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Role of Shodhana (purification) in the pharmaceutical processing of Tamra Bhasma and its physico-chemical characterization

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Abstract

Tamra Bhasma is a metallic Ayurvedic formulation which has been therapeutically used for the treatment of various ailments like Kustha (Skin diseases), Parinam sula (Duodenal ulcer), Arsha (Haemorrhoids), Pandu (Anaemia), Swasa (Dyspnoea), etc[1]. for a long time. But the scientific basis of the cause of detoxification of metallic copper is not well documented. More over different pharmaceutical procedures like shodhana(Purification), Marana (Inceneration), and Amritikarana(Nectarisation), etc[2], leads to removal of free copper, starts synthesis of new metallic complexes and detoxifying the Bhasma. Among these process Shodhana is very important, which eliminate adherent impurities, detoxifying he drugs, hard material converted into brittle and also enhance the drug efficacy. So improperly prepared Tamra Bhasma may cause various illness like Vanti(Vomitting), Bhrama(Halucination), Murchha(Fanting), Daha(Burning sensation), etc[3]. It is therefore very important to prepare Tamra Bhasma of best quality for Ayurvedic therapeutic uses by using classical references and standardized by following scientific quality control procedures and characterization by investigating the physico-chemical changes in the products.

Keywords: Tamra Bhasma, Shodhana, Bhasma pariksha, Quality control

Introduction

In Ayurvedic therapeutics “Tamra Bhasma” has been in use since long for many types of liver and gastro-intestinal tract disorders. If it is not prepared properly or shodhan(purification) procedure is not done properly, it acts as a poison. To indicate its toxic potential, Astamahadoshas[4] (eight major ill effects) have been quoted in classics and due emphasis have been given to its shodhan procedure. Safety and efficacy depends upon the methodology adopted for the preparation and any deviation from the classical preparation method will not yield desired result

In the present study attempts have been made to find out the importance and justification of necessity of shodhan of Tamra before preparation of Tamra Bhasma, which is a generic Ayurvedic metallic formulation for therapeutic use.

Keeping above view in mind Tamra bhasma prepared from shodhita(purified), Ashodhita(Unpurified) and water purified Tamra was taken for scientific basis of study to ascertain the role of Shodhan process on safety profile of Tamra bhasma and characterization by investigating the physico-chemical changes in the products.

Materials and Methods

Pure 99% copper turnings wire purchased from the Science world, Cuttack, Odisha supplied by oster chemical and pharmaceutical works, pvt. Ltd. for the

preparation of Tamra Bhasma. Then it was subjected to *Samanya shodhan* (General purification), *Vishesha Shodhan* (Special purification / Detoxification), *Marana*(Calcination/Incineration) and *Amriti karana*(Nectarisation) procedures as per the classical reference.

To find out the importance of shodhana as per classical texts, three different samples of Tamra Bhasma have been prepared by purifying it by different ways and the Bhasmas are prepared by these following methods :-

i) Tamra Bhasma prepared with traditionally shodhit(purified) Tamra – sample-I
ii) Tamra Bhasma prepared with water shodhit(purified) Tamra – sample-II
iii) Tamra Bhasma prepared with Ashodhit(unpurified) Tamra – sample-III

a) *Shodhan(Purification)*[5]

Pure 99% Tamra(copper turning) was heated upto red hot and then treated with different solvents. First of all copper was heated up to 700-800^{0}C and put it in *Til oil* (*Sesamum indicum*), *Takra*(Butter milk), *Gomutra*(Cow's urine), *Aranala* (Sour gruel) and *Kulattha kwatha*(Decoction of *Dolichos Biflorus*) sequence wise 7 times in each solvent. Lastly the tamra procured from end of the above procedures was boiled with gomutra(cow's urine) for 3 hours. At the end of these 6 stapes the copper turning became black and brittle and are termed as traditionally shodhit tamra (purified copper) which was used for preparation of bhasma. Like wise 2nd sample was treated 35 times with plain tap water and taken as water purified Tamra for preparation of Bhasma. 3rd sample of Tamra was taken directly without purification for preparation of Bhasma.

