

# Physicochemical characterization and antibacterial activity of *Rajata Bhasma* and silver nanoparticle

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## Abstract

**Introduction:** *Bhasma*, an Ayurvedic metallo-mineral preparation, is claimed to be biologically produced nanoparticles. *Rajata* (silver) is a noble metal known for its antimicrobial activity. *Rajata Bhasma* (RB) is expected to be composed of nanoparticles. With all these facts in place, this study was conducted to evaluate RB for the presence of silver nanoparticle (SNP) and its antimicrobial effect. **Aim:** The aim of this study is to analyze the physicochemical characterization, antibacterial activity of RB, and SNP. **Materials and Methods:** RB was commercially ordered and SNP was prepared by *Turkevich* method. Characterization of RB was carried out by scanning electron microscopy (SEM) and inductively coupled plasma-atomic emission spectroscopy (ICP-AES). SNP was characterized by ultraviolet (UV)-visible spectroscopy, transmission electron microscopy (TEM), and ICP-AES. Antibacterial activity of RB and SNP was carried out by well-diffusion method. **Results:** Analysis of RB by SEM revealed particles in range from 10 to 60 nm. UV-visible spectrum of the aqueous medium containing SNPs showed absorption peak at around 423 nm. The TEM analysis showed that SNP was spherical in the range of 5–50 nm and uniformly distributed without significant agglomeration. The content of silver in RB measured with ICP-AES was found to be 70.56% whereas in case of SNP was 65.23%. *Staphylococcus aureus* was found to be sensitive to both RB and SNP. *Escherichia coli*, *Pseudomonas aeruginosa*, and *Enterococcus faecalis* were found to be resistant to RB as well as SNP. **Conclusion:** The current study shows that RB does have silver particles in the size of nanometers and also has mild antibacterial activity.

**Keywords:** Antibacterial activity, physicochemical characteristics, *Rajata Bhasma*, silver nanoparticles

## Introduction

*Ayurveda* is as old as Indian civilization. Silver called as *Rajata* is an important metal having high therapeutic value. *Bhasmas*, metallo-medicine, are basically made from metals and minerals. The process of *Bhasmikaarana* is used to transform metals and minerals into bioassimilable form, i.e., *Bhasmas*. The metals and minerals obtained from ore have to undergo various processes (*Sansakaras*) such as *Shodhana* and *Marana*. These combinations are then used to treat illness.<sup>[1]</sup> In this process, raw metal is converted into therapeutic form through the classical processes by repeated incineration and grinding with some herbal juices and other specified matters. The product of the above method is called as “*Bhasma*.” Thus, *Bhasmas* are metallo-medicines in powdered form which consist of nanocrystalline particle with submicron size.<sup>[2]</sup>

*Bhasma* is claimed to be biologically produced nanoparticles, which are prescribed with several other medicines of

*Ayurveda*.<sup>[3]</sup> The concept of using metals in the form of reduced sized particles is age old since *Charaka Samhita* (1500 BC).<sup>[3]</sup> *Rasayana* (immunomodulation and antiaging quality) and *Yogavahi* (ability to target drugs to the site) are the characteristics of a properly made herbo – mineral/metals/nonmetals preparation, which is also nontoxic, gently absorbable, adaptable, and digestible in the body.<sup>[4,5]</sup>

There are literatures which show that *Rajata Bhasma* (RB) has anxiolytic effect, free radical scavenging effect, and can reduce infertility.<sup>[6-8]</sup> Although several reports prove the antibacterial activity of silver, however to the best of our knowledge, there are no articles which describe the antibacterial activity of RB.

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Recently, synthesis of silver nanoparticles (SNPs) has proliferative activity against many cancerous cell lines, useful interaction with HIV, and bactericidal effect.<sup>[9,10]</sup> SNPs are normally synthesized chemically and also involve the usage of toxic and potentially hazardous chemicals which may pose potential environmental and biological risks.<sup>[11]</sup> Such risks can be taken care of with the usage of *Bhasma*.

The current study focuses on whether RB does contain nanocrystals. The present study aims at to determine the content and size distribution of particles of RB available commercially and to evaluate whether RB possesses antibacterial activity as compared to the SNPs as claimed by many other research literature. The outcome of the above study will contribute immensely to the existing scientific evidence of the chemical and pharmacological properties of RB.

## Materials and methods

### Procurement of *Rajata Bhasma*

One bottle of RB was purchased from Medical Practitioners Co-Operative Pharmacy and Stores Limited, Mumbai, India, and designated as RB [Figure 1a].

