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Characterization of *Yashad Bhasma* (Zinc calx) and establishment of the importance of *Shodhan* (purification)

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Yashad bhasma (Zinc calx) is prepared through a series of pharmaceutical process namely *shodhan* (purification), *jaran* (polling), and *maran* (incineration). The careful investigation of *bhasma* at the end of each pharmaceutical process determines the completion and quality of the *bhasma.* The present study was planned to establish the importance of *shodhan* process by adopting the pharmaceutical process like *shodhan*, *jaran*, and *maran* with the characterization of *yashad bhasma* without *shodhan*, with only *vishesh shodhan* and with *samanya* as well as *vishesh shodhan* though the classical (*bhasmapariksha*) and contemporary (SEM, XRD and EDAX) parameters. Raw *yashad* tagged as 99.99% pure was purchased from the local market of Varanasi, Uttar Pradesh. It was divided into three batches. Batch 1 proceeded for *jaran* and *maran*, no *shodhan* was done. Batch 2 proceeded with *vishesh shodhan*, *jaran* and *maran* while batch 3 proceeded with *samanya* as well as *vishesh shodhan* followed by *jaran* and *maran*. Though all the batches passed the *bhasma pariksha* and established as ZnO, their characteristic appearance were different in terms of time, structure, particle size, composition, and concentration of ZnO. *Samanya* and *vishesh shodhit yashad* was best among the three as the long series of pharmaceutical processing were followed for batch 3 of *yashad*.

Keywords: *Bhasma*, Classical parameters, *Jaran*, *Maran*, *Shodhan*, *Yashad*.

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Introduction

Rasa Shastra, a branch of Ayurvedic pharmaceutics deals mainly with the conversion of mercurials, metals, and minerals into bioassimiable form through the very specified pharmaceutical processing. These pharmaceutical processes are different for different metals or minerals. Variability of these procedures depends completely upon physical properties, chemical properties of metals and minerals and their capacity to cure the disease-specific condition. *Yashad* (zinc) is one of the *dhatus* (metals) mentioned in the authoritative textbooks of *Rasa Shastra* (Ayurvedic Pharmaceutics) mentioned in Schedule I of Drug and cosmetic Act 1940 and Rule 1945[1]. It is considered as *puti loha* (having a low melting point). Acceptable variety of *yashad* shows lustre on cutting (*chhede samujjwala*), heavily weighted (*mahabhar*), soft *(mridu*), clear (*nirmala*) and melted at very low temperature (*drutadrava*)[2]. *Yashad bhasma* posses wide therapeutic applications and is used for both internal and external application viz. *Drishtiroga* (eye disorders), *Nishasweda* (night sweats), *Shwas* (bronchial asthma), *Vicharchika* (eczema-external, application) *Pakshavadha* (paralysis), *Kampavata* (Parkinson's disease), *Yoshopsmar* (hysteria), *Shwetapradar* (leucorrhoea), *Suryavarta* (vestibular migraine), etc. when taken with suitable *anupana* (adjuvant)[2]. Concurrently, nanomaterials containing zinc, in particular zinc oxide nanoparticles (ZnO NPs), are becoming increasingly attractive as innovative agents for medical applications. Zinc oxide is characterized by good biocompatibility which allows the exploitation of its antibacterial, antifungal, antiviral, antitumour[3-6] activity. *Yashad bhasma* is proved effective against diabetes[7]. A hypothesis is made that a combination of chloroquine or hydroxychloroquine with zinc, and an antibiotic like azithromycin will be an effective remedy against COVID-19[8-9].

Significant *shodhan*

Ayurvedic *shodhana* process is one of the powerful methods of detoxification and purification[10]. *Shodhan* means the purification of material by various pharmaceutical processes[11]. In the broad sense, it is

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the procedure that is the base of all the pharmaceutical processes. It clears blemishes of material, makes it brittle, imparts the specific therapeutic property, and facilitates the material for further pharmaceutical processing[12].