b) *Bhasmikarana (Incineration*)

The purified copper was triturated with equal amount of samaguna *Kajjali*(Black Sulphide of mercury-HgS) in addition with *Nimbu Swarasa* (Juice of *Citrus limon*) and make pellets, dried on sun light and subjected to heat by horizontal electric muffle furnace(EMF) keeping inside two closed earthen pots in gradually rising temperature up to 700 ^{0}C. Repeated the procedures for 10 times and lastly obtained 3 samples of Tamra Bhasma which were subjected to Amriti karana.

c) Amriti karana(necterasion)

All the 3 samples of Tamra Bhasma were separately triturated with equal quantity of purified sulphur in addition with required quantity *panchamrit*(Cow's milk, curd, ghee, honey & sugar candy) and make pellets, dried on sun light and subjected to heat by horizontal electric muffle furnace(EMF) keeping inside two closed earthen pots in gradually rising temperature up to 700 ^{0}C. Lastly obtained bhasma was taken for physico-chemical study.

Analysis of the final product

Tamra Bhasmas were analysed by using different organoleptic parameters as per classical tests like *Rekha purnatwa, Varitaratwa, Apurnabhavatwa, Unam, Niruttha, Amla pariksha,curd test and Avami*[6] were performed. Modern physico-chemical parameters[7] like loss on drying, ash value, acid insoluble ash, and water soluble extractive were performed on 3 samples of Bhasmas. Modern instrumental analytical techniques like X-ray diffraction(XRD), Scanning Electron Microscopy(SEM), Particle size distribution(PSD) were carried out.

Classical Bhasma pariksha

1. *Rekhapurnatva* :-On rubbing between thumb and fore finger, Bhasma was found completely entering the furrows of the fingers.

2. *Varitarattva* :-On gently sprinkling the bhasma over the still surface of water taken in a glass, bhasma particle were found floating over the surface.

3. *Avami* :-Very little amount of bhasma after ingestion did not produce nausea.

4. *Niruttha test :*-5 gm of Tamra bhasma was taken and small weighed amount of silver piece was kept together in sarava (earthen crucible) and subjected to heat. The silver was reweighed. The weight of silver could not found having increased weight.

   This test was done for three samples of bhasma separately and found having no change in weight of silver.

5. *Apunarbhava* :-Ingredients :- Tamra bhasma – 10 gm,Mitra panchaka – 10 gm (*2gm each of Guda,Guggulu*, *Gunja, Madhu & Tankana*)

   Bhasma and mitra panchaka were triturated, pellets were prepared , kept in sarava and subjected to heat on which the bhasma was prepared and on self cooling there was nothing any trace of conversion of bhasma into copper forms. This test was done for all the 3 samples separately.
6. *Curd test :*-A small amount of bhasma was sprinkled over curd taken in a petridish and left for

24 hours. There was no appearance of blue or green colour in the curd.

7. *Amla pariksha :-*Fresh and filtered lemon juice was kept in two test tubes. A little amount of bhasma was put in a test tube and compared with that of other test tube after few hours. There was nothing any bluish or greenish colouration present in lemon juice.

x-ray diffraction analysis of Tamra Bhasma[8]

X-ray diffraction analysis is a powerful technique for detecting the presence of a substance, as that substance actually exists in the sample contains the compound A_xB_y, the diffraction method will disclose the presence of A_xB_y as such, where as ordinary chemical analysis would show only the presence of elements A and B. further more of the sample contained both A_xB_{2y} and both of these compounds would be disclosed by the XRD methods.

