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Research Article

PHYSICO- CHEMICAL STANDARDIZATION OF TRADITIONAL

"MEDICINE", MUKTA BHASMA IN NANOPARTICLES

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ABSTRACT

Mukta or Pearl, a valuable gem is included in Ratna varga in most of the Ayurvedic texts. As far as the Ayurvedic Pharmaceutical Science is concerned, selection of raw material is the nucleus of the present study which is important step in standardization. Pearl is available in natural and cultured form. However the scarcity and price has limited the use of natural Pearl in medicine. Thus cultured Pearl was used for the study after thorough analysis using ancient as well as modern techniques like Microscopic study. Pearl was subjected to simple and special purification by Swedan in freshly prepared juice of lemon (Citrus acida Linn) and leaves of Jayanti (Sesbania sesban (Jacq.) W. Wight) respectively. Rose water was prepared by a classical method of distillation so as to preserve its essential volatile oils. Mukta Bhasma (MB) was prepared by triturating purified Pearl with Rose water. It was then subjected to heat in 3 laghu puta (puta system of heating) and for firing in each puta, approximately 1.5kg cow dung cakes were used. To assure the quality of MB, Rasa-shastra quality control tests like Nishchandratva, Rekhapurnatva and Varitaratva were used. After MB was found to be in compliance with these tests, it was further analyzed using X-ray diffraction (XRD) analysis. Analysis of raw Pearl and MB revealed that raw Pearl contain $CaCO_3$ in Aragonite form and $CaCO_3$ in Calcite form respectively. Determination of particle size was done by Dynamic light scattering (DLS) and Nanoparticle tracking analysis (NTA) for raw Pearl and MB respectively. The particle size was reduced greatly from114.019 µm to 156 nm. It may be concluded that closed oxidation of Pearl at the temperature up to 700 °C resulted in transformation of Aragonite form into Calcite form. Also thorough trituration, levigation and heat treatment can reduce particle size up to nanometers. Thus it can validate the sukshmata of Bhasma and can be correlated with concept of nanomedicine, essential for its quick and cellular activity.

KEYWORDS : *Mukta Bhasma, X-ray Diffraction, Dynamic light scattering, Nano-particle tracking analysis.*

INTRODUCTION

Variations in the raw material used as well as failure in following standard operating procedures (SOP) for bhasma preparation leads to alteration in properties of the finished product. Standardization is a measurement for ensuring the quality and is used to describe all measures, which are taken during the manufacturing process and quality control leading to reproducible quality. Thus for quality assurance, there is an urgent need of standardization of preparation process and end product. MB, a unique herbo-mineral preparation has been used in large proportion in diseases like Tuberculosis, Diabetes, Asthma, cough and various diseases due to vitiated Vata [1] owing to its miraculous results. Although classical texts of Rasa-shastra negate preparation of bhasma using gems, their methods have also been described. MB is prepared by triturating pearl powder

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with rose water. It was then subjected to heat in 3 laghu putas and for firing in each puta, approximately 1.5 kg cow dung cakes were used. Trituration, levigation and heat treatment greatly reduce the particle size of pearl. Various milling parameters (Milling temperatures, nature of products and number of phases present during mechanical milling and alloying) have a pronounced influence on limiting attainable grain size and product phases [2]. Ayurvedic concept of Mardan (Trituration) and Bhavana (Levigation) to reduce particle size is an ultimate result of these processes [3].The studies have confirmed that Bhasmas, which are unique Ayurvedic metallic/mineral preparations are biologically produced nanoparticles prescribed with several other medicines of Ayurveda. Studies have also established that manufacturing methods of Bhasma are in tune of nanotechnology of modern era and bhasmas are nearer to nanocrystalline materials, similar in physico-chemical properties. Till date no valid data is available which can confirm its physico-chemical parameters and activities mentioned in various Ayurvedic literatures. So an attempt was made to assess structural form as well as particle size to confirm its properties and to validate it as nanomedicine. The Analytical presentation of bhasma on the basis of modern parameters has become the need of time as it allows us to study the quality, potency and assists in negating toxicity, thus allowing the study to achieve worldwide acceptance. This attempt was made to standardize raw Pearl and MB by ancient and sophisticated modern tools such as microscopic examination, XRD and particle size by DLS and NTA.