There are various *shodhan* processes, ranging from very simple to vary rigorous like *prakshalan* (washing with water), *bhavana* (levigation), *mardan* (trituration), *nirjalikaran* (dehydration), *swedan* (fumigation), *nirvapa* (heating and quenching) etc. These all are selected on the virtue of the physical property and chemical composition of the drug and media used for the *shodhan*[12].

Specifically for the metals, there are two subtypes of *shodhan*, i.e., *samanya shodhan* and *vishesh shodhan*. *Samanya shodhan* is a common method of purification for all metal classes described in *Rasa Shastra* where all the metals are heated to the red hot condition and quench in the five different media i.e., *tila taila* (sesame oil), *takra* (buttermilk), *gomutra* (cow's urine), *kanji* (rice gruel), *kullatha kwath* (horse gram decoction) repeatedly. For the metals having a low melting point frequency of heating and quenching is three times[13]. *Vishesh shodhan* is specific for specific metal. Specific media (lime water, lime juice, aloe vera juice, milk, various decoctions etc.), specific procedure (melting and pouring, heating, roasting, washing, levigation etc.), specific heating temperature (depending upon the properties of metals) and frequency (repetition of the same process) is so specific for a particular metal.

Justified *Jaran*

Yashad (Zinc) is a metal belonging to the class of "*putilauha*" (metals having low melting point). *Putiloha* is processed with an additional process called "*jaran*". *Jaran* can be comparable with poling as a material is heated with the herbals on high heat for its reduction[12]. Media of *jaran* is generally the alkaline herbal ashes. To reduce the alkaline nature of the finally prepared material, it is repeatedly washed with water until pH becomes neutral. This process is responsible for the solidification of metal and facilitates the process of incineration[14].

Methodical *maran*

The third step for the preparation of *bhasma* is "*maran*" i.e., incineration. Incineration is again classified into the sub-process like *bhavana* (trituration), *chakrikanirman* (pelletization), *sharavsamputikaran* (sealing the pellets between the earthen saucers), and *puta* (providing that optimum heat which is necessary for the *bhasma* to be prepared) or *bhasmikaran*. In *bhavana process*, the material is triturated with the indicated media and converted into dough form to prepare the pellets of uniform size (same diameter and thickness). These pellets are then allowed to dry and sealed in between two earthen saucers with mud smeared cloth. This arrangement after proper drying is transferred to the electric muffle furnace and heated to the optimum temperature needed to complete the formation of *bhasma*. In ancient time, in place of Electric muffle furnace, the cow dung cakes were used for the heating procedure. Number of cowdung cakes, dimensions of puta, heating pattern were different for different materials.

The basis of transformation of natural substances into potent medicine is explained as *gunantaradhan* which means an alteration in biochemical properties of the substance by specific *sanskar* (process) on the material in the presence of water/ liquid and heat[15].

With this background, this study was undertaken to establish the Importance of *shodhan* on account of differences observed in particle size, shape, composition, compound formation, and early or delayed attainment of chief desired classical characteristics (*bhasma pariksha*) in all three batches.

Materials and Method

Procurement of raw materials

Raw *yashad* tagged as 99.99% pure and weighing 1283 g was purchased from local market of Varanasi, Uttar Pradesh. All the necessary ingredients required for *shodhan, jaran*, and *maran* were arranged from the Department of Rasa Shastra, Faculty of Ayurveda, Institute of Medical Sciences, Banaras Hindu University.

Batches of *yashad*

Raw *yashad* was divided into three batches. Batch 1 was processed for *jaran* and *maran*, no *shodhan* was done. Batch 2 was processed with *vishesh shodhan, jaran*, and *maran* while batch- 3 was processed with *samanya* as well as *vishesh shodhan* followed by *jaran* and *maran*. In each batch, 400 g of the sample was taken.