Diffraction analysis is therefore, useful whenever it is necessary to know the state of chemical combination of the elements involved or the particular phases in which they are present. The basic principle of the phase analysis using power XRD technique is the presence of diffraction peaks corresponding to various interlunar (d_{hkl}) spacing's which are characteristics of a given material. The relative intensities of various peaks occurring at different "d" spacing's are also different for different phases. The joint committee on powder diffraction standards (JCPDS) index, provides the relative intensities and corresponding 'd" spacing's of various known patterns of very large number of compounds to characterize the pattern of an unknown compound.

Scanning electron microscopic study of Tamra Bhasma[9]

Microscopy is the technique of examining the samples at high magnifications, so that minute details which are not visible to the unaided eye are made clearly visible. The earliest and commonly used light microscope in which a beam of visible light is made to pass through a system of convex lenses, yields magnifications upto 155x only. Another limitation of the light microscope is that it has a very limit depth of focus i.e. small elevations and depressions on the surface of the specimen can lead to haz, unsharp pictures due to focusing problem. Hence the specimen to be studied under light microscope must have a flat and smooth surface.

In recent years, Electron Microscopes have enhanced the capability of the microscopic technique. The electron microscope uses fast moving beam of electrons as the source of imaging instead of a light beam as in the light microscope. Since the wave-lengths of these electrons are in the order of a fraction of an Angstrom (A^0) compared to about 500A^0 for the light waves used in the light microscope and the resolution of the electron microscopes and hence their limits of magnifications are much higher, going upto 300, 000_x. Additional, the narrow beam of electrons (100A^0) give much greater depth of focus compared to the light microscope. In conclusion, the electron microscope has higher magnification limits and greater depth of focus.

Particle size Distribution[10]

Particle size distribution is known as grain size distribution and it can be important in understanding the physical and chemical properties of a material. PSD is a list of values or a mathematical function that define the relative amount of particle present.

Principle :_When a particle passes through a laser beam it causes light to be scattered at an angle that is inversely proportional to size. The scattered light is collected by a detector and analysis of the ensuring diffraction pattern enables calculation of the size distribution of the particle in a given sample.

Results and Discussion

The raw Tamra of 99.88% w/w was taken for the preparation of Bhasma which indicates the high purity of raw material. After *samanya shodhana and vishesa shodhan* the percentage of copper content was reduced where as iron content was found increased after samanya shodhan which is shown in the following table.

Table-1 showing percentage of copper and Iron after *samanya* and *vishes shodhana* of tamra

Sl. No.	Samples	Percentage of Copper	% of Iron
1	Raw Tamra	99.88	0.09
2	After samanya shodhan	91.69	9.56
3	After vishes shodhan	91.08	6.86
4	Tamra Bhasma – I	59.8	3.2
5	Tamra Bhasma – II	60.12	5.86
6	Tamra Bhasma – III	60.89	0.85

During shodhan process red hot copper is decomposed to cupric oxide on its surface. This leads to decrease in copper content after shodhana procedure. Where as iron percentage increased during procedure due to the process performed in iron pan. The ferroso-ferric oxide formed by the reaction with atmospheric oxygen or steam, when heated to red hot during the process get mixed with copper and the iron percentage is increased.

The colour of Tamra Bhasma is black as per classical texts. The cupric oxide(CuO) and copper sulphide(CuS) are black in colour. Therefore the Tamra Bhasma is chemically one of these two or combination of both.

Organoleptically Tamra Bhasma is black, soft, smooth and tasteless powder, which was clearly mentioned in the (Table -2) of 3 samples of tamra Bhasma.