MATERIALS & METHODS

Pearl Processing

This was done using standard procedures and includes steps, namely Purification and preparation of Bhasma.

Raw Pearl Procurement

The raw Pearl was procured from the local gem dealer, Mumbai. Lemon and Rose were procured from local market. Leaves of Jayanti were collected from Keshav srushti, Bhayander, Mumbai. These were subjected to ancient and modern selection criteria. The raw herbal drugs were authenticated in Pharmacognosy Department of Nicolas & Piramal, Mumbai. Their identification is summarized in [Table1]

Table 1: Identification of raw drugs

Sr. No.	*Raw drug*	*Part used*	*Latin name*
1.	Lemon	Fruit juice	*Citrus acida* Linn
2.	Jayanti	Leaves	*Sesbania sesban* (Jacq.) W. Wight
3.	Rose	Flower (Petals)	*Rosa centifolia* Linn

Instruments

Mortar and pestle of Motiya stone, steel vessels, Arka yantra (classical instrument for distillation), gas stove, thermometer

Simple Purification of Pearl

Simple Purification was done by swedan in Lemon juice for 3 hours [4]. It was then washed with Luke warm water and dried.

Special purification of Pearl

Special purification was done by processing pearls obtained in previous method in freshly prepared juice of Jayanti for 3 hours [5]. It was then washed with Luke warm water and dried. The final product obtained is called as purified pearls. It was crushed in an iron mortar and pestle and used in preparation of Bhasma.

Preparation of Rose Water

Rose water was prepared by classical method of distillation [6]. The fresh rose petals were soaked overnight in four times luke warm water and transferred in Arka yantra (distillation apparatus). It was subjected to heat at the temperature of 100° C for 30 minutes. 10% of total amount of water is collected as a distillate. It was then stored in an air tight glass jar.

Preparation of MB

MB prepared by triturating purified Pearl with Rose water per day 6 hours for 3 Days till a homogenous paste was formed. After triturating, small pellets of uniform size (3×3 cm) and thickness were prepared and dried in sunlight [7]. Pellets were kept inside a sharava (shallow earthen disc) and another sharava was inverted over it. The joint between the two discs was sealed with mud smeared cloth to ensure proper closure. The properly sealed and dried samputa was subjected to puta system of heating with approximately 1.5 kg cow dung cake. Maximum temperature was 600-700 °C with total duration of 8 hours. The process was repeated for 2 more times to obtain bhasma of desired quality. MB obtained from above process was subjected to analytical tests.

Analysis using parameters described in Ayurveda texts

The final bhasma was analyzed for quality control as described in ancient texts & results found were as follows:

- Nishchandratva: MB was taken in a petri dish and observed for any luster in daylight through magnifying glass. No luster was observed in the MB.
- Rekhapurnatva: A pinch of MB was taken in between the thumb and index finger and rubbed.
- Varitartva: A small amount of the prepared MB was sprinkled over the still water in a beaker. It was found that the MB particles floated over the surface of the water.

Thus MB was found suitable as per Ayurvedic texts and further subjected to modern parameters of standardization.

Analysis using modern parameters

The MB as well as the raw Pearl was also analyzed using the following techniques:

- Microscopic analysis
- XRD
- Particle size by DLS
- Particle size by NTA

Microscopic Analysis

Microscopic analysis was performed at Gems and Diamond Testing Laboratory, Mumbai.

Instrument: Electron Microscope

The whole pearl was studied under electron microscope for its properties like Isotrophism, Fluorescence under UV light spectrum and its characteristic. Gemological microscopic study of the internal structure is used to determine whether a gem is synthetic or natural by revealing natural fluid inclusions, and included partially melted exogenous crystals to demonstrate evidence of heat treatment to enhance colour [8]. Results are summarized in [Table 2].

