DEVELOPMENT OF QUALITY STANDARDS OF ANCIENT SILVER BASED NANOMEDICINE: RAUPYA (SILVER) BHASMA

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ABSTRACT
Health practitioner afraid to use metal as medicine due to reported severe toxicity. However Ayurvedic system of medicine particularly Rashshastra described about metal based medicine to cure various ailments. From ancient time bhasma is used in various disease and found to be free from toxicity. As per Ayurvedic physician bhasma may be toxic if it is not prepared as per standard method mention in Rashshastra. Raupya bhasma is silver based nanomedicine of ancient Ayurveda which is used to strengthen brain, liver, heart and memory. It is also used as immunomodulator and aphrodisiac. Due to lack of scientific data over Raupya bhasma it is not as popular as other silver nanomedicine. To consider above mention fact an attempt has been taken to prepare Raupya bhasma according to ancient literature and their characterisation by modern analytical techniques. In this work, we present a systematic characterization of this traditional drug using various techniques like inductive coupled plasma mass spectroscopy (ICP-MS), X-ray diffraction (XRD), thermo gravimetric analysis (TGA), scanning electron microscopy (SEM), Fourier transform infrared spectroscopy (FT-IR) and zeta sizer. The silver content in bhasma was found to be 63.054%. The nature of bhasma was found to be microcrystalline irregular having particle size 323.8 nm. TGA analysis indicates about loss of weight with temperature. The results obtained were found to satisfactory and confirm the traditional evaluation process by modern method. In addition, some specific findings were also made which could be used as standard data for quality control of Raupya bhasma.

Keywords
Bhasma, Nanoparticles, silver nanoparticles, Raupya bhasma, Rajat bhasma, Nanomedicine

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INTRODUCTION

Modern era is of nanomedicine owing to their various therapeutic applications with more efficacies and lesser side effects. The popularity is due to their potential for achieving specific process and selectivity in pharmacological action. [1,2] Bhasma the ancient concept of nanomedicine is used treament of various chronic ailments since 7th century BC. [3,4] Bhasma literally means anything inorganic or organic burnt to ash. However metal based medicine (Ash of metal) of Ayurveda is known as bhasma. The metals used in ayurvedic system of medicine include silver (Rajata), gold (Swarna), copper (Tamra), mercury (Parada), iron (Lauha), lead (Naga) and zinc (Yasada) etc.[5] The antimicrobial activity of silver were utilized as early as 1000 BC to keep water safe. [1,6] One of silver based nanomedicine used in Ayurvedic system of medicine is raupya bhasma. The literal meaning of raupya is silver and bhasma means fine powder. Raupya bhasma is therapeutically used in neuropsychological disorder. It strengthens brain, liver and heart. It also possesses analgesic, aphrodisiac, nerve tonic and general tonic and anti-inflammatory activity. [6,7,8,9,10] The pharmacological activity and adverse effect of bhasma totally depends upon the proper preparation, dose and doses regimen. Actually the bhasmas can be toxic to humans only if they are not prepared in the correct manner. [11] The bhasma is a mystery as a medicine because different compound of a metal resulting from different methods of preparation. As per one of study tamra bhasma is composed of copper oxide while other research proves tamra bhasma is composed mainly of copper sulphite. [12,13] At a fundamental level, the bhasma as a medicine needs a detailed scientific scrutiny for their acceptance in modern society. To consider these fact raupya bhasma is prepared as per ayurvedic literature and evaluated by traditional method and by using modern analytical technique. The bhasma can be evaluated by traditional method described in ancient literature but it does not give universal acceptance, so an attempt has been taken to characterise the bhasma using traditional as well as modern analytical techniques.

MATERIALS AND METHODS

Preparation of bhasma

RB was prepared by method described in Rasendrasara Samagrahaha: an Ayurvedic text. Pure silver leaves and sulphur were taken equally by weight and mixed with half quantity of arsenic sulphide and then soaked in lemon juice and subjected to calcination process in sealed earthen container. The material was scrapped after cooling, mixed with lemon juice in mortar, pulverized and calcined again. The procedure was repeated 14 times to obtain the ash of silver. [14]

Traditional method of evaluation

The quality of bhasma was evaluated by traditional method for Nishchandratvam, Rekhapurnata, Varitara test and Unama test [3]. The procedure of traditional method of evaluation is mentioned in table-1