Table -2 showing Organoleptic parameters of Tamra Bhasma(3 Samples)

Sl. No.	Parameters	T.B.(I)	T.B.(II)	T.B.(III)
1	Shabda(sound)	(Soundless) No metallic sound when crushed between teeth	Soundless	Soundless
2	Sparsha(Touch)	(Soft) & fine No course particle by touch	Soft & fine	Soft & fine
3	Rupa(Colour)	Deep black	Black	Slight faded black
4	Rasa(Taste)	Tasteless	Tasteless	Tasteless
5	Gandha(Odour)	Not specific	Not specific	Not specific

The classical tests are very important for finalizing therapeutically effectiveness of Tamra Bhasma because when Apakwa(improper) Bhasma is taken internally it produces excessive salivation, nausea, metallic taste and headache etc. *Dadhi pariksha* is an important classical test to verify the quality of Bhasma. No discoloration noticed surrounding area of Bhasma is considered as proper Bhasma and bluish green discoloration is considered as improperly prepared Bhasma.

Values of physico-chemical parameters of different samples were found identical with some reports of previous scholars. Less value of loss on drying indicates almost absence of moisture in tamra bhasma. High ash value indicates presence of high inorganic content in tamra bhasma where as lower value of acid insoluble ash indicates greater bioavailability of the drug.

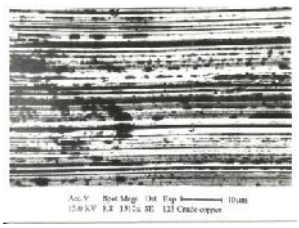
Table -3 showing Physico-chemical Parameters of three sample of Tamra Bhasma

Parameters	Samples		
	T.B.(I)	T.B.(II)	T.B.(III)
Loss on drying	1.05%	0.98%	0.87%
Ash value	98.28%	88.56%	79.27%
Acid insoluble ash	2.38%	3.82%	4.57%
Water soluble extractive	0.36%	0.25%	0.21%
Carbon disulphide soluble extractive	1.13%	1.28%	1.32%

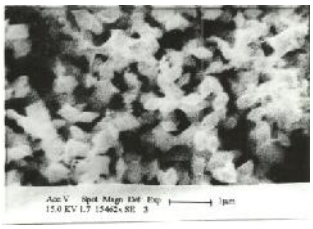
The advanced analytical techniques like scanning electron micrographs of copper turnings used in preparing the Tamra bhasma and the fileing marking are clearly shown in the figure. The grainy structure shown at the surface should be of importance and relevance in the processing, because this will decide diffusion rate of various ingredient.

The grainyness should be of concerned to decide the activity of the Bhasmas. The graining morphology of particles shape in Tamra bhasma prepared with traditionally purified copper are similar, but the size of the particles vary between 2.27 to 2.49 micron which is not significant, as seen in Figure the particles are found uniform and homogeneous.

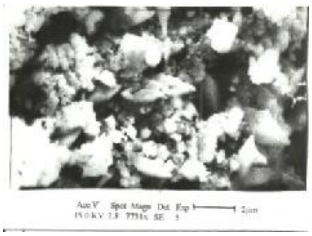
The shape and size of the particles in Tamra bhasmas prepared with water purified copper and unpurified copper are very irregular and heterogeneous as found on Figure. The particles size vary between 0.99 to 2.99 micron and 0.66 to 7.26 micron in bhasmas. These variations of particles size are highly significant.



SEM photograph of copper turning



SEM photograph of T.B-I



SEM photograph of T.B.-II

SEM photograph of T.B.-III

Particle size distribution of a powder or granular material is a list of values which indicates the relative amount of particle present according to size. PSD also indicates the physical and chemical properties of a material. PSD analysis of different tamra bhasma is shown in the table -5

Table 4 Showing result of particle size distribution analysis of 3 samples of Tamra bhasmas

Samples	VMD (inμm)	X 10 (inμm)	X 16 (inμm)	X 50	X 90 (inμm)	X 99 (inμm)
T.B. – I	27.76	2.38	4.58	21.35	83.43	147.15
T.B. – II	96.24	3.99	8.62	85.28	368.12	434.29
T.B. – III	108.43	7.82	13.15	121.17	432.16	502.63

VMD : Volumetric mean diameter

X10, X16, X50, X90 and X99 indicate the percentage of particles are below the mentioned micron value. The reduced particle size of the Bhasma may facilitate absorption and assimilation of the drug into the body system.