Table 2: Microscopic study of Pearl

Sr. No.	*Characteristics*	*Observations*
1.	Isotropic / Anisotropic	Isotropic
2.	Fluorescence under UV light spectrum	Chalky fluorescence
3.	characteristic inclusions under magnification	Smooth nacreous layers with uneven growth pattern

X-ray Diffraction

X- ray diffraction studies were performed at Indian Institute of Technology (IIT), Powai, Mumbai.

Instrument: Rigaku D-max IC X-ray diffraction unit with a wide angle goniometer

The powdered sample was spread onto a double side tape with a spatula, which was then placed on an aluminum sample holder. All the peaks were recorded on the chart, and the corresponding 2 theta values were calculated [9]. Results are summarized in [Figures 1 and 2] as well as [Table 3].

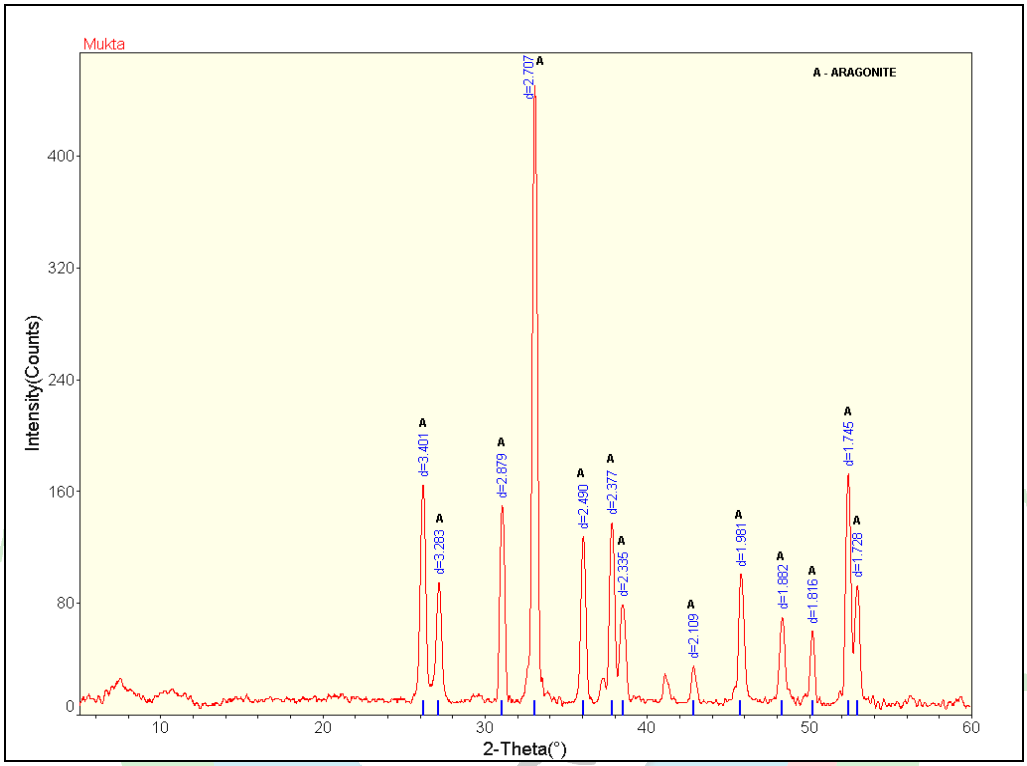

Figure 1: XRD of raw Pearl

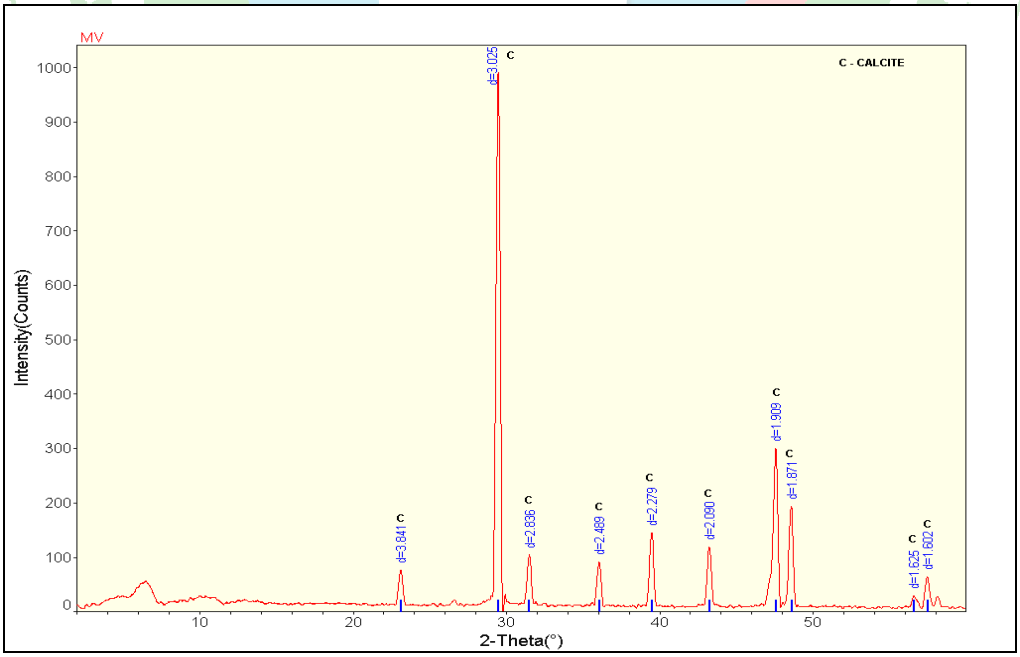

Figure 2: XRD Analysis of Mukta Bhasma

Table 3: XRD of the Raw Pearl and Mukta Bhasma

Compound	*Raw Pearl*		*Compound*	*MB*	
	Intensity counts: 2- Theta values	*d(A)*		*Intensity counts: 2- Theta values*	*d(A)*
	26.180	3.401		23.140	3.841
	27.140	3.283		29.500	3.025
	31.040	2.879		31.520	2.836
	33.060	2.707		36.061	2.489
	36.040	2.490		39.500	2.279
$CaCO_3$	37.820	2.377	$CaCO_3$	43.260	2.090
Aragonite	38.520	2.335	Calcite	47.600	1.909
	42.840	2.109		48.619	1.871
	45.761	1.981		56.581	1.625
	48.320	1.882		57.480	1.602
	50.199	1.816			
	52.400	1.745			
	52.940	1.728			

The strongest peak identified in the raw material was $CaCO_3$ in Aragonite form whereas in final product $CaCO_3$ was found in Calcite form.

Particle size by DLS

DLS studies were performed at Shraddha Analytical Services, Ghatkopar, Mumbai for determination of particle size of raw Pearl.

Instrument: Malvern Mastersizer

The raw Pearl was coarsely powdered and subjected to Dynamic light scattering. When light hits small particles, the light scatters in all directions (Rayleigh scattering) as long as the particles are small compared to the wavelength (below 250 nm). If the light source is a laser, and thus is monochromatic and coherent, then one observes a time-dependent fluctuation in the scattering intensity. This fluctuation is due to the fact that the small molecules in solutions are undergoing Brownian motion, and so the distance between the scatterers in the solution is constantly changing with time. This scattered light then undergoes either constructive or destructive interference by the surrounding particles, and within this intensity fluctuation, information is contained about the time scale of movement of the scatterers. The dynamic information of the particles is derived from an autocorrelation of the intensity trace recorded during the experiment[10]. [Figure 3 and Table 4]

