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SYSTEMATIC STUDY OF TAMRA (COPPER) BHASMA PREPARED BY TRADITIONAL AYURVEDA METHOD

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ABSTRACT: Bhasmas, the formulations, which can be prepared from metals, minerals or even some marine products, are being used in Ayurvedic system of medicine. Copper (Tamra) is one such metal, used to prepare a bhasma, called "Tamra Bhasma" (TB) which is claimed to cure various diseased conditions. Though bhasmas are prepared since centuries, the exact mechanism of conversion of metal to its bhasma, especially by the use of modern scientific tools is not established. Here is an attempt to prepare TB as per the text "Ayurved Sar Sangraha" and analyze the stepwise changes in terms of chemistry. TB was prepared from the copper wires. Steps involved in the preparation were shodhan, maran and post maran treatment. Raw materials, intermediates and finished product were analyzed as per Ayurvedic pharmacopeia. Modern scientific techniques such as XRD, XPS and SEM were employed to assess the changes taking place during the steps of preparation and thus characterization of the intermediates and finished drug was accomplished. Chronological study of TB preparation, showed that conversion of copper metal to its compounds starts right from shodhan process. Further changes occurring during maran and post maran treatment, gave fair idea about the complex mechanism of TB formation. Sixteen cycles of incineration (puta) were required to get the final product which fulfilled the Ayurveda tests. At the end of puta 16, XRD indicates Cu_2O and CuO as major phases in TB, which subsequently transforms into CuO after puta 22. This research also highlights the contribution of post maran process on the Bhasma.

INTRODUCTION: Rasa-shastra, is a branch of Ayurvedic pharmaceutics. It deals with the preparation of various dosage forms namely, bhasma, kupi pakva rasayana, parpati, pottali and khalvi-rasayan [1]. Bhasma, which literally means ash, (residue after incineration), is a therapeutic agent, prepared from metals and/or minerals.

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Copper bhasma (Tamra bhasma, TB) is widely prescribed for the treatment of diseases such as ascites (Udar), jaundice (Kamala), haemorroides (Arsha), obesity (Medoroga) and worm infestation (Krumi) etc [2]. The metal is converted to its bhasma, through a series of processes which are called as Ayurvedic sanskars. These sanskars are intended not only for the removal of unwanted properties, but induction of desirable qualities also. Accordingly, the metals are treated with herbs and other materials to make them bio- compatible and easily absorbable in the body [3].

In ancient times bhasmas were prepared by the Ayurvedic physicians as per patient's requirement.

The concept of metal pharmacology based on Ayurvedic literature is discussed by Sarkar et al[4a]. Now a day, due to increased demand of Ayurvedic medicines, various companies have started preparing bhasma on a large scale. Bhasma preparation being a tedious and time consuming process, the tendency to skip some important steps, is observed. Moreover, different pharmacies follow varied procedures for bhasma formation resulting in final product with varied particle size, bulk density, copper content etc[4b-4e]. It is claimed that if bhasma is not prepared as per classical text, deposition of administered bhasma in the vital organs may be observed leading to serious consequences. Reproducibility is also very important from the viewpoint of standardization. Thus, it is essential to study critically the process of bhasma formation and optimize the parameters without compromising with Ayurvedic norms.

The literature survey showed that little work is being done in the standardization of TB. More emphasis is given to the therapeutic action and nontoxic nature of TB. The hepato-protective activity of TB is studied by several researchers[5]. A study of effects of tamra bhasma, on gastric ulcers and secretion was examined by Goel[6] and Pandey et al[7]. Study of therapeutic action of TB prepared by two methods, proved lipid lowering action (lekhana karma) of TB[8]. Toxicology[9a] and free radicals scavenging property of tamra bhasma was studied on albino rats by Pattanaik et al[9b]. He observed that tamra bhasma given for longer period (90 days) in 5mg/kg dose, induced lipid peroxidation without any effect on the survival.

Although, Jagtap et al.[10] mentioned standard method of preparation of TB, they used muffle furnace instead of traditional gaja puta bhatti. Effect of Shodhan process was established in the tamra bhasma by preparing with and without shodhan process. TB was subjected to oral toxicity studies to ascertain the role of Shodhan on safety profile of the bhasma on subchronic administration to albino rats[11]. Rai and Jha[12] pointed out that, TB samples prepared by using various media like kajjali, mullaika, gandhakadi at the stage of maran, were studied for their relative anti-hyper-lipidemic efficacy in male albino rats. The results indicate that the lipid lowering capacity of TB, prepared using mullika is the best and that using kajjali is of second grade. Maran using gandhakadi is not effective. Wadekar et al.[13] established the composition of TB by using various characterization techniques. The results were compared with those of standard copper oxide samples. Major phase of TB was observed to be copper in +2 oxidation state and the particle size was seen to increase due to agglomeration of small particles due to repeated calcinations processes.

An attempt has been made to characterize various bhasmas such as Swarnamakshika, vanga and naga bhasma by several researchers[14a-c] while physicochemical changes during the TB preparation were investigated by Tripathi[14d]. The results clearly indicate that the complete process of bhasma preparation (shodhan and maran) leads to the removal of free copper from the sample. Copper bhasma is selected for the present research work, as it is used for the liver disorders, biliary duct diseases and ascites and even for diabetes mellitus. Interestingly, in the European community and Australia/ New Zealand, copper recommendations for adults, range from 1.1 to 1.2 mg/d, as one of the necessary micronutrient for the normal functioning of vital organs. Inadequate copper produces adverse effects on the metabolism of cholesterol and glucose, blood pressure control, heart function, mineralization of bones and immunity[15].

