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Original Article

Preparation and Characterization of Suvarna Bhasma Parada Marit

- Characterization of Suvarna Bhasma Parada Marit -

Kapil Thakur[1]*, Ramacharya Gudi[2], Mahesh Vahalia[1], Shekhar Shitut[3], Shailesh Nadkarni[4]

[1]Department of Research and Development, Shree Dhootapapeshwar Limited, Panvel, Maharashtra, India
[2]Department of Production, Shree Dhootapapeshwar Limited, Panvel, Maharashtra, India
[3]Department of Biomedical services, Shree Dhootapapeshwar Limited, Panvel, Maharashtra, India
[4]Department of Healthcare services, Shree Dhootapapeshwar Limited, Mumbai, Maharashtra, India

Key Words
atomic absorption spectroscopy, ayurvedic formulation, bharat bhaishajya ratnakar, fourier transform infrared spectroscopy, suvarna bhasma, X-ray diffraction

Abstract

Objectives: The goal of this study was to characterize Suvarna Bhasma Parada Marit by using the Ayurvedic test parameters, physico-chemical tests, and various instrumentation techniques.

Methods: Suvarna Bhasma, an Ayurvedic formulation manufactured as per Bharat Bhaishajya Ratnakar 5/8357 (BBR), has been studied using various instrumentation techniques: X-ray diffraction (XRD), scanning electron microscopy (SEM), energy dispersive X-ray analysis (EDAX), laser particle size distribution (PSD) analysis, fourier transform infrared spectroscopy (FT-IR), and atomic absorption spectroscopy (AAS), and physico-chemical parameters, such as the loss on drying (LOD), loss on ignition (LOI), and acid insoluble Ash (AIA) were determined. In addition, Ayurvedic tests, such as Rekhapurnatva (enterable in the furrows of the fingers), Varitaratwa (floatable over water), Nirdhoomta (smokeless), Dantagre Kach-Kach (gritty particle feeling between the teeth), were performed.

Results: The XRD study showed Suvarna Bhasma to be crystalline in nature and to contain more than 98% gold. The mean size of the gold crystallites was less than 10 microns, and the morphology was globular and irregular. Suvarna Bhasma contains gold as its single and major element, with EDAX and FT-IR spectra showing that it is more than 98% pure gold. The moisture content (LOD) is less than 0.5%, the LOI is less than 2%, and the AIA is not less than 95%. The Ayurvedic tests, as specified above, helped to confirm the quality of Suvarna bhasma prepared as per the text reference (BBR).

Conclusion: This chemical characterization of Suvarna Bhasma performed in this study by using modern instrumentation techniques will be helpful in understanding its pharmacological actions and will help in establishing quality protocols and specifications to substantiate the safety, efficacy & quality of Suvarna Bhasma.

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1. Introduction

Suvarna Bhasma has a unique place in the Ayurvedic system of medicine. It is an integral part of Ayurved, which describes its usage for the treatment of patients with various chronic disorders, such as rheumatoid arthritis, anemia, cough, and nervous diseases. It is known

*Corresponding Author
Kapil Thakur. Shree Dhootapapeshwar Limited, Veer Sawarkar Chowk, Panvel, Dist. Raigad, Maharashtra 410-206, India.
Tel: +02-23-969-6457 Fax: +02-22-388-1308
E-mail: kst@sdlindia.com

for its anti-aging properties [1-3], and it acts as Rasayan, Balya, and Ojovardhak in Jeerna Vyadhi [4].

Bhasmas commonly integrate metals and minerals into herbal formulations, which is usually done for their endorsed medicinal properties and enhanced potency as defined by the world health organization (WHO) [5]. Metals, such as copper, iron, mercury, lead, and arsenic, are used in Ayurved therapeutics and are known to play important roles in biochemical processes [6-7]. In spite of the known evidence regarding the beneficial effects of Bhasmas, i.e., better healing and potency [8], many details regarding the interactions of Bhasmas with biological systems are still not known. Now, concerns that traditional medicines need to be examined along the lines of modern pharmaceutical products have been raised [9]. Therefore, if the promising clinical benefits associated with Bhasmas are to be understood, information about their chemical constituents and structures are necessary.

Very few studies have been done to characterize Suvarna Bhasma [10], especially Suvarna Bhasma Parada marit prepared as per Bharat Bhaishajya Ratnakar 5/8357 (BBR). In this research, this material was chemically characterized using various techniques: X-ray diffraction (XRD), scanning electron microscopy (SEM), energy dispersive X-ray analysis (EDAX), laser particle size distribution (PSD) analysis, fourier-transform infrared spectroscopy (FT-IR), and atomic absorption spectroscopy (AAS). The results of those characterizations are reported here.