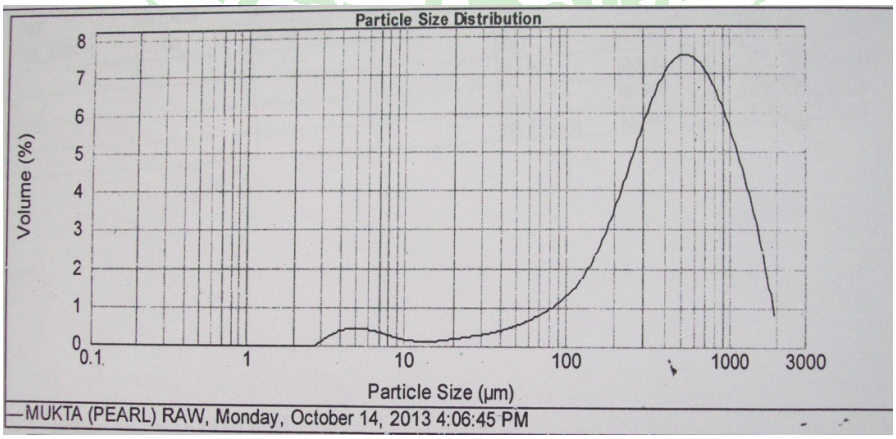

Figure 3: DLS of Raw Pearl

Table 4: Particle size of raw Pearl by DLS

Sample	*Observations*	
Raw Mukta powder	10% particles below	114.019 µm
	50% particles below	466.819 µm
	90% particles below	1170.290 µm
	100% particles below	1998.590 µm

Particle size by NTA

NTA for particle size of MB was performed at Institute of Science, Churchgate, Mumbai.

Instrument: Nanoparticle Tracking Analysis (NTA) Version 2.3 Build 0013

The powdered sample was dissolved in 1cc distilled water and subjected to sonication for 1 minute. One drop of solution was injected in the column of ultra microscope.

NTA is a method for visualizing and analyzing particles in liquids that relates the rate of Brownian motion to particle size. The rate of movement is related only to the viscosity and temperature of the liquid; it is not influenced by particle density or refractive index. NTA allows the determination of a size distribution profile of small particles with a diameter of approximately 10-1000 nanometers (nm) in liquid suspension.

The technique is used in conjunction with an ultra microscope and a laser illumination unit that together allow small particles in liquid suspension to be visualized moving under Brownian motion. The light scattered by the particles is captured using a CCD or EMCCD camera over multiple frames. Computer software is then used to track the motion of each particle from frame to frame. The rate of particle movement is related to a sphere equivalent hydrodynamic radius as calculated through the Stokes–Einstein equation. The technique calculates particle size on a particle-by particle basis, overcoming inherent weaknesses in ensemble techniques such as dynamic light scattering. Since video clips form the basis of the analysis, accurate characterization of real time events such as aggregation and dissolution is possible [11]. The result is summarized in [figure 4 & table 5].

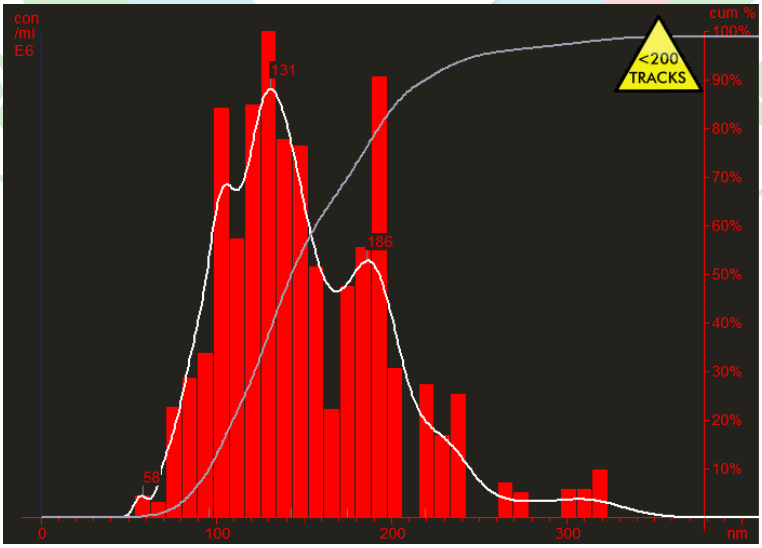

Figure 4: Particle size of Mukta Bhasma

Table 5: Particle size of MB by NTA

Sr. No.	*Sample*	*Average Particle size (in nm)*
1.	MB	156

The particles in the final product were found less scattered than that in raw material. The particle size of the raw material was between 114.019 and 1998.59 µm while that for the MB particles was 156 nm.