### Characterization of *Rajata Bhasma*

Scanning electron microscopy (SEM) was performed to find the particle size of RB. The colloidal solution containing RB was centrifuged at 5000 rpm for 20 min, and the resulting suspension was redispersed in 10 ml sterile distilled water. The centrifuging and redispersing process was repeated three times. The supernatants were discarded and the final pellets were dissolved in 0.1 ml of deionized water. The pellet was mixed properly and carefully placed on a glass coverslip followed by air-drying. The size distribution and surface topology of particles from commercial sample of RB were qualitatively assessed using a field emission gun-scanning electron microscope (FEG-SEM) (JEOL JSM-7600F). SEM was performed in the Department of SAIF, IIT-Mumbai. The commercially procured RB was analyzed for its silver

content by inductively coupled plasma-atomic emission spectroscopy (ICP-AES) (Model No: ARCOS, Simultaneous ICP-AES) with the wavelength range of 130 nm to 770 nm. The known amount (5 mg) RB was dissolved of in 25 ml Aqua regia (3:1 of HCl and HNO<sub>3</sub>) for the analysis. A volume of 1 ml from the preparation was taken and made up to 25 ml with D/W.

### Preparation of silver nanoparticles

SNPs were prepared by the well-known *Turkevich* method; the method pioneered by *Turkevich et al.*<sup>[12]</sup> It involves the chemical reduction of metal salts and is the simplest and most commonly used bulk synthesis method. The particle synthesis usually makes use of a metal salt, a reducing agent, and a stabilizer. In the experiment, silver nitrate (AgNO<sub>3</sub>) was used as a metal salt and trisodium citrate was used as a reducing agent. AgNO<sub>3</sub> in deionized water was heated until it began to boil. Sodium citrate solution was added to the AgNO<sub>3</sub> solution as soon as the boiling commenced. The color of the solution slowly turned into pale yellow, indicating the initiation of reduction of the Ag<sup>+</sup> ions. Heating was continued for an additional 6–8 min that showed a gradual color change from pale yellow at 3 min to golden yellow at 4 min, then to reddish brown at 6 min. The solution was cooled to room temperature for further experiments and was designated as SNP [Figure 1b].

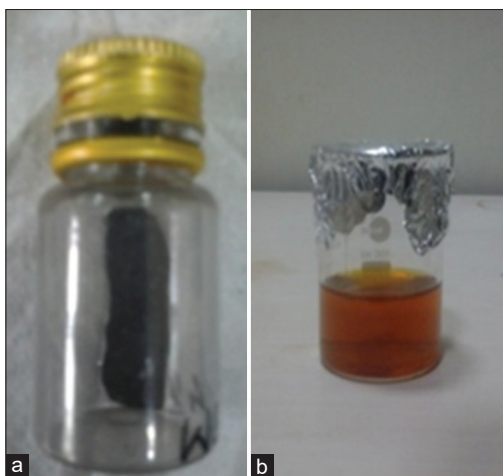
### Characterization of silver nanoparticles

Ultraviolet (UV)-visible spectra of colloidal solution were evaluated on Shimadzu's UV-visible spectrophotometer from 350 to 600 nm. The absorption phenomenon shown by the nanoparticles is due to surface plasmon resonance. The position and shape of the plasmon absorption of nanoparticles are strongly dependent on the particle size, dielectric constant, and surface-adsorbed species. Further characterization was done, and transmission electron microscope (TEM) (JEOL, JEM-1011; Japan) was used to determine the size, shape, and the size distribution of the SNPs. Samples were prepared by placing a drop of working solution on a carbon-coated standard copper grid (300 meshes) operating at 80 kV. FT. Contents of silver were obtained by ICP-AES and it was performed in the Department of SAIF, IIT-Mumbai.

### Antibacterial activity of silver nanoparticles and *Rajata Bhasma*

Aqueous extraction of *Bhasma* was carried out by decoction process.<sup>[13]</sup> This was carried out by boiling in hot water; in this process, one part of dried powder *Bhasma* and 5 parts of sterilized water were taken in a boiling water flask and boiled for 15–20 min. In the present study, 100 mg of RB was dissolved in 500ml of distilled water; after boiling, the sample was filtered through Whatman filter paper no. 1, autoclaved at 121°C for 15 min and kept in clean and sterilized test tubes, and stored at 4°C till further use.