As per Ayurved, different methods can be used for preparing Suvarna Bhasma: Ariloha marit, Parad marit, Vanaspati marit and Gandhak marit. These different methods yield the same Bhasma, but with different physico-chemical properties [11]. Therefore, the Bhasma must be standardized with reference to the classical method of preparation. In this study, we selected Parada marit Suvarna Bhasma for chemical characterization as this has been referred to as having superior quality by the seers of Ayurved.

2. Materials and Methods

The procedure for preparation was as per BBR (Fig. 1). Shuddha Suvarna (purified gold) and Shuddha Parada (purified mercury) were triturated in a Khal (instrument used for trituration) to form an amalgam, and a bolus of the amalgam was prepared. In a Sharav (earthen crucible), Erand Patra (Castor Leaf) was placed. Then, the bolus was placed with Shuddha Gandhak (purified sulphur) in equal proportions. Over this, another Erand Patra (Castor Leaf) was placed and covered with another Sharav (earthen crucible). The joined Sharav (earthen crucible) was sealed with a mud-smeared cloth, a "maatkapad"; the assembly is known as "Sharav Samputa". The assembly was placed in a Kukkut Puta Bhatti (calcination furnace) using about 30 cow-dung cakes in a 4-tier bed. The temperature in the Bhatti (furnace) was continuously monitored using a pyrograph and reached 800°C. The Bhatti (furnace) was allowed to cool on its own, which normally took three hours. The process was repeated 13 more times (Total: 14 times), which included heating with Shuddha Gandhak (purified sulphur) and cooling to obtain a fine homogenous powder. Further, the Bhasma was taken in a Khal (instrument used for trituration) and triturated with Korphad (Aloe vera) juice until the mixture was homogeneous, after which it was dried at a temperature not exceeding 70°C. Three batches of Suvarna Bhasma were prepared at the manufacturing unit of Shree Dhootapapeshwar Ltd: batch numbers P130400287, P130600028, and P121000388. All chemicals and reagents (HNO , HCL, etc.) used in the analyses were of analytical reagent grade and were purchased from Merck.

Physico-chemical parameters, such as the loss on drying (LOD), loss on ignition (LOI), and acid insoluble ash (AIA), were checked for all three batches of Bhasma [12]. The three batches were also analyzed using various Ayurvedic tests: Rekhapurnatva (enterable in the furrows of the fingers), Varitaratwa (floatable over water), Nirdhoomta (smokeless), Dantagre Kach-Kach (gritty particle feeling between teeth). An elemental assay of the gold content in Suvarna Bhasma was carried out by using a gravimetric method as per Pharmacopoeial Standards for Ayurvedic Formulations [13].

An atomic absorption spectrometer (AAS; Perkin Elmer, USA) was used for trace elemental analysis of the Suvarna Bhasma. Suvarna Bhasma was dissolved in the required quantities of acids, and after complete dissolution, the volume of the solution was brought to 25 mL by adding distilled water. Appropriate dilutions were made, and the concentrations of the elements present in the sample were determined by using flame AAS [14].

SEM and EDAX (KARL ZEISS - EVO18) were used to investigate the morphologies and the elemental compositions of the Bhasma samples. The Suvarna Bhasma (50 mg) was placed on a double-sided carbon tape and mounted on an aluminum stub. The powder sample was placed in the chamber, 10 kV was applied, and the sample was scanned at different magnifications from 1000 x to 5000 x. For the EDAX, the sample was packed into a hole in an aluminum stub (9-mm diameter, 9-mm depth). The operating parameters were 30 keV, a count rate of 1,500 ± 500 counts/s, a working distance of 10 mm, a chamber pressure set to < 2.2 10^{-4} Torr, a tilt angle of 0°, and an accumulation time of 50 s.

A Malvern Mastersizer Hydro 2000 S (A) laser particle size analyzer (Malvern Instrument Ltd, U.K.) was used for particle size distribution analyses. The prepared sample was suspended in water, and the measurement was done in an instrument that had a minimum detection limit of 0.01 microns (10 nanometers) [15]. A FT-IR spectrometer (Bruker) was used to record the FT-IR spectra of the three batches of Suvarna Bhasma, as well as that of pure gold as a reference [16]. The XRD powder diffraction patterns of the three batches of Suvarna Bhasma were recorded using an X-ray diffractometer (Shimadzu, 6000, Japan) with Cu Kα radiation, l = 1.5406 Å, filtered by using a nickel foil over the range from 20.0 to 80.0° at a scanning rate of 3°/second [17]. Pure gold was also analyzed as a reference sample.