Pharmaceutical procedures

The method adopted for *samanya shodhan* was heating and quenching in *tila taila*, *takra*, *kanji*, *gomutra* and *kullatha kwath* three times for batch 3. The method adopted for *vishesh shodhan* was melting and pouring in the *churnodak* (lime water) for batch 2 and 3[16]. The media *ardranimbadanda* (wet neem stick) was taken for the *jaran* process for all three batches[2]. *Bhavana* was given with the *kumari swaras* (aloe vera juice)[17] and pellets were formed having average diameter 2-2.5 cm, average weight 11.5 g and average thickness 1.2 cm and subjected to temperature 600 ^{0}C for one hour in an electric muffle furnace. Repetition of *puta* was done for seven times. This process was common for all three batches of *yashad*.

Bhasma pariksha

The careful observation of each batch was done by certain parameters laid down in classics for completion test of *bhasma*. These tastes includes i) *varitar* (floating of *bhasma* over the surface of stagnant water), ii) *uttam* (in *varitar* condition if rice grain particle is dropped but still it remains floating) iii) *rekhapurnatva* (on rubbing between the thumb and index finger *bhasma* sticks to the furrows of fingers), iv) *apurnbhav* (*yashad bhasma* when triturated with the combination of five herbals which includes resin of *Commiphora mukul*, seeds of *Abrus precatorius*, honey, ghee and borax when subjecting it to same heating temperature, then no any shining or weight gain is observed)[18]. The details of the pharmaceutical process of *yashad* are described in Table 1 along with the observations.

Characterization technique

The classical *bhasma pariksha* is enough to judge the completion of *bhasma* but the size, shape nature of the compound, the concentration of elements could be determined only by SEM, EDAX, and XRD.

XRD analysis

The crystallite size, structural identification, phase and purity of raw metal and *yashada bhasma* sample were determined by X-ray diffractometer (PANalytica X'Pert Pro) using Cu Ka radiation and xenon detector. It was scanned over 2 theta range of 20-80^{O} using ½ ^{O} fixed divergence slit and ¼ ^{O} receiving slit with a step size of 0.0250,1/20 s/step and total run time of 56 minutes at 45 kV and 40 mA.

SEM and EDAX analysis

The nanoparticle morphology, particle size, and elemental analysis of raw metal and *yashada bhasma* sample were carried by using SEM-EDAX instrument, Penta FET Precision OXFORD instrument-X act ZEISS model no 51-1385-046 after Gold grid coating by coated- sputter QUORAM -150 RES. A small portion of the sample was sprinkled onto a double-sided carbon tape and mounted on aluminium stubs, to get electron image for SEM and EDAX analysis.

Results and Discussion

SEM analysis revealed that the shapes of particles of *bhasma* were rectangle, spherical, triangle, radial, and spheres. The particles were heterogeneous and agglomerated Fig. 1b, d & f.

The average particle size of *jarit yashad* was 1000.211±111.86 nm, 667.68±43.73 nm, and 314.95±75.26 nm for batch 1, 2, and 3 respectively as depicted in Fig. 1a, Fig. 1c, and Fig. 1e. The average particle size of finally prepared *yashad bhasmas* was 375.24±34.79 nm, 232.51±15.74 nm and 206.06±16.35 nm for batch 1,2 and 3 respectively as depicted in Fig. 1b, Fig. 1d and Fig. 1f. Average size of particles in *jarit* sample was large as compared to *bhasma* sample in all three batches. *Yashad bhasma*

Table 1 — Summary of comparative observations of *Yashad Bhasma* of all three batches

Batch no	Sample (g)	Weight after *Shodhan* (g)	% loss after *Shodhan*	Weight after *Jaran* (g)	% loss after *Jaran*	Weight after *Maran* (g)	% loss after *Maran*	Completion tests passed
1	400	Not done	NA	320	25	310	22.5	*Varitar* *Rekhapurnata* *Utttam* *Apurnarbhav*
2	400	*Vishesh*-383.7	*Vishesh* -4.075	316	21	309	22.75	*Varitar* *Rekhapurnata* *Utttam* *Apunarbhav*
3	400	*Samanya*-383 *Vishesh*-364.50	*Samanya*-4.24 *Vishesh*- 8.87	307	23.25	307	23.25	*Varitar* *Rekhapurnata* *Utttam* *Apunarbhav*

