The particles in CYB sample are heterogeneous and agglomerated ranged between 200 to 702 nm and in PYB, particles are homogenous and well dispersed ranged between 20nm – 263nm as depicted in Fig.7 & 8.

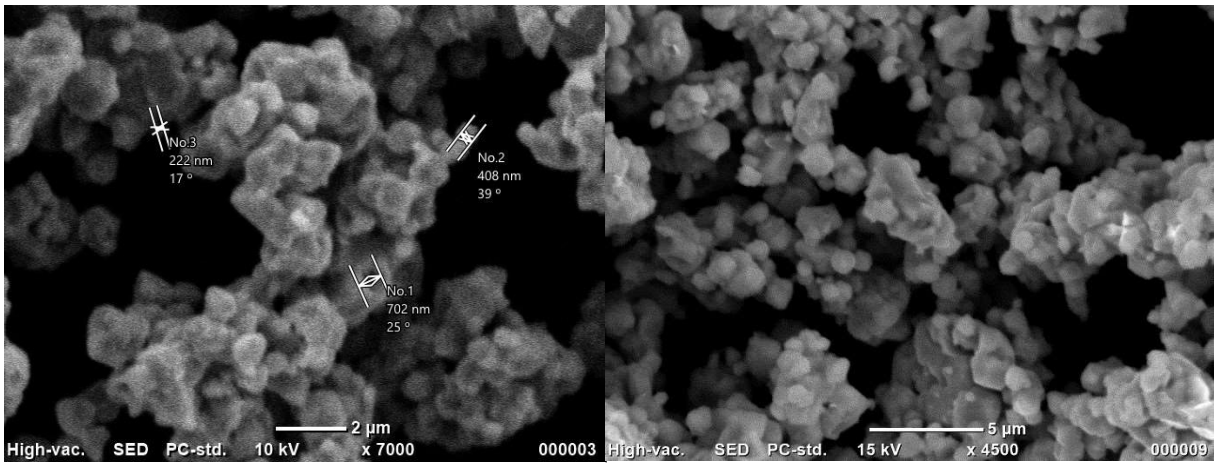

Fig. 7. SEM micrograph of CYB

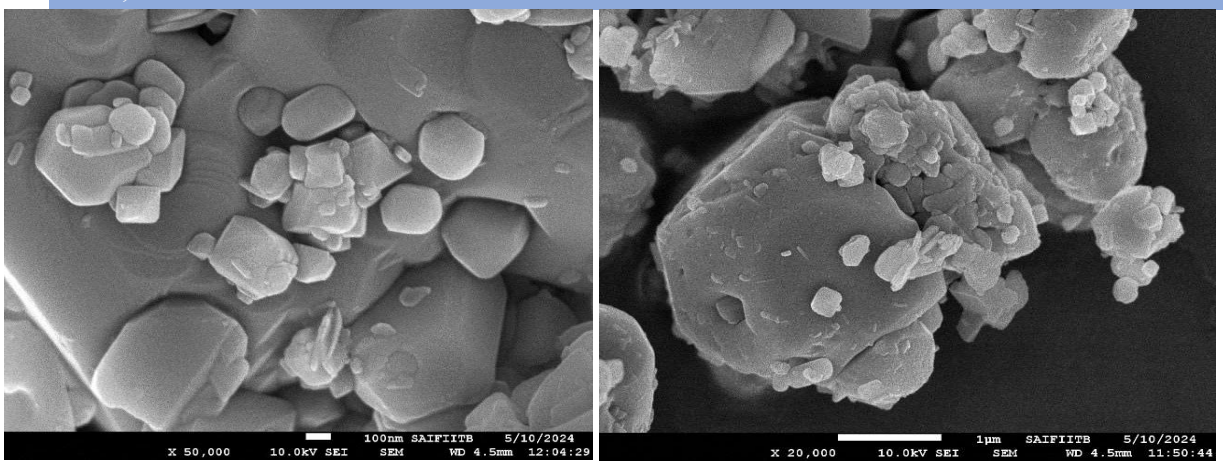

Fig. 8. SEM micrograph of PYB

DISCUSSION:

Ayurvedic method of *bhasmikarana* (process of making nano-sized particles) is a well-known and validated procedure to obtain metallic and mineral based nano-particles. The ideology of size reduction being the most basic principle of *Rasashastra* (Ayurvedic alchemy), application of the same to already nano-sized particles raises the question of possibility to get increased bioavailability owing to even more small particle size and invariably better therapeutic outcome in lesser doses than recommended. Hence the present study aimed at accelerating further size reduction to classically prepared yashadha bhasma and analyze it through instrumental techniques appropriate.