Physicochemical characterization of roupya bhasma

Determination of silver content

Silver content in raupya bhasma was estimated using ICP-MS [Elan-DRCe (Perkin Elmer)]. Instrument condition was as follows:

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Power</td>
<td>1100Watt</td>
</tr>
<tr>
<td>Nebulizer gas flow</td>
<td>0.93 litre/min</td>
</tr>
<tr>
<td>Auxiliary gas flow</td>
<td>1.5litre/min</td>
</tr>
<tr>
<td>Plasma gas flow</td>
<td>15 litre/min</td>
</tr>
</tbody>
</table>

Sample preparation

Silver bhasma (0.1g) was weighed accurately and transferred to a 100 ml volumetric flask, than 5 ml AR grade nitric acid was added. Flask containing silver bhasma and nitric acid was heated for 10 min on hot plate or until the sample digested. After digestion the flask was allowed to cool and volume was make up to 100 ml. The sample was filtered through 0.45 μ filter paper. Final solution was subjected to ICP-MS for quantitative estimation.

FT-IR

Samples were prepared in KBr disk (2mg sample in 200 mg KBr) with a hydraulic press by applying a pressure of 8-9 tons for 2 min. The disks were scanned over a wave number range of 4000–400 cm-1 and the resolution was kept as 4 cm-1.

SEM

Shape and surface characteristics of bhasma were investigated using SEM. The SEM samples were prepared by lightly sprinkling the bhasma on a double adhesive tape, which was stuck on an aluminum stub. The stubs were coated with gold using a gold sputter coater in a high vacuum evaporator, and samples were observed by SEM at 15 kV.

Particle size analysis

Particle size was determined using a laser diffraction particle size analyzer (Zetasizer Nano ZS90). Bhasma were suspended in the chamber of the particle size analyzer containing distilled water, and subjected to dynamic light scattering (DLS) to determine the particle size.

XRD (X-ray diffraction analysis)

The XRD powder diffraction pattern of raupya bhasma was recorded on X-ray diffractometer (X’pert pro Netherland) using CuKα radiation, l = 1.5405980 Å, filtered by nickel foil over the range 20.0–80.0°. Tension and current applied were 40 KV and 30 mA respectively.

TGA

TGA tool was utilized to determine degradation behaviour of drug at exposure of temperature, and the drug amount with no effect of temperature using Perkin Elmer TGA7.
RESULTS

Prepared bhasma was found to be satisfactory and given all positive result as described in ancient literature. The result of evaluation of bhasma by traditional method is shown in Table-1. The silver content determined using ICP-MS was found to be 63.054%. Interpretation of FT-IR spectra (Figure 1) shows bhasma is free from organic substance. The particle size of bhasma was found to be 323.8 nm by zeta sizer (Figure 2). Shape of bhasma was found to be microcrystalline irregular, using SEM (Figure 3,4). The thermogram (Figure 5) of the drug sample was recorded at temperature range of 30–960°C which showed 20.515% weight loss at temperature between 60 to 624.63°C, 6.784% weight loss in between 624.63 to 811.71°C and 2.740% weight loss in between 811.72 to 995.83 °C. The XRD pattern (Figure 6) of bhasma indicate nature of bhasma is crystalline in nature and mainly composed of argentous and argentic oxide.

<table>
<thead>
<tr>
<th>Test</th>
<th>Observation</th>
<th>Inference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nishchandratvam</td>
<td>Bhasma was observed and bright sunlight to detect the presence and absence of lustre.</td>
<td>No metallic lustre were observed even in minute.</td>
</tr>
<tr>
<td>Rekhaapurnata</td>
<td>A little amount of bhasma was rubbed in between the index finger and thumb to observe either particles can fill furrows of fingertips or not.</td>
<td>Particles of bhasma fill in furrows of finger.</td>
</tr>
<tr>
<td>Varitara test</td>
<td>A small amount of bhasma was sprinkled over the stagnant water surface.</td>
<td>Bhasma float over surface of water.</td>
</tr>
<tr>
<td>Unama test</td>
<td>It is further assessment of varitara test. A grain of rice was kept carefully on floating bhasma.</td>
<td>Rice grain float over film of bhasma on surface of water.</td>
</tr>
</tbody>
</table>

Silver is completely converted into its compound.

Size of prepared bhasma is fine.

Size of prepared bhasma is very fine.

Further confirmation of size of prepared bhasma is very fine.