With the advent of modern tools of materials characterization, it is an opportune moment to use these tools to study the transformation of Copper metal into its bhasmas. Although modern tools of characterization have been used in recent publications, it is rarely attempted to understand the detailed mechanism of bhasma formation including the analysis of intermediate steps involved. Incidentally, such a study would prove to be ‘a model’ for the further developments in the field of bhasmas in general. On this background, the present endeavor is the first attempt which would help in understanding the mechanism of bhasma formation, in view of the necessity and usefulness of various steps involved in the traditional process.

MATERIALS AND METHODS:

Raw Materials: A] Cu wire: For this study, copper wires having purity 99.8 % were procured from

local market, Nagpur, India. The impurities were analyzed using the spot test and showed presence of Cd, Sn, Zn and Fe.

B] Following materials used for bhasma preparation process, were analyzed using criteria mentioned in the Ayurvedic pharmacopeia[16] and given in **Table S1** (supporting information).

1. TilTaila: (sesame oil)
2. Takra: (buttermilk)
3. Gomootra: (cow's urine)
4. Kanji: (Sour gruel)
5. Kulith kwath: (decoction of *Dolichos biflorus)*
6. Gandhak: (Sulphur), pure (shudha) and raw (ashudha)
7. Tamrind juice: (juice of *Tamarindus indica*)
8. Saindhav: (Rock salt)

Preparation of TB:
TB can be prepared by various Ayurvedic methods. Process of bhasma preparation using mercury is said to be environmentally hazardous. Hence sulphur route mentioned in "Ayurveda Sarsangraha"[17] was followed here for preparation of TB. TB was prepared by following steps, namely 'Shodhan', 'Maran' and 'Post maran processes'

A] Shodhan:
Shodhan process is aimed to eliminate the demerits (doshas) of metals[18]. Thin copper wires were heated until red hot in an iron pan and quenched in sesame oil (Tiltaila). The wires were then taken out of the oil, heated to red hot and again quenched to fresh quantity of oil. This process was repeated for three times. The heating and quenching process was performed further with butter milk (Takra), cow's urine (Gomootra), sour gruel (Kanji) and decoction of Dolichos biflorus (Kulith kwath), 3 times in each liquid chronologically. The physical and chemical changes occurred in the copper wires were analyzed.

B] Maran:
Maran process is the heat treatment of the shodhit metal in an appropriate traditional heating mechanism called as puta in order to destroy demerits in raw material. For the maran of copper, traditional furnace (gaja puta) with cow dung cakes was used[19]. The Shodhit copper wire pieces were mixed well with equal proportion of rock salt (Saindhav) and Sulphur (shuddha Gandhak) (w/w). The mixture was adequately wetted with juice of Tamarindus indica (Imali swaras) and kept in earthen clay pot (sharava-samputa) sealed at the joint by using clay (Multani mitti) and cloth. This sharava-samputa was then subjected to gaja puta. Next day the material was taken out and ground well with Tamarindus indica (Imali swaras) taking care that the solid part is completely immersed in it. Small circular discs (chakrikas) were prepared out of this mixture, kept in the sharava-samputa and then subjected to Gaja puta. A puta is said to be completed when it goes through the cycle of heating (maximum temperature attained was $750^{O}C$) and natural cooling to room temperature (swanga-sheet).

The whole process was repeated three times and checked for ayurvedic bhasma completion test such as 'Varitara', 'Rekhapoorna', 'Nischandra', 'Nishkalank' and 'Apunarbhava'. The material could not pass these tests. Moreover, in curd test, greenish blue coloration was observed within 30 minutes. Hence, it was inferred that further puta treatment is necessary. The chakrikas were made into fine powder, weighed and Sulphur (25% by weight) was added after 5^{th} and 10^{th}puta. Total 16 putas were required in order to fulfill Aurvedic bhasma tests. Analysis of intermediates during the whole process was done by XRD. The samples at each stage of puta are referred as p-1 to p-16 according to the respective cycle of heating.

Completion of the bhasma was confirmed by a characteristic test for TB (vishesh bhasma pariksha: Dadhi pariksha) [20]. The absence of free metal in the prepared bhasma was also confirmed by chemical displacement reaction by $AgNO_3$. After complying with these tests, TB was further analyzed by advanced analytical techniques like XRD, XPS and SEM. In order to confirm the possibility of further oxidation of this bhasma, sample P-16 was heated in muffle furnace at $600^{o}C$ for 1hr, and simultaneously in traditional bhatti (gaja puta) upto 22 heating cycles.

C] Post maran processes: As TB is known to have toxic effects[21], Ayurveda texts recommend post maran processes to minimize the hazardous properties.

- Washings (Dhavan vidhi): After completion of 16 putas, the TB was ground well in pestle mortar (khalva yantra), water was added in it and allowed to settle. The supernatant liquid was filtered with the help of muslin cloth, for 3-4 times per day until green color and bad odor of water is removed.

- Curd treatment (Dadhi vidhi): After dhavan vidhi, TB was mixed with curd in a pot and kept for 4-5 days. Water was added in it, decanted 3-4 times a day, for at least 3-4 days. The final product was dried well.

- Oil treatment (Taila-bharjan): The above TB was added with sufficient amount of sesame oil (tiltaila) and heated in a pan with continuous stirring till the oil gets evaporated. After cooling, the bhasma was ground well and sieved (120 Mesh size). The product after this treatment is referred to as TB-final and was analyzed by XRD.