3. Results

Physico-chemical analyses of Suvarna Bhasma were carried out to determine the moisture content (LOD), LOI, AIA, and elemental gold. The Ayurvedic parameters and the results of the organoleptic tests are tabulated in Tables 1, 2, 3. An elemental assay of gold in Suvarna Bhasma showed that it contained gold of about 98% w/w. Trace elements, silver (Ag),

Figure 1 Manufacturing process of Suvarna Bhasma as per text reference - Bharat Bhaishajya Ratnakar 5/8357.

iron (Fe), copper (Cu) and zinc (Zn), were detected by using AAS, and the results are listed in Table 4.

The infrared spectra of Suvarna Bhasma show major transmittance peaks at wave numbers of 2346 cm^{-1}, 2114 cm^{-1}, 1991 cm^{-1} and 1107 cm^{-1}. These transmittance peaks were compared with those in the spectrum for the reference pure gold sample (Fig. 2). The comparison of the spectra showed that the major transmittance peaks of the Suvarna Bhasma sample matched those of pure gold. The X-ray powder diffraction (XRPD) technique was used to characterize the compound through crystalline phase identification. The XRD patterns of Suvarna Bhasma samples from the three batches are shown in Fig. 3. Sample identification was done by matching the d-spacing with the standard JCPDS database (Table 5). The XRD peaks of Suvarna Bhasma were observed at 2 Theta values of 37.88°, 44.08°, 64.42° and 77.15°, showing that the samples were crystalline in nature. These 2 Theta values match the 2 Theta values for standard gold (Au°). Only four major peaks were observed, and the peak intensity was highest at 37.88°. The EDAX data also confirmed that the major phase contained in Suvarna Bhasma was pure gold (Figs. 4A - 4C).

The average particle sizes of the gold crystallites in the three batches of Suvarna Bhasma were calculated by using a laser particle size analyzer and were found to be less than 10 microns for all three batches (Table 6). Suvarna Bhasma P130400287 showed an average particle size of 8.44 microns, Suvarna Bhasma P130600028 an average size of 9.97 microns, and Suvarna Bhasma P121000388 an average size of 7.55 microns (Figs. 5A - 5C). The PSD showed that 10% of the Bhasma particles had sizes below 1 micron and 50% had sizes below 6 microns [18].

High-resolution images of Suvarna Bhasma captured from SEM are shown in Figs. 6A - 6C. The powder samples in the chamber were scanned at magnifications ranging from 1000 x to 5000 x, and the maxima in the PSDs for the three batches of Suvarna Bhasma occurred at particles sizes less than 9 microns. The morphologies of these particles were noted to be globular and irregular and to be agglomerated clusters of particles. Some nanoparticles were also observed to have been formed.

4. Discussion

Ayurvedic medicines did not undergo stringent quality tests in earlier days [19, 20]. However, with increased usage and consumption of Ayurvedic medicines, concern for safety, efficacy and quality has increased [21]. Suvarna Bhasma was prepared and studied with this objective - safety efficacy, and quality. Suvarna Bhasma is one of the ancient powdered Ayurvedic formulations prepared from pure gold. The metals/minerals used in Bhasma preparations are subjected to a detoxification process, thereby making them bio-compatible with strict adherence to a textual reference and thus allowing an efficacious and safe medicine to be delivered [22]. The advantages of a herbo-metallic Bhasma over a pure herbal formulation are stability and a lower dose to achieve the same effect, thus a higher efficacy [23]. For that reason, we performed a characterization of Suvarna Bhasma by using physico-chemical analyses, Ayurvedic tests, and various modern techniques.

The physico-chemical analyses showed that for all batches, Suvarna Bhasma's moisture content (LOD) was less than 0.5%, its LOI was less than 2% and its AIA was not less than 95%. The LOI measures the amount of moisture or impurities lost when the sample is ignited under the specified condi-

Table 1 Loss on drying, loss on ignition, acid insoluble ash and elemental assays of Suvarna Bhasma

No	Sample Name	Batch No.	Loss on Drying	Loss on Ignition	Acid Insoluble Ash	Gold (Au)
1	Suvarna Bhasma	P130400287	Nil	Nil	99.95%	98.00%
2	Suvarna Bhasma	P130600028	Nil	Nil	99.09%	98.33%
3	Suvarna Bhasma	P121000388	Nil	Nil	96.08%	98.41%

Table 2 Ayurvedic pariksha of Suvarna Bhasma as per Bharat Bhaishajya Ratnakar 5/8357

No	Sample Name	Batch No.	Varitaratva	Rekhapurnatva	Nirdhoo matva	Dantagre kachkach
1	Suvarna Bhasma	P130400287	Varitara	Rekhapurna	Nirdhoom	Kachkach abhav
2	Suvarna Bhasma	P130600028	Varitara	Rekhapurna	Nirdhoom	Kachkach abhav
3	Suvarna Bhasma	P121000388	Varitara	Rekhapurna	Nirdhoom	Kachkach abhav

