ABSTRACT

Introduction: In Ayurveda, metals and minerals are converted into biocompatible forms through specified classical procedures like Shodhana, Marana, etc. before their therapeutic use. Their frequent use without any noticeable side effects since ages is the ultimate proof of safety. But, safety and toxicity concerns are being raised by conventional researchers considering the presence of heavy metals in them. Noncompliance of pharmaceutical procedures as described in Ayurvedic classics is one possible cause for toxicity. At the same time, no standard analytical profiles are available for many metallic preparations.

Materials and methods: The present study aims to prepare and develop preliminary analytical profiles for Tamra bhasma (calcined copper) with and without Amritikarana on structural and elemental basis to address the role of the raw materials used during the preparation, compound form, nature and particle-size of both samples.

Results and conclusion: The study revealed that Tamra Bhasma contains copper in a nano-crystalline structure having standard mean diameter ~12.72 μ in Tamra bhasma without Amritikarana and ~8.83 μ in Amritikarana sample. Copper sulfide form (CuS) associated with elemental sulfur was found to be a structural component in both samples.

Keywords: Amritikarana, Characterization, Copper, Standardization, Tamra bhasma

How to cite this article: Chaudhari S, Ruknuddin G, Prajapati PK, Rao MM. Analytical Specifications of Tamra Bhasma (Calcined Copper). J Drug Res Ayurvedic Sci 2018;3(2):65-70.

Source of support: Nil

Conflict of interest: None

INTRODUCTION

Ayurveda uses natural resources including metals and minerals in therapeutics since ages. Knowing the possibilities of toxic effects; seers emphasized on following exclusive pharmaceutical procedures like Shodhana, Marana, Amritikarana, etc. that converts the metals and minerals into Bhasma (calcined powders), which are devoid of toxic nature when used judiciously. Copper is, one such metal essentially required in many metabolic functions. Ayurveda advocated the use of Tamra bhasma (calcined copper) in the management of Udara (ascitis), Pandu (anemia), Shvasa (dyspnoea), Amlapitta (hyper-acidity), etc.1 It is reported to be safe through acute, sub-chronic, chronic and genotoxic studies.2-4 Ayurvedic texts provided a few qualitative tests to ensure the quality of Bhasmas. Some of them are fineness, having no metallic shine, etc.5 However, these qualitative tests do not provide any quantitative information about the composition and structure of the final drug. Structural information is must for any drug having the presence of metallic content. Considering the raising concerns on the traditional formulations for containing heavy metals, it becomes essential to understand in which form the heavy metal is present in the formulation.6-8 This will help in understanding the possible nature of metabolism. Considering this, an attempt has been made to explore the structure and composition of Tamra bhasma through sophisticated instrumental facilities like thermo gravimetric analysis (TGA), X-ray diffraction (XRD), inductive coupled plasma with atomic emission spectroscopy (ICP-AES), scanning electron microscope coupled with wavelength dispersive spectroscopy (SEM-WDS) and particle size analyzer.

MATERIALS AND METHODS

Preparation of Tamra Bhasma

Tamra bhasma was prepared by adhering guidelines described in Rasashastra classics (Fig. 1). It involves the below procedures.

Samanya Shodhana

Copper scraps (99% pure) were taken in Loha darvi (iron spoon) and heated in gas stove up to the red-hot stage and quenched in Tila taila (oil of Sesamum indicum Linn.) in a stainless steel vessel. After cooling, Tamra was taken out from the vessel. Quenching in Taila was done for six times more times. This process of quenching was repeated in other specified liquids, i.e., Takra (buttermilk), Gomutra (cows urine), Kanji (sour gruel) and Kulattha kwatha (decoction of seeds of Dolichos biflorus Linn.) respectively.9 Every time fresh, gravimetrically same amount of media was taken. The product obtained at the end of this procedure is Samanya shodhita tamra.