Physico-chemical characterization:

The structural characterization of the TB is done by X-ray diffraction using Phillips PW 1730 system with Cu-Kα radiation. The surface studies of the TB was also done by X-ray photoelectron spectroscopy (XPS) analysis using an ESCA-3000 (VG Scientific Ltd., UK) with a base pressure of better than 1.0×10^{-9} Pa. Mg Kα radiation (1253.6 eV) was used as a X-ray source and operated at 150 W. All the binding energies were calibrated by using the contaminant carbon (C1s = 284.6 eV) as a reference. The surface morphology of TB particles was carried out using Leica Stereoscan model 440 SEM. Atomic Absorption spectrometer (AAS) was used for finding the composition of the raw materials (Varian Spectra AA 220FS).

RESULTS AND DISCUSSION:

Preliminary observations:

A] Shodhan Process:

Chemical purity of raw copper wires was 99%, measured by AAS. The effect of various steps of shodhan process on starting copper metal has been reported by Jagtap et.al[10]. According to these authors, copper undergoes a treatment alternatively in acidic-basic medium during chronological shodhan process.

However, the present study reveals that most of the steps of shodhan process consist of heating the precursor Cu wires in acidic or near neutral medium followed by quenching. As the heating/quenching time is very short, the changes occurring would be confined to surface only. Due to the treatment given in the shodhan process, the luster of metal wires was diminished with tarnishing the color; wires became brittle and broken into small pieces. Peculiar observation was noted during the treatment with Kanji, where the metal wires appear to be cleaned and shinning.

Of course, the topology/morphology of the surface would change which could not be observed by bare-eyes. Such changes also may have their effect on the rate of bhasma formation (required number of putas) in the over-all process.

Some composite formations on the surface become apparent. Since these composites formation is likely to have an intermediates consisting of an organic functional group from plant or otherwise origin, it is likely to be burnt in the subsequent heating process and form carbon coating on copper wire pieces. These organic parts may act as surface active agents, helping dispersion of copper intermediates in liquids used for the shodhan process, and consequently exposing fresh surface for further treatment.

B] Maran process:

1. Ayurveda Bhasma tests:

Ayurveda believes that metals, if not processed properly, may prove hazardous. Hence following tests for the quality control of the final bhasma are prescribed, namely varitar and unam (floatability), rekhapurna (fineness), apunarbhava (irreversibility), nirdhuma (fumeless), nishchandra (shine less) and Nishkalanka (no coloration to curd).

TB powder completely floats on water and fills the ridges of the finger tips. For apunrbhava tests TB was mixed with mitra panchak (equal amounts by weight of honey, seeds of Abrus-Precatorious, borax, ghee, jaggery) and heated in muffle furnace

at 750 ^{0}C. The ash obtained did not indicate any shinning metallic particles. TB showed neither white nor yellow fumes during heating in a test tube or any shinning particles in the sunlight, thus fulfilling the Nirdhuma and nishchandra test respectively. Nishkalanka (discoloration) is a specific test for copper bhasma, where a pinch of bhasma, when sprinkled on curd should not show any coloration even after a day. Our TB satisfies this test also.

2. Ayurvedic pharmacopeia test: [16]

The final bhasma was examined with the tests recommended in ayurvedic pharmacopeia.

3. Chemical tests:

The presence/absence of possible copper compounds ($CuSO_4$, CuS, Cu_2O, CuO and Cu metal) formed during synthesis of TB was tested by common laboratory chemical tests[22].

C] Post maran process:

1. Dhavan vidhi: After this treatment the resulting bhasma was found to be free from Sodium chloride, confirmed by $AgNO_3$ test. Also, the washings show no blue color indicating that bhasma is free from $CuSO_4$.

2. Curd treatment:

The unconverted-free copper if present develops green color when immersed in the curd and subsequently can be removed by washings.

3. Sesame oil treatment:

Bhasma when subjected to the bharjana process, the fine brownish-black powder was obtained. The Brown color can be attributed to the presence of Cu_2O as against black CuO.

Instrumental analysis:

In today's modern era, it is the need of the hour, to standardize bhasma formation process, by coupling the classical Ayurvedic tests with the advanced analytical techniques. Thus the sophisticated methods of physico-chemical characterization are applied to map the phase transformation of Copper metal to its Bhasma through its various intermediate stages.

A] Shodhan Process: The whole shodhan process is critically screened by XRD technique.