Table 3 Organoleptic Ayurvedic Pariksha of Suvarna Bhasma

No	Sample Name	Batch No.	Mfd. Date	Varna / Color	Sparsh / Texture	Rasa / Taste	Gandha (Odor)
1	Suvarna Bhasma	P130400287	Apr-13	Light brown	Fine powder, smooth	Tasteless	Odorless
2	Suvarna Bhasma	P130600028	Jun-13	Light brown	Fine powder, smooth	Tasteless	Odorless
3	Suvarna Bhasma	P121000388	Oct-12	Light brown	Fine powder, smooth	Tasteless	Odorless

Table 4 Trace elements in Suvarna Bhasma as detected by using atomic absorption spectroscopy

No	Sample Name	Batch No.	Ag (%)	Fe (%)	Pb (%)	Cu (%)	Hg (%)	Zn (%)
1	Suvarna Bhasma	P130400287	0.05	0.17	< 0.005	0.021	< 0.001	0.020
2	Suvarna Bhasma	P130600028	0.04	0.10	< 0.005	0.020	< 0.001	0.019
3	Suvarna Bhasma	P121000388	0.04	0.12	< 0.005	0.013	< 0.001	0.021

tions. The AIA method signifies the percentage of inorganic matter, which is insoluble in 2-N HCl. In the case of Bhasmas, this method helps identify the particular Bhasma as the AIA value is product specific. The AAS results indicated the presence of gold (Au), along with trace elements such as silver (Ag), zinc (Zn), iron (Fe) and copper (Cu); the presence of these elements might be responsible for the therapeutic activity of the Bhasma. The elemental assays of the three batches of Suvarna Bhasma consistently showed that they had gold (Au) contents in range of 98% w/w. All these physico-chemical results demonstrated batch-to-batch consistency for Suvarna Bhasma.

The organoleptic tests and Ayurvedic Bhasma Pariksha (Ayurvedic tests) confirmed the descriptions of Suvarna Bhasma's properties in classical texts: i.e., Varitar - float over still water, indicating lightness & reduced particle size; Rekhapurna - enter the furrows of the fingers, indicating that the Bhasma has a fine particle size; Dantagre Kach Kachabhav - no gritty feeling between teeth, indicating that the particles present in the Bhasma are small; Nirdhoom – smokeless, indicating the presence of no fumes on ignition of the sample and the complete conversion of the Bhasma after calcination. The results of these Ayurvedic tests have now been validated with the use of modern sophisticated techniques, such as SEM-EDAX,

Table 5 Values of 2 Theta with d-spacing obtained from the XRD patterns for Suvarna Bhasma and for pure gold

No	I		II		III		IV	
	2 Theta	d-value	2 Theta	d-value	2 Theta	d-value	2 Theta	d-value
Pure Gold	37.9136	2.37121	44.1708	2.04873	64.4045	1.44546	77.4087	1.23188
P121000388	37.8821	2.37311	44.0866	2.05245	64.2156	1.44926	77.1578	1.23526
P130400287	37.9960	2.36626	44.2067	2.04715	64.4264	1.44502	77.3909	1.23212
P130600028	37.8905	2.37260	44.0945	2.05210	64.3106	1.44734	77.2763	1.23366

XRD, X-ray diffraction.

Table 6 Particle sizes for Suvarna Bhasma as obtained by using a Malvern Mastersizer Hydro 2000S (A) unit

No	Sample Name	Batch No.	Average Particle Size
1	Suvarna Bhasma	P130400287	8.44 um
2	Suvarna Bhasma	P130600028	9.97 um
3	Suvarna Bhasma	P121000388	7.55 um