**TABLE 1: Traditional method of evaluation of bhasma**

![Figure 1: FT-IR spectra of Raupya bhasma](image-url)
**FIGURE 2:** Particle size using Zetasizer

**TABLE: Zetasizer Measurements**

<table>
<thead>
<tr>
<th>Diam. (nm)</th>
<th>% Intensity</th>
<th>Width (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Z.Average (d.nm): 323.8</td>
<td>Peak 1: 631.8</td>
<td>100.0</td>
</tr>
<tr>
<td>Pd: 0.557</td>
<td>Peak 2: 0.000</td>
<td>0.0</td>
</tr>
<tr>
<td>Intercept: 0.024</td>
<td>Peak 3: 0.000</td>
<td>0.0</td>
</tr>
</tbody>
</table>

Result quality: Refer to quality report

**FIGURE 3:** SEM of raupya bhasma (X 2500)

**FIGURE 4:** SEM of raupya bhasma (X 5000)

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DISCUSSION

Heating of silver foil during sodhana may leads to increase in tension causing expansion of silver foil followed by cooling in liquid media leads to decrease in tension and increase in compression force. Repeated heating and cooling process may leads to brittleness, reduction in hardness and finally reduction in particle size. [3] Repeated calcination during marana may leads to oxidation of metal. During these oxidation, the zero valent metal state gets converted into a form with higher oxidation state and the most important aspect of this synthesis (known traditionally as ‘bhasmikarana’) is that the toxic nature (i.e. systemic toxicity causing nausea, vomiting, stomach pain, etc.) of the resulting metallic compound is completely destroyed while inducing the medicinal properties into it. [12] Decrease in particle size may leads to increase in bioavailability and decrease in dose which is directly related to reduction in toxicity. The colour of prepared bhasma was found to be black that is similar to reported colour of standard raupya bhasma. As silver sulphide and oxide are black in colour so raupya bhasma may be the mixture of both. [11] The prepared bhasma was found to be odourless, tasteless, fine in touch without any metallic sound. Metal is characterised by its metallic lustre. When this prepared bhasma was seen under day light as well as sun light it did not show any metallic lustre indicating that all silver is completely converted into bhasma (Ash). Rekhapuran test was found to be positive as bhasma fills in between the furrows of finger which indicate that bhasma is very fine in particle size. Varitara test was found to be positive as the bhasma starts floating on surface of water, again it indicate that the metal is completely converted into ash. Even the better quality of bhasma was insured by unama test as rice grain also started floating over bhasma. The result of traditional method of evaluation was summarised in table-1. There is some limitation of traditional method of evaluation as it do not give information about silver content in prepared bhasma, presence of organic substance, particle size, shape and crystalline nature. To consider this an attempt has been taken to characterise the bhasma by modern analytical techniques. Silver content was determined using ICP-MS method and found to contain 63.054% silver by ICP-MS. The absence of organic matter is confirmed by FT-IR spectra (Figure 1) which is further proof of proper incineration during the preparation of raupya bhasma. The particle size of bhasma was found to be 323.8 nm by zeta sizer (Figure-2). Shape of bhasma was found to be microcrystalline irregular, obviously due to aggregation of particle as the particle size was found to be of 1µm (1000 nm) however the average particle size determined by laser diffraction analyser was found to be 323.8 nm.

As the preparation of bhasma involves repeated calcination cycle so it was necessary to perform thermo gravimetric analysis. The thermo gram of the drug sample was recorded in air atmosphere in the temperature range of 30–960°C which showed 20.515% weight loss at temperature between 60 to 624.63°C, 6.784% weight loss in between 624.63 to 811.71°C and 2.740% weight loss in between 811.72 to 995.83 °C. The TGA thermo gram (Fig-5) can be used as standard for quality of raupya bhasma. The XRD pattern of raupya bhasma is shown in figure-6. Diffraction peak at 2 Theta = 33° and 38.5°, were obtained. The high intensity of XRD lines in the XRD pattern suggests that the drug is present as crystalline material and bhasma is composed of oxide of silver [15].
CONCLUSION

Current finding again confirm that bhasma is ancient concept of nanomedicine which is free from any organic content. Present finding provide supplementary data which can be used as standard for insuring quality of raupya bhasma. Further studies should be carried out to insure pharmacological aspects of raupya bhasma.

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Authors’ Statements

Competing Interests

The authors declare no conflict of interest.

REFERENCES