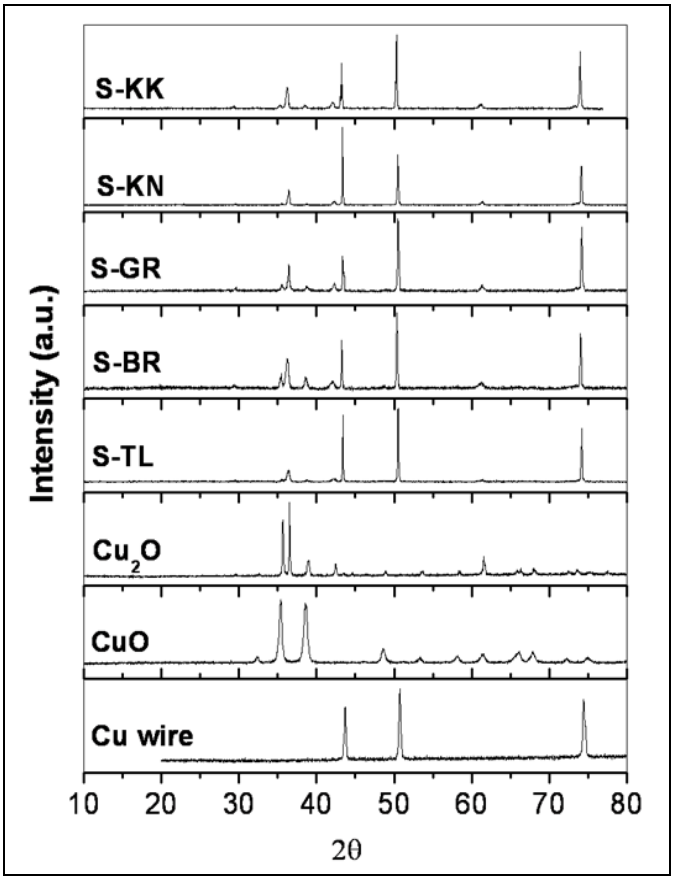

FIG. 1: XRD PATTERN OF TB INTERMEDIATES DURING SHODHAN PROCESS

Fig.1 shows the powder X-ray diffraction pattern of the raw materials (Cu wire) at different stages of shodhan. The XRD of CuO, Cu_2O (aged) and copper metal precursor (wire pieces) used in the present study are also shown for comparison. XRD indicates that the free metal continues to be present as major phase during the shodhan process; however there are signatures for the presence of Cu_2O/CuO phases (2θ =34 - 37 degree). It should be noted that oxidation process occurs right from the first step of shodhan process (i.e. Tila taila treatment). With the subsequent steps of the shodhan process, conversion to copper oxides goes on increasing and the metal peak around 44 degrees starts broadening and deforming. However, after the treatment with Kanji, the metal wire appear to be cleaned and shinning which is also supported by the presence of sharp metal peaks in the XRD.

The rates of the processes of oxidation and/or etching along various planes of copper metal vary considerably, developing cracks during the shodhan process and thus metal surface becomes brittle.

B] Maran process:

The Maran process is critically examined by XRD, XPS and SEM to trace the path of bhasma formation, the nature of the intermediates and chemical composition of final bhasma.

XRD studies: Phase changes occurring during each stage of maran process (puta) were recorded by XRD. Sulphur was added after 5th and 10thputa. The changes developed due to this addition were represented in **Fig. 2**.

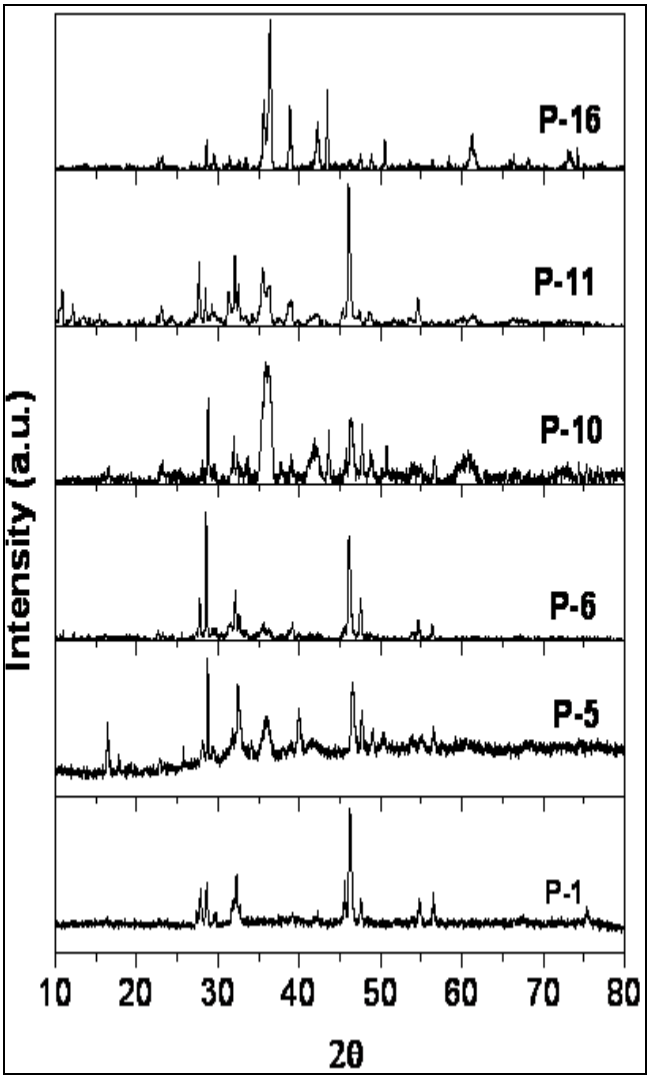

FIG.2: XRD PATTERN FOR PUTA 1, 5, 6, 10, 11, 16.

Fig. 2 shows indication of Cu_2SO_4 formation for p-1. Cu_2O phase starts appearing as we progress from p-1 to p-5, and Cu metal lines disappear. After p-5, sulphur was added and its effect was reflected in XRD of TB after p-6 by the presence of predominant peaks of Cu_2SO_4. Then onwards, from P-6 to P-10, simultaneous formation of both oxides take place (CuO, Cu_2O). After puta 10, Sulphur was again added and subsequently the XRD of P-11 shows increase in intensity of peak at around 43 degrees, corresponding to copper sulphide (CuS). Copper sulphate/sulphide phases are decreasing and simultaneously that of copper oxides (Cu_2O/CuO) is increasing from p-13 to p-16.

The intermediates showed the presence of the copper sulphide, copper sulphate, cuprous oxide and cupric oxide till 16 putas.

The possible reactions are,

$2Cu + O_2$ ---------- $2CuO$

$2CuO + S$ ------ $Cu + SO_2$ ------- On surface

$2Cu + O$ -------- Cu_2O

$2Cu_2O + S$ ---- $Cu + SO_2$ ------- On surface

$Cu + S$ --------- CuS

$2Cu + S$ -------- Cu_2S

$CuS + 2\ O_2$ ------ $CuSO_4$

$Cu_2S + 2\ O_2$---- Cu_2SO_4

$CuS + O$ ------- CuO/Cu_2O

$Cu_2S + O$ ----- CuO/Cu_2O

$Cu_2O + O$ ----- CuO

It may be noted that there would be various simultanious chemical processes occuring during bhasma formation and the rate of these reactions would also, affect the composition at intermediate steps. Therefore, the mechanism suggested above is a primary speculation.