Fig.1 — a) SEM photograph of *Jarit Yashad* (batch 1), b) SEM photograph of *Yashad Bhasma* (batch 1), c) SEM photograph of *Jarit Yashad* (batch 2), d) SEM photograph of *yashad bhasma* (batch 2), e) SEM photograph of *jarit yashad* (batch 3), and f) SEM photograph of *Yashad Bshasma* (batch 3)

of the 3rd batch is having a smaller particle size than 2nd batch while *yashad bhasma* of the 1st batch is having a larger particle size than the 2nd batch. The difference in the size of particles in *jarit* and *marit* samples are due to the involvement of *bhavana* and *puta sanskaras* in *maran* process. Mechanical energy originated due to the *bhavana* process is responsible for breaking the order of the structure, producing cracks to generate new surfaces. At the point of impact of the edges, the solids deform, forming hot points where the molecules can reach very high vibrational excitation leading to bond breaking and ultimately small particles are generated[19]. It is a known fact that due to repetitive burning, ultra-structural changes occur in terms of size reduction. SEM results evaluate the morphological changes in terms of size[20].

Energy dispersive X-Ray analysis (EDAX) of raw *yashad* showed 98.83% of Zn indicating the authenticity of sample. A major amount of Zn i.e. 87.45% was present in *samanya* and *vishesh shodhit yashad* (batch 3). Along with zinc oxide 12.55% while in *churnodak shodhi tyashad* (batch 2) 22.33% zinc oxide present along with a minor percentage of calcium (0.81%) which might have come from media of *shodhan*. In *jarit* sample of batch 1, 2, and 3, the ZnO was observed to be 9.09, 34.56, and 14.20% respectively. In finally prepared *bhasma*, the amount of ZnO was observed as 17.82, 36.46, and 49.89% respectively in batches 1,2 and 3. Concentration of ZnO was observed highest in batch 3 than batch 2 and batch1. Many other trace elements such as Fe, Si, C, and Ca are incorporated in all three samples, these might have come from the media like *shodhan* media i.e., *churnodak* (batch 2 and batch 3) *jaran* media i.e., *neem danda* batch 1,2, and 3) and *maran* media i.e., *kumari swaras* (batch 1,2 and 3) used for preparation of *bhasmas*. Details are depicted in Table 2.

In the XRD study for raw *yashad* Sample, the major diffraction peaks located at 36.74, 39.03, 43.60, 54.74, 70.27 and 77.25° correspond to the 002,100, 101, 102, 103, and 004 reflection plane of hexagonal structure of Zn (ICSD: 65791), respectively. Characteristic peaks were detected in the XRD pattern confirming the Zinc in the sample Fig. 2a.

In *chunodak shodhit* sample (batch 2), the major diffraction peaks located at 36.74, 39.03, 43.60, 54.47° correspond to the (002), (100), (101), and (102) reflection plane of hexagonal structure of Zn (ICSD: 25007), respectively Fig. 2d.

Table 2 — Elemental analysis of *Yashad* samples by EDAX

S. No.	Sample	Batch no.	Elements	Weight %	Atomic %
1	Raw *yashad*	-	OK	1.17	4.60
			ZnK	98.83	96.40
2	*Samanya* and *Vishesh shodhit yashad*	3	OK	12.55	36.95
			ZnK	87.45	63.05
3	*Churnodak shodhit yashad*	2	CK	15.29	35.05
			OK	22.33	38.45
			CaK	0.81	0.56
			ZnK	61.57	25.94
4	*Jarit yashad*	1	CK	13.35	38.79
			OK	9.09	41.40
			ZnK	77.56	19.81
5	*Jarit yashad*	2	CK	54.70	65.80
			OK	34.56	31.21
			KK	1.50	0.56
			CaK	2.82	1.02
			ZnK	6.42	1.42
6	*Jarit yashad*	3	OK	14.20	39.09
			FeK	27.00	21.29
			ZnK	58.80	39.61
7	*Yashad bhasma*	1	OK	17.82	46.85
			SiK	0.25	0.37
			FeK	0.66	0.49
			ZnK	81.27	52.28
8	*Yashad bhasma*	2	OK	36.46	54.48
			MgK	11.75	11.25
			SiK	26.17	22.27
			CaK	8.24	4.91
			FeK	6.81	2.91
			ZnK	10.58	3.87
9	*Yashad bhasma*	3	CK	17.87	26.09
			OK	49.89	54.68
			Alk	0.64	0.41
			SiK	28.99	18.10
			CuK	2.62	0.72