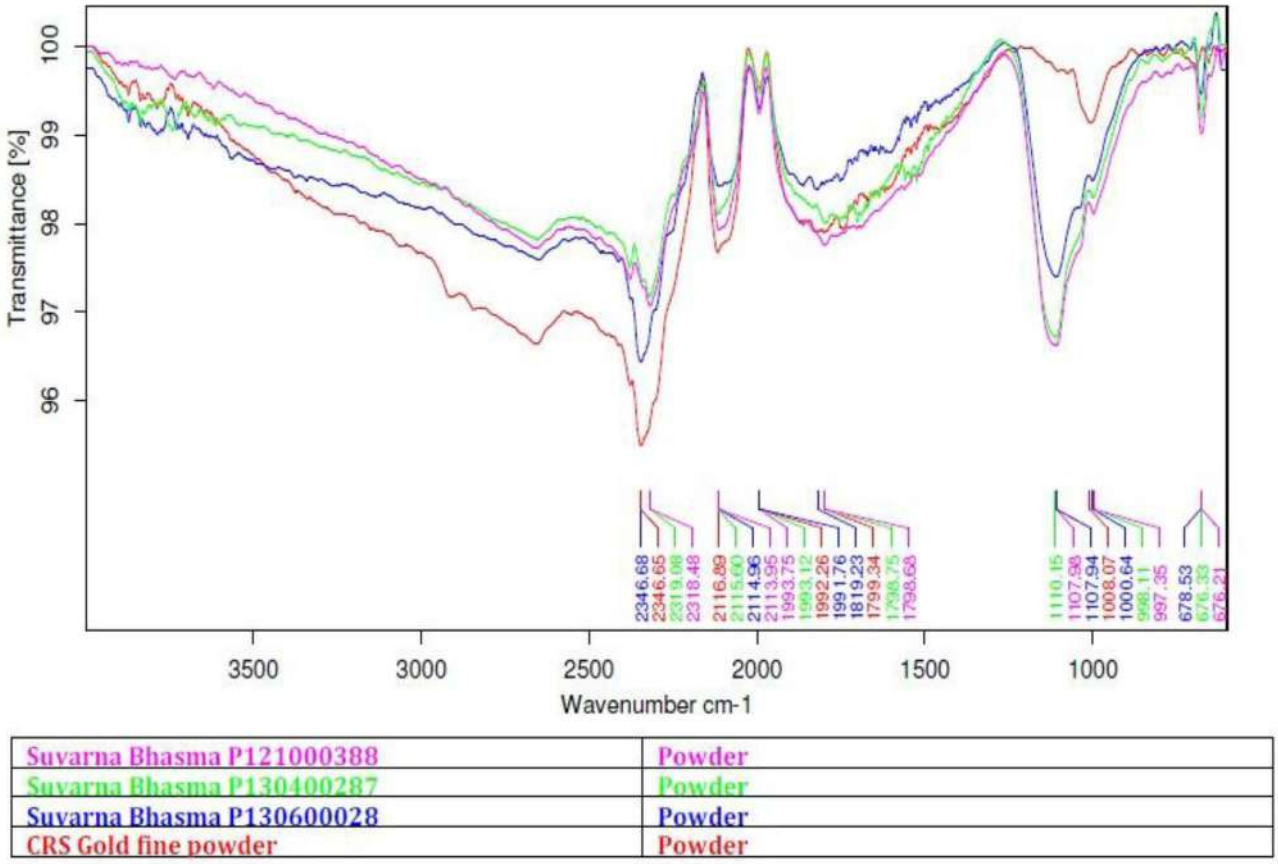
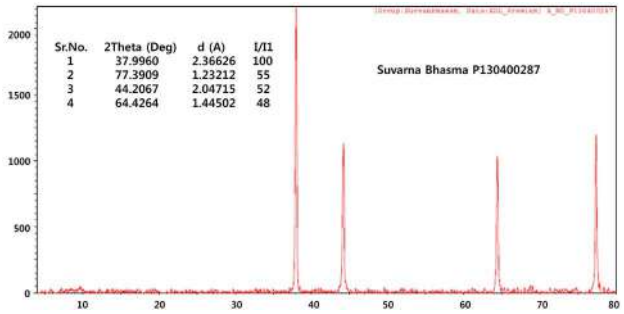


Figure 2 Infrared spectra of Suvarna Bhasma - transmittance showing similarity with that of gold (Au).



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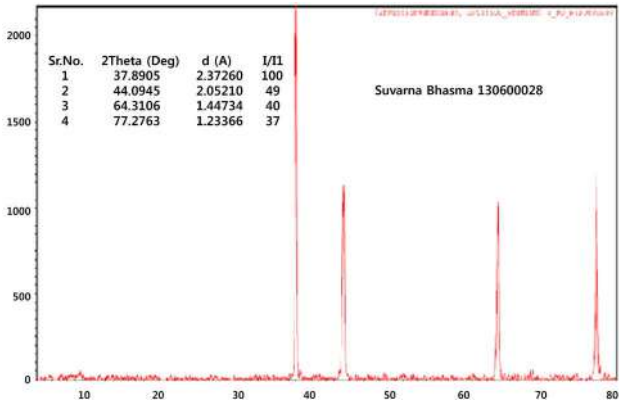

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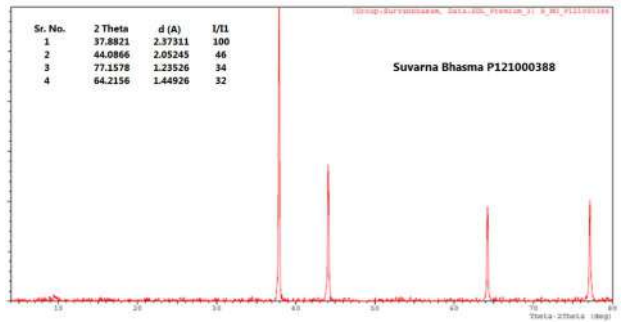