When the maran process is continued up to 4th puta, it may be noted that the XRD bands are broad, suggesting lower crystallite size. Addition of sulphur at this stage, apart from chemical changes, brings about a change in the rate of crystal growth. Then onwards, the XRD peaks start to show sharper line-width, indicating better crystallization. In the present case, the addition of sulphur after puta 10 and puta 15 shows pronounced effect on the crystallite size, which in turn may determine chemical as well as therapautic properties of the final product. This can be attribted to the flux effect of sulphur.

Although, the oxidation of Cu could have been brought about by heating, without sulphur addition, the process would be diffusion controlled and

therefore slow. Also, this oxidation process is a "batch process" as conceived by aurvedic pandits, the intermediate pulverization of powders would be difficult for oxides as against sulfides. Thus, the purpose of adding sulphur can be understood as enhancing the rate of oxidation and exposing fresh surface of copper for bhasma formation.

As CuO is more stable than Cu_2O, the bhasma at the stage of P-16 was further heated in muffle furnace at 600^{o}C for 1hr (P-16H), and simultaneously in traditional bhatti (gajputa) upto 22 heating cycles (P-22). Observations are summarized in **Fig.3**, which indicates the successive conversion to higher oxidation state of Copper (i.e. CuO) from P-16 to P-22. Subsequent to samples P-16, CuO peaks were getting sharper. Broadening of the peaks of CuO was observed in P-22 sample, indicating smaller particle size due to traditional heating pattern.

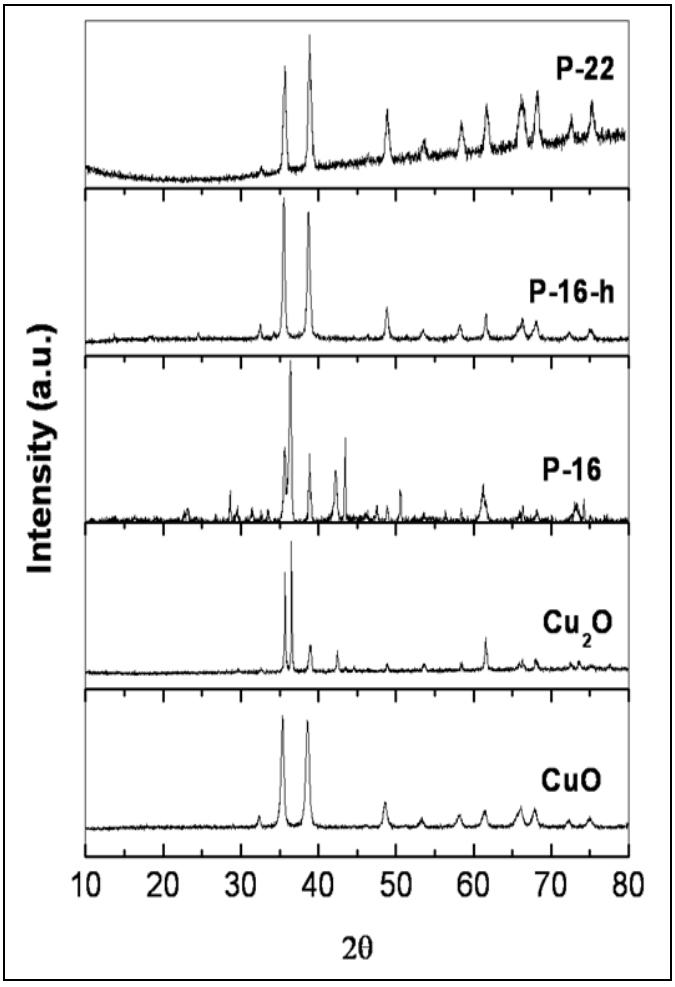

FIG.3: XRD PATTERN FOR P-16, P-16-H AND P-22 WITH STANDARD CuO AND Cu_2O

XPS studies:

The surface oxidation states of copper bhasma were analyzed by X-ray photoelectron spectroscopy. **Fig 4** shows the XPS spectrum exhibiting Cu2p, O1s and S2p lines for P-16 sample. From the figure, it is observed that the sample shows a broad Cu2p peak between 930 and 936 eV. This broad peak can be deconvoluted into three peaks corresponding to different copper phases, namely, Cu_2O (932.7 eV) and CuO (933.5 eV) and Cu_2SO_4 (934.8 eV). A characteristic satellite peak of Cu^{+1} and Cu^{+2} was observed between 938 and 945 eV for TB sample. The sample has the S_{2p} binding energy of 168.8 eV, which indicates some sulphate species on the surface of TB. The broad peak of O1s also suggests the formation of Cu_2O / CuO phase. These values well support XRD data.