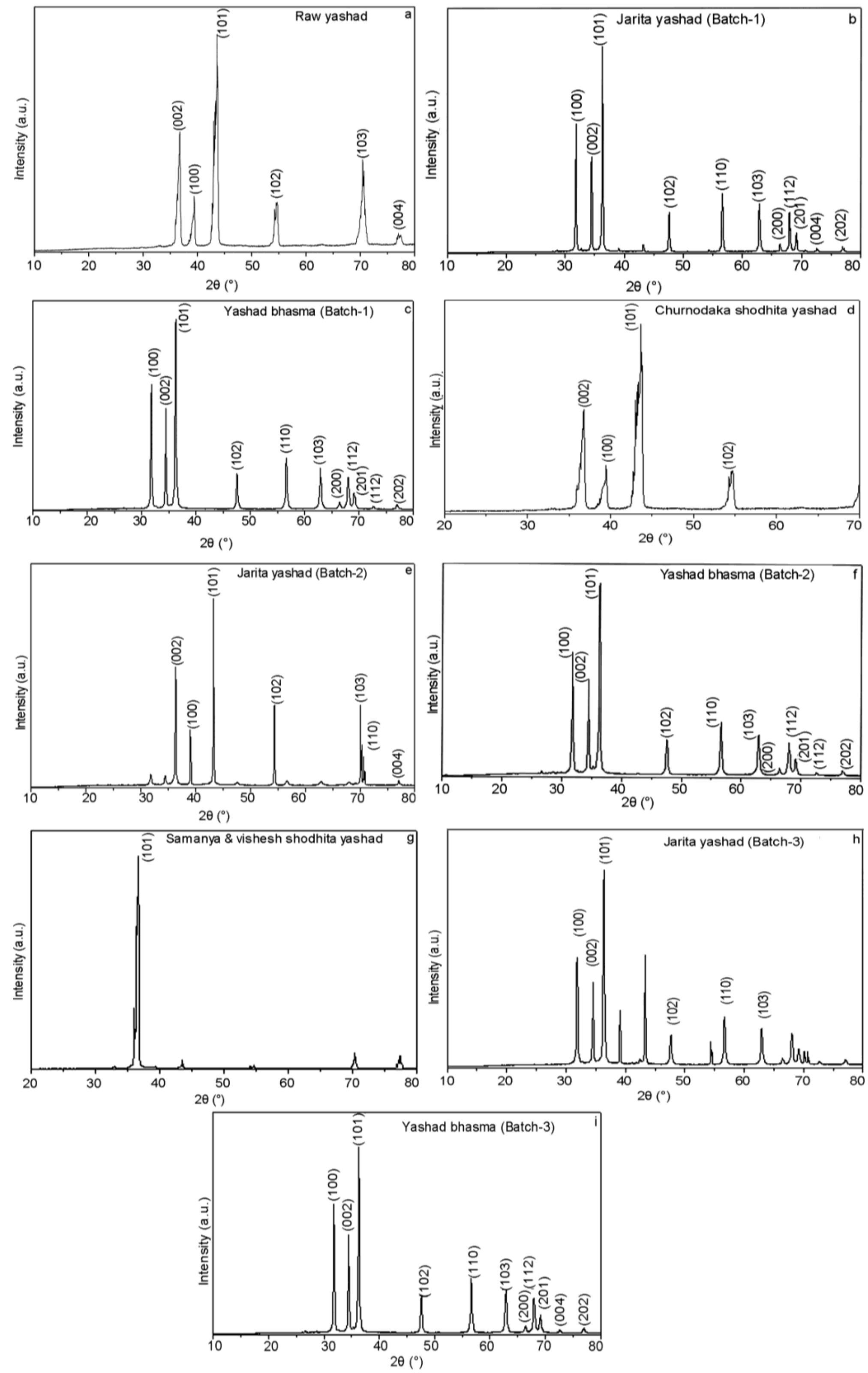

Fig. 2 — a) XRD spectra of Raw *Yashad*, b) XRD spectra of *Jarit Yashad* (batch 1), c) XRD spectra of *Yashad Bhasma* (batch 1), d) XRD spectra of *Churnodak Shodhit Yashad* (batch 2), e) XRD spectra of *Jarit Yashad* (batch 2), f) XRD spectra of *Yashad Bhasma* (batch 2), g) XRD spectra of *Samanya* and *Vishesh Shodhit Yashad* (batch 3), h) XRD spectra of *JaritYashad* (batch3), and i) XRD spectra of *Yashad Bhasma* (batch 3)

In the *samanya* and *vishesh shodhit* sample (batch 3) the major diffraction peaks located at 36.49° correspond to the (101) reflection plane of hexagonal structure of Zn (ICSD: 67591) Fig. 2g.

In the *jarit* samples of all the three batches of *yashad*, the diffraction peaks located at 31.81, 43.25, 34.53, 36.36, 43.30, 56.71, 62.96, and 68.13° correspond to the (100), (002), (101), (102), (110), (103) reflection planes of hexagonal structure of ZnO (ICSD: 67591), respectively. Characteristic peaks related to impurities were not detected in the XRD pattern confirming the high purity of the sample (Fig. 2b, e, and i).

X-ray diffraction study (XRD) of *yashad bhasmas* confirms that the sample was ZnO with wurtzite phase and the entire diffraction peaks are in agreement with the standard JCPDS data (card No. 36-1451). An examination of the spectrum of raw *yashad* (Fig. 2a) shows a characteristic peak of zinc metal at d= 2.09 and 2θ = 43.60° was observed in *churnodak shodhit yashad* (Fig. 2d) but Not observed in *samanya* and *vishesh shodhit yashad* (Fig. 2i) indicates the importance of repeated quenching in different media that helps in phase transfer of metal.