It is observed that, where traditional pharmaceutical processing are adopted, the shape and size of the particles are similar and homogeneous, but where traditional pharmaceutical processes have not been adopted the particles shape and size are very irregular and heterogeneous.

During the preparation of bhasma, different vegetable drugs treatment at different stages like, shodhan, maran and Amritikaran are responsible for getting the particle size homogeneous and also due to its chemical compound formation, like that of organo-metallic compounds of the bhasma.

Both SEM and PSD analysis show the significant reduction in particle size in bhasma, which is correlated with the classical tests like Rekhapurnatwa, Varitaratwa and Sukshmatwa etc. The decrease in particle size and increased surface area enhanced the dissolution rate which helps in rapid absorption and bioavailability of the drug.

XRD pattern of crude copper, which is taken as starting material for the preparation of the Tamra bhasma clearly shows that it is quite pure. The XRD pattern of traditionally purified copper shows that, after purification copper is converted into copper oxide compounds as CuO and Cu_2O and also these two compounds are found from XRD of water purified copper.

In XRD pattern of tamra bhasma –I (bhasma made with traditionally purified copper) reasonable evidence for Cu_7S_4 is observed.

In fact other peaks were also observed and indicated in the figure.

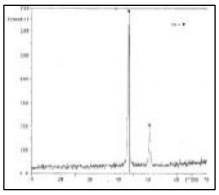


Fig.-1 XRD of Copper turning

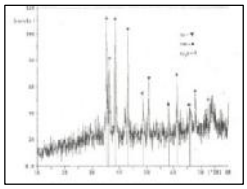


Fig.-2 XRD of traditionally purified Cu turning

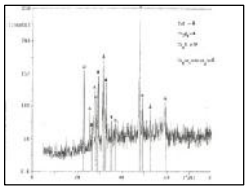


Fig.-3 XRD of water purified Cu turning

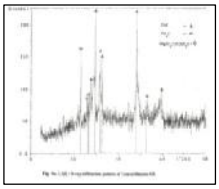


Fig.-4 XRD of Tamra bhasma -I

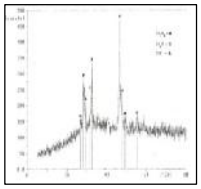


Fig.-5 XRD of Tamra bhasma -II

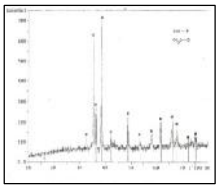


Fig.-6 XRD of Tamra bhasma -III

Understanding the intensities of the peak carefully we infer that Cu_7S_4 is more dominant in Tamra bhasma –I. tamra bhasma-I contains trace amount of CuS which is much lesser than in comparison to those in Tamra bhasma-ii and iii

In the process of preparation of tamra bhasma major part of the copper is converted to its sulphide form because, sulphur is added in the processing. During repeated heat treatment for the preparation of bhasma some sulphide may get converted to oxide. Therefore some copper oxide are also found in tamra bhasma. Metallic oxides are toxic in comparison to sulphide of metal which are generally less toxic compound. So internal use of cupric oxide(CuO) causes different unwanted toxic symptoms in the body which is similar to Apakwa or improperly prepared bhasma.

We therefore attribute the superior action of the bhasma –I over the other two due to the presence of Cu_7S_4.

It is suggested that whatever parameters have been mentioned in Ayurvedic texts should be followed as such. There should not be any change and alteration of the process. There are several methods mentioned for preparing the bhasma. One should try to adopt such method, which is easier, cheaper and efficacious as per therapeutic need.

Conclusion

All the classical methods mentioned for Shodhana and Marana have scientific values. So these methods should not be avoided. Chemically the tamra bhasma prepared by classical method is in the form of copper sulphide(CuS), which is less toxic than other two. The particle size and SEM images show reduced particle size which helps in absorption and assimilation of the drug in the body.

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