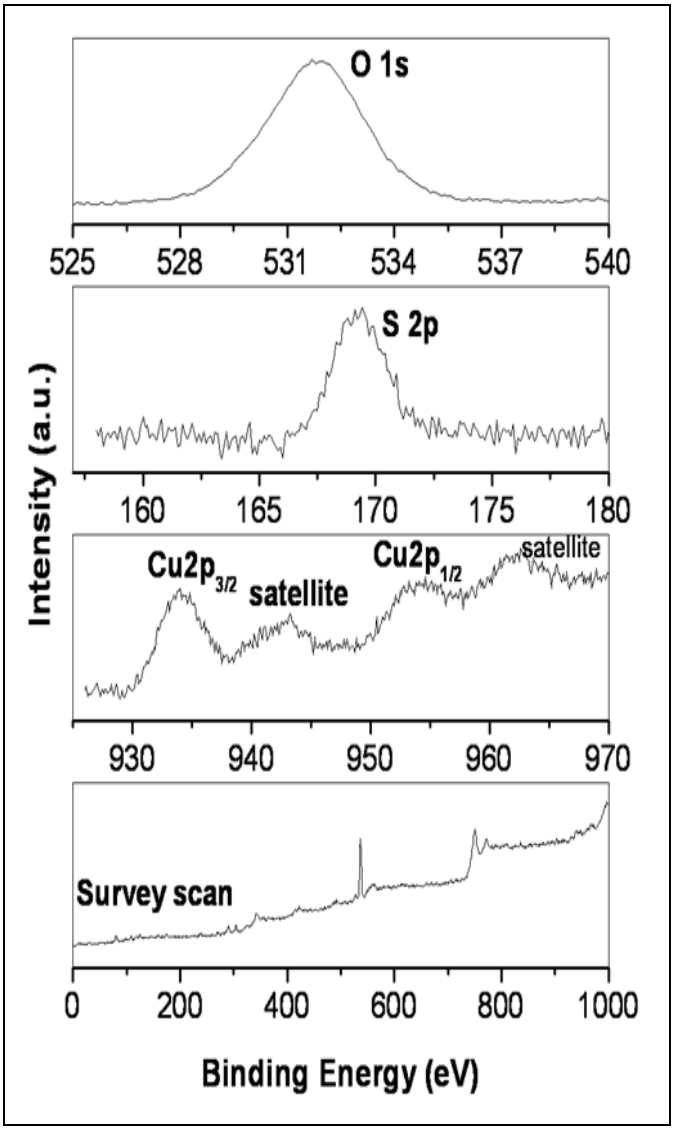

FIG. 4: X-RAY PHOTOELECTRON SPECTROSCOPY FOR THE SAMPLE P-16

SEM studies: SEM shows that the material is uniform nano scale size (30-50 nm) for puta 16, however heated sample shows agglomerated particles **(Fig 5)**.

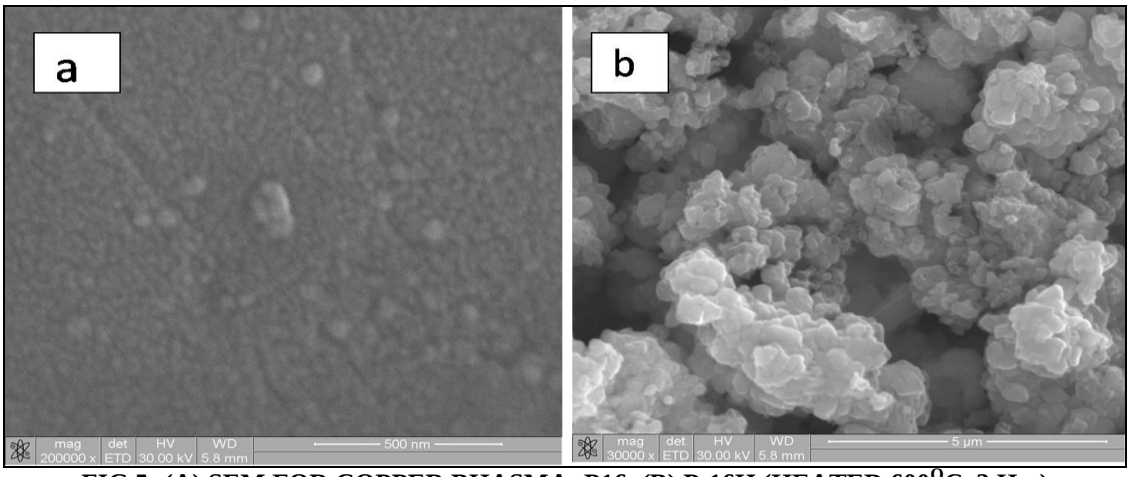

FIG 5: (A) SEM FOR COPPER BHASMA: P16, (B) P-16H (HEATED 600^{O}C, 3 Hrs)

C] Post maran process:

XRD was used to evaluate the value addition to TB occurred during Curd treatment and oil treatment **(Fig. 6)**. After curd treatment (dahi wash), the sample becomes free of Cu_2SO_4 and whatever free Cu is present is likely to be removed as water soluble lactate.