The antibacterial property of the SNP and RB was evaluated by well-diffusion method.<sup>[14]</sup> The test organisms used were two Gram-positive organisms such as *Staphylococcus aureus* and *Enterococcus faecalis* and two Gram-negative organisms



**Figure 1:** (a) Commercially purchased *Rajata Bhasma*. (b) Silver nanoparticles synthesized using sodium citrate

such as *Escherichia coli* and *Pseudomonas aeruginosa*. The inoculums were matched with the McFarland turbidity standard. The inoculums were then spread on Mueller-Hinton agar by a sterile cotton swab. Wells were punched using a 6mm cork borer. Different concentration of the samples of both RB and SNP (100 µg/ml) were prepared. After solidification, the filter paper wells (5 mm in diameter) impregnated with the samples were placed on test organism-seeded plates. Streptomycin sulfate (10 µg/ml) was used as positive control and distilled water (100 µg/ml) was used as negative control. The antibacterial assay plates were incubated at 37°C for 24 h. The diameters of the inhibition zones were measured in millimeter.

## Results

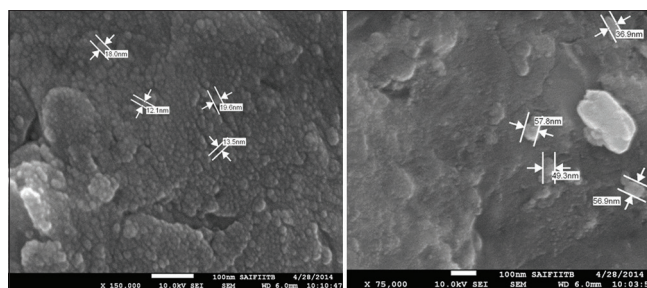
Analysis of the RB by SEM revealed highly heterogeneous particles. The study revealed regular and uniform arrangement of cluster of granules in RB. They were found to be of many irregular shapes such as round, elliptical, elongated. The sizes of the particles ranged from 10 to 60 nm as shown in Figure 2.

The content of silver in the RB was measured with inductively coupled plasma-atomic emission spectroscopy. The content ranged from 70% to 98% and the silver content of the RB was found to be 70.56%, whereas in case of SNPs, it ranged from 60% to 70% and the exact content was 65.23%.

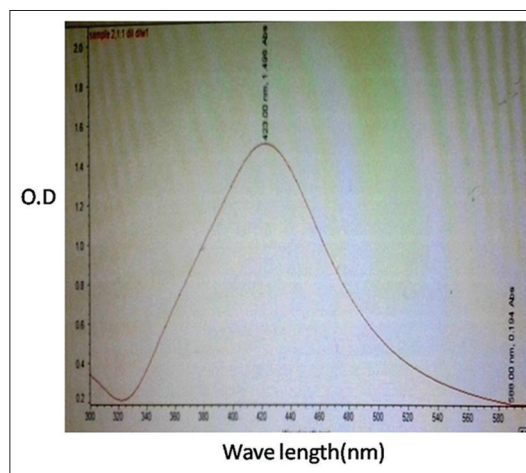
The SNPs were prepared as per the standard procedure of *Turkevich*. The color of the solution turned into pale yellow initially, and on further, boiling gave a golden yellow color indicating the formation of SNPs as shown in Figure 1. SNPs exhibit yellowish color in aqueous solution due to excitation of surface plasmon resonance band in the UV-visible region. It is generally accepted that UV-visible spectroscopy could be used to examine size- and shape-controlled nanoparticles in aqueous solution.<sup>[13]</sup> Figure 3 shows the UV-visible spectra recorded from the reaction medium. The absorption spectra of silver solution consists a single sharp surface plasmon resonance band at 423 nm. The most characteristic part of silver solution is a narrow plasmon absorption band observable in the 350–600 nm regions.

TEM provided further insight into the morphology and particle size distribution profile of SNPs. The data obtained from transmission electron micrograph showed distinct shape and size of nanoparticles. The particle was spherical in the range of 5–50 nm and uniformly distributed without significant agglomeration [Figure 4].

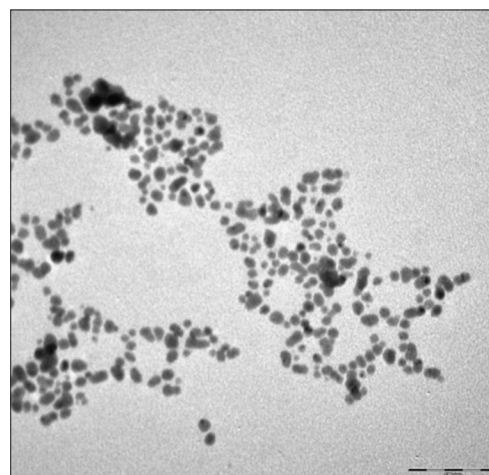
To study the antibacterial activity of both SNP and RB, each was introduced at concentrations as mentioned earlier in all agar plates and incubating for 24 h; *S. aureus* was found to be sensitive to both RB and SNP. In case of RB, a clear zone of inhibition of 8 mm was evident only in *S. aureus* [Figure 5] while *E. coli*, *P. aeruginosa*, and *E. faecalis* were found to be resistant to RB as well as SNP's.