The characteristic Zn metal peak was not observed in diffractogram of all three *Jarit* samples of *yashad* (Fig. 2b, 2e and 2h) and also in all three batches of *yashad bhasma* (Fig. 2c, 2f, and 2i), this indicates that *jaran* process is useful for phase transfer of metal. The results shows that ZnO (hexagonal) is the major crystalline phase present in *yashad bhasms* of all three batches.

The above-mentioned differences in the characterization of raw sample, *jarit* sample, and *bhasma* sample in all the three batches are due to their different pharmaceutical procedures i.e. No *shodhan*, only *jaran* and *maran* for the first batch, *vishesh shodhan*, *jaran*, and *maran* for the second batch, and *samanya shodhan*, *vishesh shodhan*, *jaran*, and *maran* for the third batch.

Physico-chemical analysis of *yashad bhasma* revealed no significant differences in colour, odour, taste, state. The classical parameters of *bhasma pariksha* was done until the achievement chief desired characteristics in all three batches. All three batches represented creamish coloured, odourless, tasteless fine powdered state of *yashad bhasma*. Loss on drying was observed as 2.65±1.22%, 2.21±1.09% and 1.89±1.25% in the 1st, 2nd and 3rd batch respectively. Ash value was observed as 98.28±1.66%, 98.46±2.13% and 99.00±1.20% in 1st, 2nd and 3rd batch respectively, Acid insoluble ash value shown as 2.54±2.32%, 2.13±1.67$^{\%}$ and 2.10±1.34% in 1st, 2nd and 3rd batch respectively