ORIGINAL ARTICLES

Analytical Specifications of Tamra Bhasma

1Swapnil Chaudhari, 2Galib Ruknuddin, 3Pradeep K Prajpati, 4Meda M Rao

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Vishesha Shodhana

Samanya shodhita tamra was bundled into a Pottali, hanged in stainless steel vessel (diameter 19 cm, depth 20 cm and breadth 18 cm having capacity 3.2 lit.) containing Gomutra. Due care was taken so that the Pottali does not touch the boundaries of the vessel and immerse completely in the liquid media. Approximately, 2.5 L of Gomutra was needed for complete immersion of 500 gms of Tamra. The contents were boiled (Swedana) for three hours maintaining the temperature around 120° C. Increments of Gomutra were added to facilitate complete immersion of Pottali. After three hours of Swedana, Pottali was taken out, Tamra was collected carefully, washed with warm water and dried. The product obtained at the end of this procedure is Shuddha tamra.

Tamra Marana

Samaguna kajjali (black sulfide of mercury) equal to the amount of Shuddha tamra was taken in Khalva yantra (mortar and pestle) and levigated in the presence of Jambiri nimbu swarasa. When paste-like consistency appeared, Shuddha tamra scraps were spread on it and triturated well. It was dried in shade, placed in Sharava (earthen saucer) and covered by another Sharava. The junctions were sealed with double folded cloth smeared with Multani mitti (fullers earth). This apparatus was dried in the shade, subjected to heat in electric muffle furnace (EMF) by following a specific temperature pattern. On the next day after Swangasheetikarana (self-cooling), the material was collected and ground to make powder. In subsequent Puta; an equal amount of Samaguna kajjali was added and triturated well by giving Bhavana of sufficient quantity of Jambiri nimbu swarasa, Chakrikas (round pellets) were prepared and dried. After drying, they were subjected to again heat in EMF. Up to the initial three cycles of heating, an equal amount of Samaguna kajjali was added. From 4th cycle, 1/4th amount of Shuddha gandhaka (processed sulphur) was added. Total five Putas were required to obtain Tamra bhasma that was coded as TB.

Tamra Amritikarana

Tamra bhasma was added with half part of Shuddha gandhaka and levigated with Jambiri nimbu swarasa. A round bolus of this levigated mass was prepared and dried in the shade. Surana kanda (Corm of Amorphophallus campanulatus L.) was cut into two halves horizontally. A dried bolus of levigated mass was placed in it and the two halves were joined together. Thick layers of mud smeared cloth were made covering entire corm and dried. After drying, it was subjected to heat in EMF at a temperature of 650° C for 30 minutes. It was removed after Swangasheetikarana, and the bolus was taken out, triturated and stored in the airtight glass container. It was coded as TBA.

Analysis of Samples

Tamra bhasma and TBA were labeled and analyzed through sophisticated instrumental facilities like TGA, XRD, ICP-AES, scanning electron microscope coupled WDS-SEM and particle size analyzer.

RESULTS AND DISCUSSION

Elemental composition of TB and TBA obtained through ICP-AES are placed at Table 1. Arsenic, lead, and mercury were within the permissible limit of heavy metals as
Analytical Specifications of Tamra bhasma

Table 1: Elemental composition of Tamra bhasma

<table>
<thead>
<tr>
<th>Element</th>
<th>Instrument detection limit (ppm)</th>
<th>TB (ppm)</th>
<th>TBA (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Iron (Fe)</td>
<td>0.0046</td>
<td>2.328</td>
<td>35.561</td>
</tr>
<tr>
<td>Arsenic (As)</td>
<td>0.0530</td>
<td>0.035</td>
<td>0.034</td>
</tr>
<tr>
<td>Lead (Pb)</td>
<td>0.0420</td>
<td>0.086</td>
<td>0.099</td>
</tr>
<tr>
<td>Mercury (Hg)</td>
<td>0.0610</td>
<td>0.301</td>
<td>0.727</td>
</tr>
<tr>
<td>Cadmium (Cd)</td>
<td>0.0027</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

Table 2: Thermo gravimetric analysis of Tamra bhasma

<table>
<thead>
<tr>
<th>No. Sample</th>
<th>Delta Y (%)</th>
<th>Area (µV x sec)</th>
<th>Peak height (µV)</th>
<th>Onset (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Tamra</td>
<td>8.329</td>
<td>1025.115</td>
<td>-8.582</td>
<td>474.90</td>
</tr>
<tr>
<td>2. TBA</td>
<td>20.501</td>
<td>698.530</td>
<td>437.58</td>
<td>408.36</td>
</tr>
</tbody>
</table>