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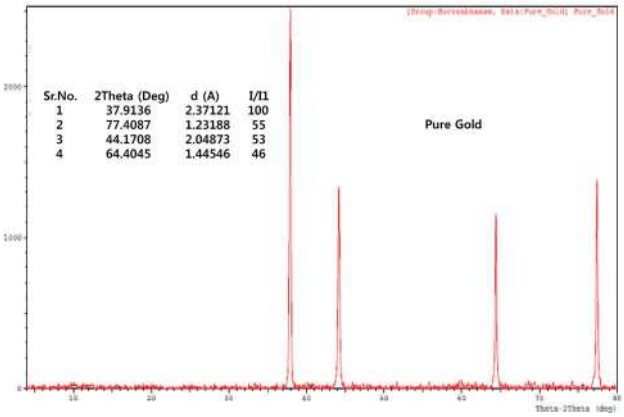
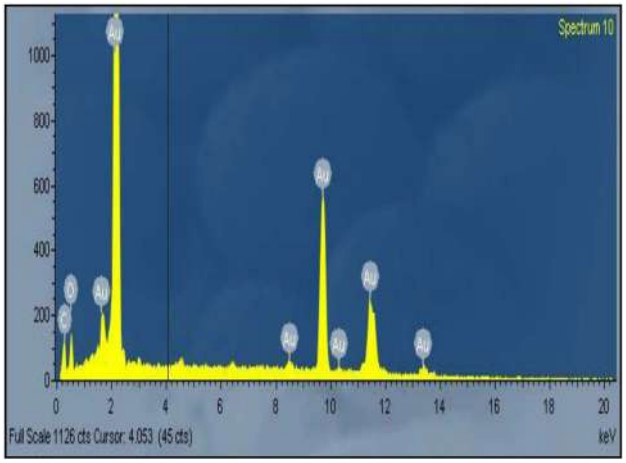
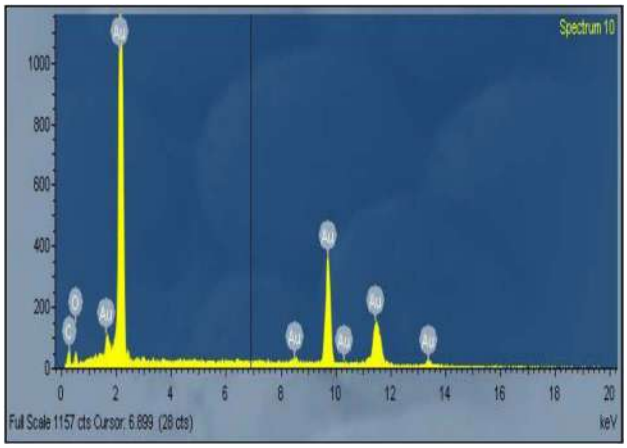


D

Figure 3 X-ray diffraction patterns of Suvarna Bhasma with d-spacing values at corresponding 2 Theta (degree), (A) P130400287, (B) P130600028, and (C) P121000388, and (D) the pattern for pure gold (Au).



A



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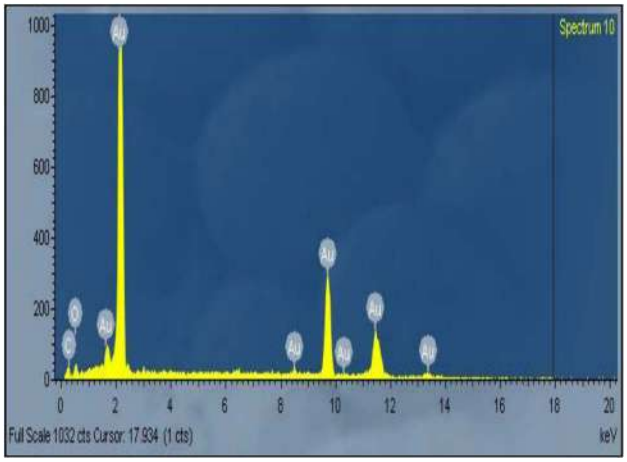
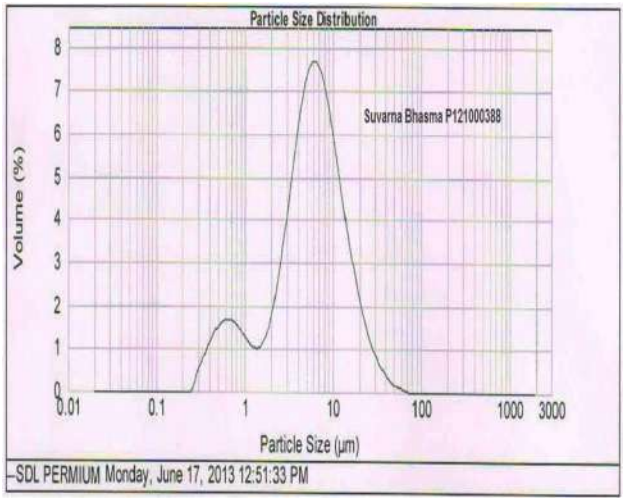


C

Figure 4 Energy Dispersive X-Ray (EDAX) analyses of Suvarna Bhasma, (A) P121000388, (B) P130600028, and (C) P130400287, showing Gold as the major peak.