Differences in classical parameters i.e., *bhasma pariksha* i.e., *Varna*- changing of ash-coloured *jarit yashad* to creamish colour was observed in all three batches after 1 puta. *Rekhapurnatva* (particles in *bhasma* should be fine enough to lodge itself between the fine lines of fingers when rubbed)- It came positive in 2 *puta* for 1st and 2nd batch, but it was observed positive after first *puta* in 3rd batch. *Varitara* (*bhasma*, when sprinkled, should float on water and not settle down)- It came positive after 5 *puta* in 1st batch, after 4 *puta* in 2nd batch and after 3 *puta* in 3rd batch. *Nischandratva* (should not shine in sunlight)-It was observed after 2 *puta* in 1st and 2nd batch while after 1 *puta* in 3rd batch. *Unnam* (grain particle along with *bhasma* should float on the surface of stagnant water.)- It was observed after 6 *puta* in 1st and 2nd batch while after 4 *puta* in 3rd batch. *Apurbhava* (on heating with ghee, honey and borax; should not lead to reappearance of the source material). -It came positive at last i.e., after 7 *puta* in 1st and 2nd batch while for 3rd batch it was observed after 6 *puta*. *Nirdhum* (smokeless) and *Niswadu* (tasteless)- both these tests came positive lastly i.e., after 7 *puta* in 1st and 2nd batch while for 3rd batch it was observed after 5 *puta*. All these changes observed in three batches especially between 1st - 2nd and 3rd batches was a result of the *shodhan* process i.e., *samanya shaodhan* of the 3rd batch of *yashad* done in different media which has facilities the conversion of *yashad* to its *bhasma* in relatively less no. of *puta*.

The significance of *shodhan* is that in each of the steps in *samanya shodhana* (normal purification), progressive increase in surface area and reduction in particle size observed. This may be due to micro-cracks formed during heat treatment. Repetition in heating and cooling disrupts compression tension equilibrium and leads to increased brittleness, reduction in hardness and finally reduction in the particle size. After removing the hydrophobic impurities using sesame oil (*tila taila*) treatment, oxide scales are formed due to atmospheric oxidation of the raw material. Oxide scales are removed by treatment with aqueous media viz., buttermilk (*takra*), rice gruel (kanji), cow urine (*gomuta*), and horse gram decoction (*kullatha kwatha*)[21]. These media possess metals chelation properties. *Tila taila* acts as metal chelator[22] Lactic acid from *takra* dissolve the minerals and leach them[23]. Cow urine contains ammonia. Ammonia can be used as a leaching reagent for extraction of Zn.[24,25] *Kullatha* contains Phytate may have a stronger ability to quench free radicals because of its metal-chelating ability, They are capable of removing free radicals and chelating metal catalysts[26]. *Kanji* prepared by fermentation of rice causes an increase in total phenolic content which are responsible for metal chelation[27]. *chunodaka* used for *vishesh shodhan* acts as alkaline media. alkalinity enhances particle–particle interactions and thereby promotes nanoparticle aggregation of Zinc[28].

The *jaran* media *neem* contains a high amount of reducing agents called terpenoids that serves as capping and stabilizing agents. The involvement of many low and high molecular weight protein molecules helps in the stabilization of biosynthesized ZnO nanoparticles[29].

Kumari swaras used for *bhavana* process have identified for the presence of 75 active ingredients. The phytochemicals responsible for the synthesis of nanoparticles are alkaloids, carbohydrates, flavonoids, protein, saponins, and terponoids[30].

Conclusion

Shodhan process is foremost important for the conversion of raw materials into *bhasma* form. It not only purifies but facilitates the material for further processes like *jarana* and *maran*. Batch 3 of *yashad bhasma* was found best on the parameters of particle size, percentage of zinc oxide, reductions in the number of *puta* and appearance of classical parameters early than the rest two batches this is a result of a long chain of pharmaceutical procedures adopted for the third batch of *yashad*.

Conflict of interest

No competing financial interests exist.

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