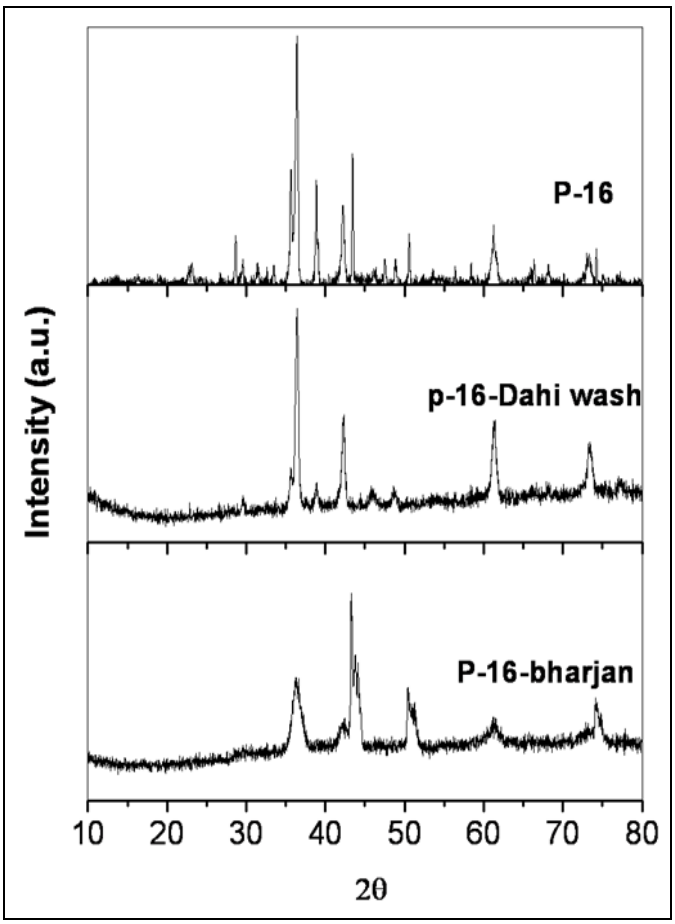

FIG 6: XRD OF COPPER BHASMA, TREATED WITH CURD AND OIL

After oil treatment (bharjana wash), XRD lines broadened remarkably, inferring lowering of particle size which can be attributed to the carbon capping of each particle of the bhasma , restricting the further growth in spite of heating. Cu_2O was observed as the major phase. As cuprous ions have been shown to be considerably more toxic to bacteria than cupric ones,[23] the post maran treatments seems to contribute to the value addition of final bhasma.

In conclusion, the present study throws light on the following points,

- Chemical composition of bhasma and nature of its intermediates.
- Exact path of bhasma Formation.
- Contribution of shodhan, maran in view of facilitating the TB formation and post maran processes to the value addition of the quality of bhasma.
- Presence of carbon capping, if any, during bhasma formation.
- Detection of free metals in the finished product.
- Possibility of presence of nano-particle and/ or aggregates.

Thus, in the present study, we co-relate Ayurvedic sanskars in terms of modern science to understand a mechanism by which the metal is transformed into its biocompatible bhasma.

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REFERENCES:

1. Dhamankar, P.V. and Puranik, G.V: Ayurvediya Aushadhikaran. Dhootapapeshwar Prakashan, 2nd edition 1964; Part 2; 43.
2. Mishra, G: 'Arthavidyotini' Hindi commentary of, 'Ayurveda Prakasha by Aacharya Madhava'. Chaukhamba Vidyabhavana Varanasi, 2nd edition 1962; Chapter 3 : Verse 145.
3. Tripathi, I: Hindi commentary Rasaprabha of ,'Rasa Ratna Samucchaya by Acharya Vagabhata '. Edited by Giri KD, Chaukhamba Sanskrit Bhavana, and 1st edition 1998.
4. (a) Sarkar, P.K., Das, S. and Prajapati, P.K: Ancient concept of metal pharmacology based on Ayurvedic literature. Anc. Sci. Life, 2010; 29: 1–6.
   (b) Honwad, S.V: Toxicity study of tamra bhasma prepared with various methods. International Journal of Research in Ayurveda and Pharmacy, 2011; 2(6):1685-1691.
   (c) Uchil, D., Rege, N. and Dahanukar, S: Modern correlates for Ayurvedic quality control test for tamra bhasma. Indian Drugs, 1996; 33(8): 384-389.
   (d) Chitanis, K.S. and Stanley, A: Chemical evaluation of Tamra bhasma. International Journal of Pharma and Bio Sciences, 2011; 2(2): 160-168.
   (e) Kapoor, R.C: Some observations on the metal based preparation in the Indian System of Medicine. Indian Journal of Traditional Knowledge, 2010; 9: 562–575.
5. (a) Kanase, R., Patil, S. and Kanase, A: Effect of hepatoprotective ayurvedic drugs on lysosomal enzymes during hepatic injury induced by single dose of CCl_4. Indian Journal Experimental Biology, 1994; 32(5): 328-332.
   (b) Tripathi, Y.B. and Singh, V.P: Role of tamra bhasma, an ayurvedic preparation, in the management of lipid peroxidation in liver of albino rats. Indian Journal Experimental Biology, 1996; 34(1): 66-70.
   (c) Patil, S., Kanase, A. and Varute, A: Effect of hepatoprotective ayurvedic drugs on lipases following CCl4 induced hepatic injury in rats. Indian Journal Experimental Biology, 1989; 27(11): 955-958.
   (d) Patil, S., Kanase, A. and Varute, A.T: Effect of hepatoprotective ayurvedic drugs on lipolytic activities during CCl_4 induced acute hepatic injury in albini rats. Indian Journal Experimental Biology, 1993; 31(3): 265-269.
6. (a) Goel, R.K. and Sairam, K: Anti-ulcer drugs from indigenous sources with emphasis on musa sapientum, tamrabhasma, asparagus racemosus and zingiber officinale. Indian Journal of Pharmacology, 2002; 34: 100-110.
   (b) Goel, R.K.,and Maiti, R.N: Gastric ulcer protective effect of tamra bhasma, an Indian ayuervedic preparation of copper and planteain banana. 'Naples Italy: Proceedings': 1st international symposium on natural drug and the digestive tract, 1992; 73-76.
   (c) Sanyal, A.K., Pandey, B.L. and Goel, R.K: The effect of a traditional preparation of copper, tamra bhasma on experimental ulcers and gastric secretion. J. Ethnopharmacol, 1982; 5(1): 79-89.
   (d) Goel, R.K., Travers, I.A. and Bennett, A: Effects of cupric chloride and tamra bhasma, a traditional Indian preparation of copper, on Eicosanoid production by human gastric and co-ionic mucosa. J. Pharm. Pharmacol., 1992; 44(10): 862-864.
7. (a) Pandey, B.L., Goel, R.K. and Das, P.K: A study of effects of tamra bhasma, an indigenous preparation of copper on experimental ulcers and gastric secretion. Indian Journal Experimental Biology, 1983; 21: 254-258.
   (b) Goel, R.K., Maiti, R.N. and Mughopadhayaya, K: Effect of tamra bhasma, an Indian indigenous preparation of copper on rat gaustric mucosal resistance. Indian Journal of Experimental Biology, 1994; 32(8): 559-561.
8. Chaudhari, S.Y., Jagtap, C.Y., Galib, R., Bedarkar, P.B., Patgiri, B. and Prajapati, P.K: Review of research works done on Tamra Bhasma [Incinerated Copper] at Institute for Post-Graduate Teaching and Research in Ayurveda, Jamnagar. Ayu., 2013; 34(1): 21–25.
9. (a) Vahalia, M.K., Thakur, K.S, Nadkarni, S. and Sangle, V.D: Chronic toxicity study for Tamra Bhasma (A generic Ayurvedic mineral formulation) in laboratory animals. Rec. Res. Sci. Technol., 2011; 3: 76-79.
   (b) Pattanaik, N., Singh, A.V., Kumar, M., Dixit, S.K. and Tripathi, Y.B: Toxicology and free radical scavenging property of tamra bhasma. Indian Journal of Clinical Biochem., 2003; 18(2): 181-189.
10. (a) Jagtap, C.Y., Prajapati, P.K., Patgiri, B. and Shukla, V.J: Standard manufacturing procedure of tamra bhasma. AYU. 2012; 33(4): 561-567.
    (b) Jagtap, C.Y., Prajapati, P.K., Patgiri, B. and Shukla, V.J: Qulity control parameters for tamra bhasma. Ancient science of life, 2012; 31(4):164-170.
11. Jagtap, C.Y., Ashok, B.K., Patgiri, B., Prajapati, P.K. and Ravishankar, B: Acute and Subchronic Toxicity Study of tamra bhasma (Incinerated Copper) prepared from Ashodhita (Unpurified) and Shodhita (Purified) Tamra in Rats. Indian Journal of Pharmaceutical Sciences, 2013; 75(3): 346-352.
12. Rai, R.K., Jha, C.B., Chansuriya, J.P.N. and Kohli, K.R: Comparative assessment of antihyperlipidaemic action of tamra bhasma. Indian Journal of Traditional Knowledge, 2008; 7(2): 335-340.
13. Wadekar, M.P, Rode, C.V., Bendale, Y.N., Patil, K.R. and Prabhune, A.A: Preparation and characterization of copper based Indian traditional drug: tamra bhasma. J. pharmaceutical and biomedical analysis, 2005; 39: 951-955.
14. (a) Mohaptra, S. and Jha, C.B: Physicochemical characterization of Ayurvedic bhasma (Swarnamakshika bhasma): An approach to standardization. International Journal of Ayurveda Research, 2010; 1: 82–86.
    (b) Nagarajan, S., Pemiah, B., Krishanan, U.M.,Rajan, K.S., Krishanaswamy, S. and Sethuraman, S: Physico-chemical characterization of lead based Indian traditional medicine-Naga Bhasma. International Journal of Pharmacy and pharmaceutical sciences, 2012; 4(2): 69-74.
    (c) Lagad, C.E., Sawant, R.S., Tayade, A.N. and Wadodkar, D.S: Physicochemical characterization of vanga bhasma. International Research Journal of Pharmacy, 2013; 4(2): 97-101.
    (d) Tripathi, Y.B., Singh, V.P., Sharma, G.M.K., Sinha, R.K. and Singh, D: X-ray diffraction and microscopic analysis of tamra bhasma: An Ayurvedic metallic preparation. Indian Journal of Traditional Knowledge, 2003; 2(2): 107-117.
15. Collins, J.F. and Klevay, L.M: Copper. American Society for Nutrition. Adv. Nutr., 2: 2011; 520–522.

16. Pharmacopeial Standards for Ayurvedic Formulations. Central Council for Research in Ayurveda and Siddha, 1987.
17. Ayurved Sarsangrah. Shri Baidyanath Ayurved Bhavan Private Limited, New Edition January 2004: 108.
18. Sharma, S: Rasatarangini. Motilal Banarasidas Delhi 11, First edition 1994; Chapter 2 Verse 52: 22.
19. Tripathi, I: Hindi commentary 'Rasaprabha' of 'Rasa Ratna Samucchaya by Acharya Vagabhata', Edited by Giri KD. Chaukhamba Sanskrit Bhavana. 1st edition, 1998: Chapter 5 Verse 52.
20. Tripathi, I**:** Hindi commentary 'Rasaprabha' of 'Rasa Ratna Samucchaya by Acharya Vagabhata ' published by Chaukhamba Sanskrit Bhavana, 1st edition 1998: Chapter 8 Verse 26-30.
21. Mishra, G: Hindi commentary 'Arthavidyotini' of 'Ayurveda Prakasha by Shri Madhava'. Chaukhamba Vidyabhavana Varanasi, 2nd edition 1962: Chapter 3 Verse 115-116.
22. Vogel, A.I: Text book of Quantitative Inorganic Analysis, 3rd edition, 1961: 323.
23. (a) Hans, M., Erbe, A., Mathews, S., Chen, T., Solioz, M. and Mucklich, F: Role of Copper Oxides in contact killing of bacteria. Langmuir, 2013; 29(52): 16160-16166.
(b) Abicht, H., Gonskikh, Y., Gerber, S. and Solioz, M: Non-enzymatic Copper Reduction by Menaquinone Enhances Copper Toxicity in Lactococcus Lactis IL1403. Microbiology, 2013; 159(6): 1190-1197.

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