Table 3: Temperature and weight changes observed in TGA analysis of TB and TBA

<table>
<thead>
<tr>
<th>No.</th>
<th>Temperature (°C)</th>
<th>Initial weight (%)</th>
<th>Final weight (%)</th>
<th>% loss/gain</th>
</tr>
</thead>
<tbody>
<tr>
<td>TB</td>
<td>800</td>
<td>100</td>
<td>91.671</td>
<td>8.329%</td>
</tr>
<tr>
<td>TBA</td>
<td>800</td>
<td>100</td>
<td>79.499</td>
<td>20.501%</td>
</tr>
</tbody>
</table>

TB: Tamra bhasma without Amritikarana, TBA: Tamra bhasma with Amritikarana

mentioned in active pharmaceutical ingredient (API),

Cadmium was not detected. Iron was present in traces in both Bhasmas that may entered into the Bhasma through iron ladle used during the pharmaceutical procedures.

During TGA, the temperature was kept on increasing rate of 20° C/minute and maximum temperature given was 800° C. Peaks obtained below 250° C represent either presence of moisture or organic compounds, which get reduced at a lower temperature. Peaks obtained in TB at 59.50° C and 102.92° C were possibly due to the presence of moisture content in the sample. Next peak obtained at 228.82° C represents oxidation of organic components. The last deviation at 500.01° C indicates the onset of decomposition in TB. The first derivative peak temperature for TBA is 437.58° C. The peak of the first derivative indicates the point of greatest rate of change on the weight loss curve. This is also known as the inflection point. The delta Y calculation was used to determine the component percentages. Increased value of delta Y indicates a reduction of organic compounds present in the sample as well as a decline in weight of sample on heating and represented by a downward deviation of the TGA curve. The observed corresponding value of delta Y in TB is 10.290 obtained at 500.01° C which may be due to the reduction of a compound of mercury and sulfur present in Kajjali (black sulfide of mercury). The value of delta Y in TBA at 437.58 is 20.501. The shape of the curve represents decomposition changes at this level, and it may be because of the presence of organic compounds added during the preparation of Tamra bhasma. In sample TB, four values of delta Y are observed in the curve which confirms that there are four distinct thermal events taking place. One value of delta Y (20.501%) is obtained in sample TBA. Fourth value of delta Y (8.329%) in TB is appeared because of some weight loss. This may be caused by chemical reactions (decomposition and loss of water of crystallization, combustion, reduction of metal oxides) or physical transitions (vaporization, evaporation, sublimation, desorption, drying). TGA analysis of TB and TBA showed 8.329 % and 20.501 % weight loss respectively (Tables 2 and 3). The difference in the TGA data of these two samples indicated a significant difference in their chemical nature as well as in their chemical properties (Figs 2A and B).

In WDS-SEM, percent value of copper and sulfur obtained in TB (80.687 % and 27.350%) is higher than TBA (75.660 % and 17.180). As Tamra Bhasma was burned in the presence of corn of Surana, multiple organo-inorganic compounds of copper and sulfur might have formed (Figs 2C and D). This may be a reason for reduction the percentage of these elements (Table 4). XRD reports revealed mixture phases of CuS along with elemental sulfur in both the samples that were supporting previous studies. Presence of sharp diffraction peaks shows the highly crystalline nature of both the samples (Figs 2E and F). In TB, it is observed with diffraction peaks at 2θ of 27.44°, 31.73°, and 32.69°. A diffraction peak at 2θ of 27.12°, 27.68°, and 31.79° was observed in TBA.

Table 5 shows that TBA sample has less minimum particle size than TB. Though, the number of heating cycles are similar in both the samples, TBA was subjected to an additional cycle of Amritikarana. This may be a reason for the decreased particle size after Amritikarana. Smallest the particle size, greatest the absorption, and fastest is the action.