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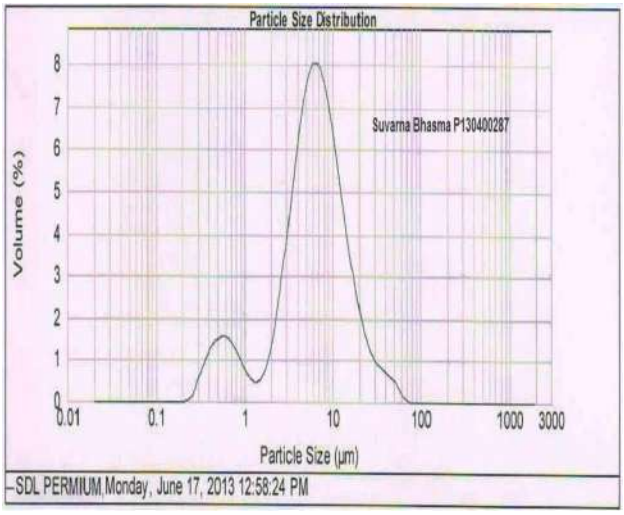

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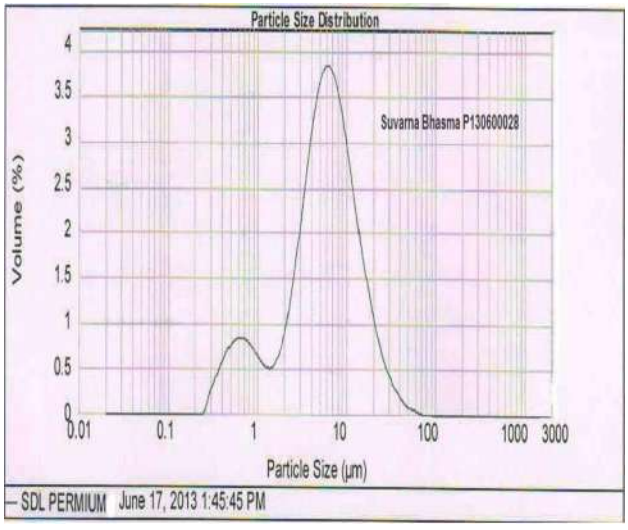
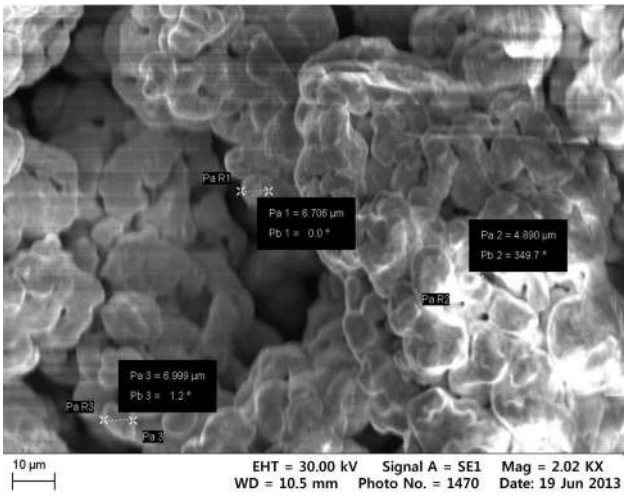


C

Figure 5 Particle size distribution of Suvarna Bhasma, (A) P121000388, (B) P130400287, and (C) P130600028. The average size is less than 10 microns.