**Figure 2:** Scanning electron microscopic images of *Rajata Bhasma* (RB1a and RB1b) of size ranges between 10 and 60 nm, at different magnification ranges from  $\times 75,000$  to  $\times 150,000$



**Figure 3:** Ultraviolet-visible absorption spectrum of silver nanoparticles prepared using sodium citrate solution

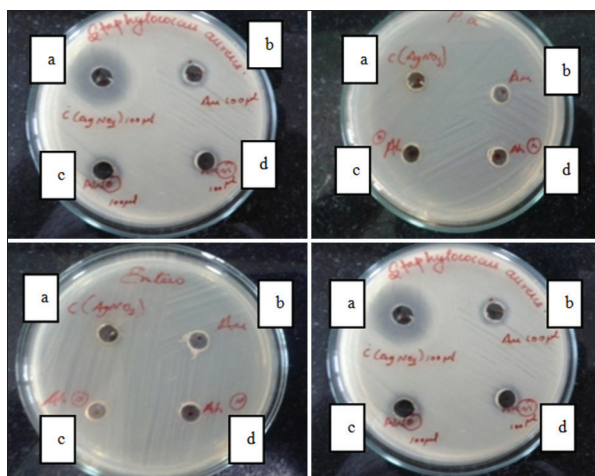


**Figure 4:** Transmission electron microscopic image of silver nanoparticles of size ranges between 5 and 50 nm

## Discussion

*Bhasma* is sole of Ayurveda metal-based preparations made by following complex pharmaceutical processes incorporating herbs, converting them into a suitable form. They are preventive and complementary alternate medicine (CAM) used in the Indian subcontinent since seventh century BC<sup>[15,16]</sup>





**Figure 5:** Antibacterial activity of silver nanoparticle and Rajata Bhasma against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Enterococcus faecalis*, and *Escherichia coli*. Control used is silver nitrate. (a) Control silver nitrate. (b) Ag silver nanoparticle. (c) Rajata Bhasma. (d) Distilled water

and widely recommended for treatment of a variety of chronic ailments. In today's modern period, traditional Indian medicine practices are now being compared with allopathic practices. As per the WHO report, 80% of the people who live in developing countries commonly use CAM.<sup>[17]</sup> RB is being extensively used in Ayurveda medical practice. It possesses aphrodisiac, antiaging, and immunomodulatory properties.<sup>[18]</sup> Structural and chemical transformation of metal into metal compounds (*Bhasma*) which are bioabsorbable is the main objective of *Marana*. One of the major concerns with these age-old practices is that there is no SNP for their production, and literature has numerous procedures for the production of a single medicine. Characterization of *Bhasmas* using scientific techniques is therefore necessary to determine the effect of the process which employs addition of unusual and exotic materials. These tests would also give a fair idea regarding its safety and efficacy. Although there are certain Ayurveda parameters (physicochemical characterization) which can be used to test the prepared *Bhasmas*, they are qualitative in nature and neither offer guidelines for characterization nor any insight into its mechanism of action. Nanotechnology is made possible by sophisticated analytical techniques such as TEM, scanning tunneling microscope, and atomic force microscope. Thus, using these instruments, nano nature of *Bhasma* can be made to reality.<sup>[19]</sup> Hence, in this study, modern analytical parameters, namely, SEM, TEM, ICP-AES, and UV-visible spectroscopy were used. The ICP-AES analysis of the *Bhasma* showed the silver content in them to be 76%–90%. The impurities which were not identified could be due to the ingredients used during the ayurvedic procedures of the *Bhasma* preparation. The SEM analysis of the *Bhasma* preparation revealed a highly heterogeneous mixture. The most important observation in the SEM studies was with respect to the size of the *Bhasma*. As seen in Figure 2, particles of 10–60 nm were found in the RB. A recent study carried out on *Jasada Bhasma* also depicted

that a clearly identifiable fraction of the particles is seen in the nanometer size range.<sup>[20]</sup> The development of easy, reliable, and eco-friendly methods helps to increase interest in the synthesis and application of nanoparticles that are beneficial for humankind.<sup>[21]</sup>

Silver and many other metals are used in modern medicines. They are well known to have antibacterial effect and are used in as life-saving drugs against infectious diseases.<sup>[19]</sup> Role of these preparations for curing various diseases such as eczema, vitiligo, and leprosy has been studied earlier.<sup>[22]</sup> Most of these medicines are mixture of compounds and because of its synergistic actions; toxicity is being diminished thereby providing promising direction for combination therapy. Many commercially available *Bhasmas* such as *Mandura Bhasma*, *Lauha*, and *Tamra Bhasmas* have been studied for their antibacterial activity and have been found effective.<sup>[23–25]</sup> In the current study, antibacterial activity of RB (500 ug) and SNP (500 ug) at the present concentration was evident only against *S. aureus*. All other organisms were found resistant to both RB and SNP. This initial study gives promising result and will require further in debt study with modern techniques to standardize and bring out high-quality herbal products in terms of RB which can have a substantial antibacterial effect on various pathogens.