Table 4: Wavelength dispersive spectroscopy results of TB and TBA

<table>
<thead>
<tr>
<th>Element</th>
<th>TB Weight (%)</th>
<th>TBA Weight (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pb La</td>
<td>-6.028</td>
<td>6.574</td>
</tr>
<tr>
<td>Hg La</td>
<td>-3.601</td>
<td>-0.525</td>
</tr>
<tr>
<td>Cu Ka</td>
<td>80.887</td>
<td>75.660</td>
</tr>
<tr>
<td>Fe Ka</td>
<td>0.521</td>
<td>0.398</td>
</tr>
<tr>
<td>Ca Ka</td>
<td>0.408</td>
<td>0.227</td>
</tr>
<tr>
<td>K Ka</td>
<td>0.221</td>
<td>0.275</td>
</tr>
<tr>
<td>S Ka</td>
<td>27.350</td>
<td>17.180</td>
</tr>
<tr>
<td>Mg Ka</td>
<td>0.440</td>
<td>0.210</td>
</tr>
<tr>
<td>Total</td>
<td>100.00</td>
<td>100.00</td>
</tr>
</tbody>
</table>
Table 5: Range of particle size of TB and TBA

<table>
<thead>
<tr>
<th>Particle size</th>
<th>TB (µ)</th>
<th>TBA (µ)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Minimum</td>
<td>5.07</td>
<td>3.13</td>
</tr>
<tr>
<td>Maximum</td>
<td>111.85</td>
<td>99.09</td>
</tr>
<tr>
<td>VMD</td>
<td>33.00</td>
<td>24.57</td>
</tr>
<tr>
<td>SMD</td>
<td>12.72</td>
<td>8.83</td>
</tr>
</tbody>
</table>

CONCLUSION

Shodhana procedure in raw copper makes it more suitable for further procedures of Mannan and Amritikarana. The use of sulfur during incineration cycles enables the conversion of Cu to CuS along with elemental sulfur in the chemical composition of both samples. Copper
content is also reduced after Amritikarana procedure. Calcination steps play a crucial role in converting the metal into nanoparticles. This work has resulted in a better understanding of the preparation protocol through different analytical characterizations. Present work can be considered as the first step toward identifying the utilized method through different advanced analytical techniques.

ACKNOWLEDGMENTS
Authors would like to acknowledge SICART, Vallabh Vidyanagar for carrying out PSD analysis; and IIT Powai, Bombay for carrying out ICP-AES, WDS-SEM, XRD, TGA.

REFERENCES
हिंदी सारांश
ताम्र भर्स का विश्लेषणात्मक विवरण

पृष्ठभूमि: आयुर्वेद में धातु और खनिज को उनके चिकित्सीय प्रयोग से पूर्व शोधन, मार्ग इत्यादि विशिष्ट प्रायोगिक प्रक्रियाओं के माध्यम से जैव अनुकूल के रूप में परिवर्तित किया जाता है। अनेक वर्षों से बिना किसी दुःखभाव के इनका चिकित्सीय उपयोग ही इसकी सुखा का एकमात्र (अतिम) प्रयाग है। लेकिन पारंपरिक शोधालों द्वारा इनमें उपस्थित मार्ग धातुओं के कारण इनकी सुखा तथा विपष्टता के विषय में प्रश्न और कौशल की जा रही है। आयुर्वेदिक ऊंचों में वर्णित चिकित्सीय प्रक्रियाओं का अनुपालन, विपष्टता का एक संबंध कारण है। साथ ही अनेक धातु भर्सों के लिए कोई मानक प्रोफाइल उपलब्ध नहीं है।

साहित्य तथा सामग्री: वर्तमान अध्ययन में दोनों नमूनों के निर्माण, वीगिक रूप, एवं कप्च एवं आकार के दौरान अपरिकृत सामग्रियों के भूमिका के निर्धारण में ताम्र भर्स (कॉपर) को अमूल्यकरण के बिना क्षेत्र उसके साथ चिकित्सक विश्लेषणात्मक प्रोफाइल के निर्माण एवं विकास का अध्ययन करना ही इस अध्ययन का उद्देश्य है।

परिणाम एवं निष्कर्ष: अध्ययन के परिणाम से यह स्पष्ट हुआ है कि नैसर्गिक क्रिस्टल आकार के कॉपर युक्त ताम्र भर्स, जिसका मानक माध्य व्यास ~ 12.72μ है वह अमूल्यकरण रिहात है एवं ~ 8.83μ व्यास के ताम्र भर्स अमूल्यकरण से युक्त है। दोनों नमूनों में सशंकप्रकार घटक के रूप में कॉपर सल्फाइड रूप (होरियूसैज) से युक्त सल्फर तथा पाया गया।

शब्दकुंजी: अमूल्यकरण, विश्लेषण, कॉपर, मानकीकरण, ताम्र भर्स.