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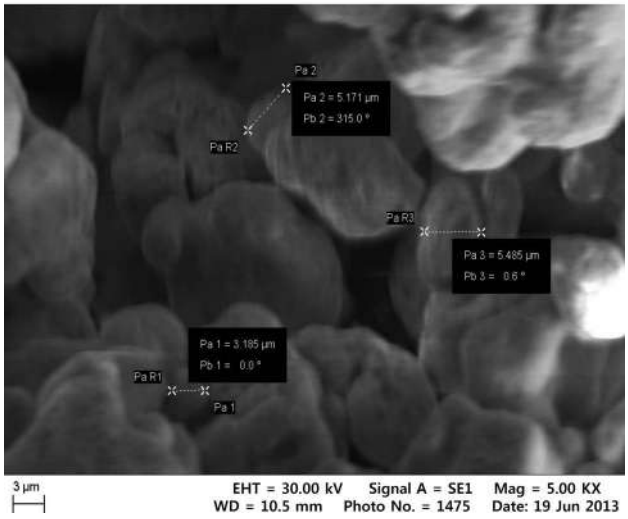

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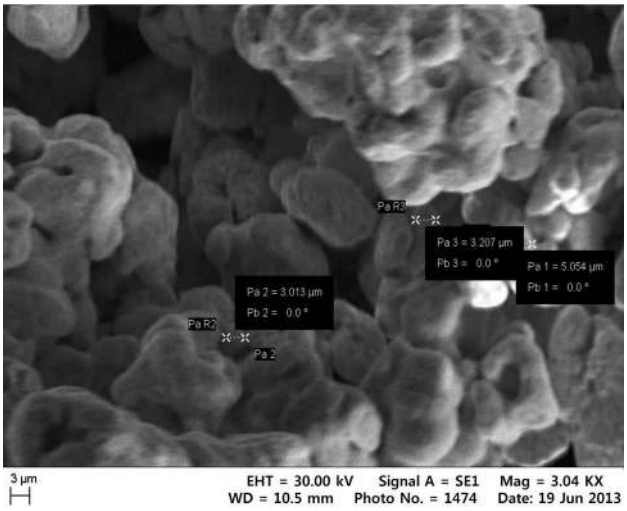

C

Figure 6 SEM photomicrographs of Suvarna Bhasma at magnifications of (A) 2000x, (B) 3000x, and (C) 5000x (30 kV).

XRD, PSD analyses, etc.

SEM helps in studies of the morphologies and the compositions of biological and physical materials. SEM on Suvarna Bhasma depicted the formation of agglomerated clusters of particles. This behavior is thought to be due to the Puta (calcination) processes involved in the manufacture of Suvarna Bhasma. High-resolution images of the morphology or the topography of a specimen, with great depth of field, at very low or very high magnifications can be obtained by using SEM.

The PSD results demonstrate that small particle sizes can be achieved by using proper levigation, trituration and incineration. The size of the particles also proves the Ayurvedic concept of Puta (calcination) for enhancing the bioavailability and the efficacy of a drug by minimizing the required dose, as well as the associated adverse effects, hence ensuring safe therapeutic use [24]. The processing of Suvarna by trituration and repeated calcinations resulted in micronizing the Bhasma. PSD graphs indicated that the volumetric mean diameter of the particles in Suvarna Bhasma was in the range from 7.55 microns to 9.97 microns. Further, the particle size distribution is also known to be linked with the physical and the chemical properties of a drug, such as its stability and chemical reactivity. SEM measurements and PSD analyses are tools to confirm objectively the Bhasma Pariksha of Rekhapurnatva.

The transmittance peaks in the FT-IR spectra for the three batches of Suvarna Bhasma correspond closely with the transmittance peaks of pure gold. This finding was also observed in an earlier infrared study [10]. XRD is used to identify crystalline compounds based on their diffraction patterns. The XRD patterns obtained from the three batches of Suvarna Bhasma confirmed their crystalline natures. The 2 Theta values and the d-values from the XRD peaks of Suvarna Bhasma corresponded closely to those from the XRD peaks for the pure gold reference sample. This reveals that the crystalline phase of Suvarna Bhasma is the same as that of the pure gold (Au^{o}) standard. EDAX, which is a less standard technique, allows a faster analysis of the sample and can be used to predict the chemical composition and moiety of a compound [3]. The EDAX results in this study suggest that the major phase of Suvarna Bhasma is pure gold, which is in agreement with the other results.

5. Conclusion

Suvarna Bhasma is an Ayurvedic formulation manufactured using a textual method that involves repeated calcinations and triturations. The mean size of these gold crystallites was less than 10 microns, and the morphology was globular and irregular. The Bhasma particles, which included a few nano-sized particles, were observed to form agglomerates, which was caused by the calcination processes. EDAX, AAS, XRD, FT-IR and elemental assay findings confirmed that Suvarna Bhasma contained gold as its single and major element, being more than 98% pure gold. Bhasmas are known to be herbo-metallic complex moieties, and such chemical characterizations will be helpful in understanding the pharmacological actions of Suvarna Bhasma and will serve as a tool for re-defining the quality specifications. Thus, the findings of this research will help in establishing well-documented specifications for the quality of Suvarna Bhasma, as well as well-documented protocols for substantiating that quality.

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Conflict of interest

The authors declare that there are no conflicts of interest.

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