## Conclusion

Average particle sizes were in the range of 10–60 nm for RB and 5–50 nm for SNP. *Bhasma* samples have varying composition from supplier to supplier. Almost 90% matters in the *Bhasma* are soluble and were analyzed using ICP-AES analysis and found to have 76%–90% silver content. Antibacterial activity of RB (500 ug) and SNP (500 ug) at the present concentration was evident only against *S. aureus*. All other organisms were found resistant to both RB and SNP. Taking inspiration from the fact that *Bhasma* contain submicronic or nanoparticles that enhance bioavailability, metal-based nanomedicines can give a scientific background. This would help in utilizing the age-old wisdom of Ayurveda for the development of newer drugs in modern medicine.

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Nil.

## Conflicts of interest

There are no conflicts of interest.

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## हिन्दी सारांश

### रजत भस्म तथा सिल्वर के अतिसूक्ष्म कणों के भौतिक, रासायनिक एवं जीवाणुरोधी प्रतिक्रिया का अध्ययन

रेवती शर्मा, अहसान भट्ट, मानसी ठाकुर

भस्म एक आयुर्वेदिक धातु विनिर्मित पदार्थ है जिसे कि अतिसूक्ष्म कणों से युक्त होने का दावा किया जाता है। रजत एक उत्तम धातु है जो रजत के अति सूक्ष्म कणों से संयुक्त है तथा इसे जीवाणुरोधी गतिविधि के लिए जाना जाता है। इन सभी तथ्यों के साथ एस एन पी (सिल्वर नैनो पार्टिकल) की मौजूदगी और इसके रोगाणुरोधी प्रभाव के लिए रजत भस्म का मूल्यांकन करने के लिए इस अध्ययन का आयोजन किया गया है अतः प्रस्तुत शोध रजत भस्म एवं एस एन पी के भौतिक लक्षणों का विश्लेषण करने और जीवाणुरोधी गतिविधि का अध्ययन करने के हेतु से किया गया है। अध्ययन हेतु पहले से ही निर्मित रजत भस्म को लिया गया था एवं एसएनपी को तुर्कोविच पद्धति द्वारा तैयार किया गया था। रजत भस्म का विश्लेषण स्केनिंग इलेक्ट्रॉन माइक्रोस्कोपी (एसईएम) और इंडक्टिव रूप से युग्मित प्लाज्मा परमाणु उत्सर्जन स्पेक्ट्रोस्कोपी (आईसीपी-ईईएस) द्वारा किया गया था। एस एन पी का परीक्षण यू वी विजुअल स्पेक्ट्रोस्कोपी ट्रांसमिशन इलेक्ट्रॉन माइक्रोस्कोपी (टीईएम) और आईसीपी-ईईएस से किया गया। रजत भस्म और एस एन पी की जीवाणुरोधी गतिविधि वेल डिफ्रूसन पद्धति द्वारा की गयी। एसईएम द्वारा रजत भस्म के विश्लेषण में १० से ६० एनएम श्रेणी में कण पाये गए, यूवी विजुअल स्पेक्ट्रम द्वारा एसएनपी के जलीय माध्यम ने ४२३ एनएम पर अधिक अवशोषण दिया। टीईएम विश्लेषण से ज्ञात हुआ कि एसएनपी के गोलाकार कण ५-५० एनएम परिसर में समान रूप से व्याप्त थे। रजत भस्म में रजत की मात्रा ७०-५०% तथा जबकि एसएनपी ६९.२३% मौजूद थे। स्टेफाइलोकॉक्स आरियस रजत भस्म और एसएनपी दोनों के प्रति संवेदनशील था। एस्चेरीचिया कोली, स्यूडोमोनास एरुगिनोसा, एंटरोकोकस फ़सलीस, रजत भस्म तथा एसएनपी के प्रतिरोधी पाये गए। अतः प्रस्तुत शोध से यह पता चलता है कि रजत भस्म और एसएनपी में मध्यम रूप की जीवाणुरोधी प्रतिक्रिया होती है।