Here potentiation was done at three different time points as detailed in the materials and methods section. Ball milling of *jarita yashada* and PYB *kajjali* respectively for 24 and 48 hrs was preferred here as the additional step of potentiation. This can be validated with the fact that ball milling has been extensively adopted to modify the supramolecular structure of any active pharmaceutical ingredient (API) into its polymorph, co-amorphous or cocrystal structures leading to increased solubility and absorption.[12] As most of the *bhasma* in *Ayurveda* are insoluble in water, currently used potentiating method with ball mill logically aids the efficacy by creating more space for the solubility of API. The second method of potentiation used in current study is wet grinding of PYB in a wet grinder with the juice of Aloe vera for 24 hrs, every time before incineration in EMF. Wet grinding techniques are reported to be significantly more efficient and effective than dry grinding, offering substantial benefits including lower energy consumption, reduced agglomeration, and finer particle sizes, ultimately leading to improved process outcomes and reduced operational costs.[13] The above described two potentiating methods are not generally followed as adopted in potentiation part of this study anywhere in classical *bhasma* preparation. Also, even while the *bhasma siddhi lakshanas* were observed at the 2nd incineration of PYB, but it was subjected to two more times of incineration in EMF with a motive to further bring down the particle size. Optimizing particle size reduction of PYB, intended for oral delivery, can substantially augment its absorption potential by facilitating seamless diffusion across physiological barriers, including the gut mucin layer and enterocyte membranes.[14]

While comparing the observations of preparation methods of both CYB and PYB, it can be seen that the quantity of *bhavana dravya* (both juices of Aloe vera and lemon) has been used extremely higher during 1st incineration of PYB than that of CYB with the only difference until the point being ball milling of *jarita yashada* in PYB method. This is highly indicative of surface area being more created in the particles due to ball milling of *jarita yashada* and this has probably led to increased quantity of liquids consumed in wet grinding before incineration. Due to addition of comparatively more amount of liquids in PYB, the time taken for *bhavana* (trituration) has also remarkably increased in PYB method thus allowing more contact time for the particles in between the grinding surfaces and liquids. Also, in PYB preparation the time of grinding was fixed to 24 hrrs, which has led to increased consumption of *bhavana dravya* compared to CYB. Classically the colour of *yashada bhasma* is found as *indu-sannibha* (colour of moon), i.e., white to pale yellow coloured. It can be noted that pale yellow colour was observed in PYB than CYB. Also, the soft feel to touch which was observed after 2nd incineration in both the samples became very soft to touch by the end of third and fourth incineration in PYB indicating successful further size reduction of particles. The same effect probably has resulted in the amorphous from of PYB while CYB was noted as only fine powder.

Total ash value of PYB is noted lesser than CYB. This reflects more incorporation of inorganic content in PYB.[15] The difference in total ash value and acid insoluble ash values can be corroborated with the methodology adopted. Present study followed the SOP outlined as per Ayurvedic Pharmacopoeia of India which specified heating of 2-3 g sample in silica or platinum crucible at a temperature not more than 450℃ until the sample is free from carbon. But the finding from Keshsun Liu (2019) is suggestive of ashing 1-4 g of samples overnight at 600℃ especially for biomass.[16] Since PYB's preparation involved more contact time with juices of Aloe vera and lemon there could be more incorporation of organic matter into PYB. ICPAES detected step wise reduction in percentage concentration of Zinc in raw *yashada, shodita yashada*, *jarita yashada*, CYB and PYB. With each procedure of *shodhana*, *jarana*, *marana* and *marana* of PYB being carried out at every step, these special techniques have only brought about such a change at the level of zinc concentration thus indicating the importance of significance of such techniques. This is also suggestive of conversion of elemental zinc into its possible oxides and sulphides upon incineration in the presence of oxygen and sulphur (from *kajjali*) at high temperatures in all the steps.

Zeta potential of both CYB and PYB are found with almost similar values. Zeta potential is a critical parameter evaluating delivery system stability by measuring particle surface charge. A threshold of 30 mV distinguishes between stable, repulsive particles and unstable, aggregating ones.[17] In present case, both the zeta potential of both CYB and PYB are of values nearing to -30 mv which is probably suggesting the stability of the drugs during its delivery.

XRD results of PYB furnished peaks that exactly correspond to the peaks of CYB which is Zinc oxide. This also coincides with the findings made by Avani Pareek et al., where *Yashada bhasma* prepared following traditional technique also yielded ZnO hexagonal structures validated by XRD.[18] This justifies the proper formation of metal oxides in the preparation method adopted for PYB.

The effectiveness of potentiation method adopted for the preparation of PYB has reflected remarkably in DLS analysis. Particle size of CYB through DLS was recorded as 696.3 nm whereas for PYB it was found as 261.1 nm (size reduction of two and half times than CYB). This ensures a greater bio-availability for PYB than CYB into the systems facilitating grater therapeutic action in lesser dosses than recommended. SEM analysis of both CYB and PYB did not show much of change except for the hexagonal wuzurite arrangement in PYB. This clearly demonstrates the ability of the method of potentiation adopted is able to alter the structural integrity of compounds formed. With such changes, more than CYB, PYB may exhibit higher therapeutic benefits with lesser doses than recommended.

CONCLUSION:

Present study has effectively come up with a newer idea of potentiation of CYB with ball milling and wet grinding logically to augment better therapeutic outcome. Basic physio-chemcical assessment and analysis with higher end instruments like SEM, Zeta Potential etc., has shown characteristic changes that have occurred in the due course of potentiation. Thus, PYB prepared using the described method of potentiation aids in further size reduction with incorporation of more quantities of organic content as justified by the findings.

SCOPE FOR FURTHER STUDY:

However present study has logically adopted various methods of potentiation, it is recommended for such modified *bhasma* preparations to screen for their safety and toxicity studies. Further animal model studies to decide the therapeutic dose of PYB in comparison to CYB for any condition specified will bring about better dose fixation to PYB.

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