DISCUSSION

Many pharmaceutical procedures and techniques have been described in Rasa-shastra texts for the effective conversion of toxic metals and minerals into a suitable dosage form like Bhasma or Pishti. The Analytical presentation of Ayurvedic Pishti and Bhasma on the basis of modern parameters has become the need of time as it allows us to study the quality, potency and assists in negating toxicity, thus allowing the study to achieve worldwide acceptance. This study is an attempt made to standardize MB. Any research work is fruitless and the end product turns out to be less efficacious unless authentic or genuine raw drug is used for study. Each of the procedure carried out was standardized starting from market survey regarding drug availability, standards and costing. The selected sample was further subjected to microscopic examination to confirm genuinity. Smooth nacreous layers with uneven growth pattern reveal that it is a cultured pearl [8].

Rose contains essential volatile oils [12]. Rose water was prepared by classical distillation process to prevent loss of essential oils. Further phase identification by diffractogram using X- ray diffraction method was carried out. In raw Pearl major peaks were of $CaCO_3$ in Aragonite form; whereas in MB peaks of $CaCO_3$ in Calcite form were detected. As per inorganic chemistry, if calcium carbonate prepared in solutions at temperatures exceeding 300°C, crystals corresponding with Aragonite are formed, and if temperatures below 300 °C, crystals of Calcite are formed. Hence, Aragonite at temperatures below 300°C is in a metastable condition [13]. If Aragonite is heated at temperatures 400 °C, it will spontaneously convert to Calcite form. Further high pressure and low temperatures, Aragonite will almost certainly alter to Calcite. Thus the transformation of Aragonite form of raw Pearl to Calcite form in MB can be attributed to the closed oxidation at the temperature above 400 °C.

Present study was carried to assess the research drug with respect to nanomedicine. Thus particle size was assessed for raw Pearl and MB. Average size of raw Pearl is 8.7× 5.4× 4.4 mm. After coarse powdering, particle size of 10% particles was below 114.019 µm. MB achieved particle size as 156 nm by rigorous trituration, levigation and heat treatment. Thus MB can be considered as a nanomedicine. It is this significant reduction of size and that allows the phenomenon of Rekhapurnatva and Varitartva to develop.

Bhasmas are prepared to formulate most assimilatory, harmless, therapeutically effectual form of herbo- mineral drug. Further sukshmata of Bhasma allows penetration of that drug at cell level. It was confirmed by transformation of Aragonite form into Calcite form and presence of nano particles in MB.

The broad spectrum of different nanoparticle–cell interactions impacts on many different cellular physiology function levels (mitochondria, ROS production, cytoskeletal, intracellular calcium, and membrane currents) and elicits a spectrum of tissue responses. These findings provide strong evidence that nanostructures per se not only passively interact with cells but also actively engage and mediate the molecular processes that, usually, are essential for regulating cell functions [14]. This can be attributed to the role of MB in various diseases related to Immune system dysfunctions which can be established by various experimental studies.

CONCLUSION

XRD studies have confirmed that Pearl contains $CaCO_3$ in Aragonite form. The manufacturing process of MB plays a significant role in transforming the $CaCO_3$ in

Aragonite form of raw Pearl to Calcite form in the final product, MB. This could be important unique selling point for MB prepared using this particular method. As a result of different stages of processing techniques like Simple purification, Special purification, Trituration, Levigation and Heat treatment, the particle size reduces significantly. This may facilitate easy absorption and assimilation of the drug into the body system. MB can be considered as nanomedicine owing to its particle size of 156 nm. This facilitates its intracellular activities, cell penetration and cell alteration. The particle size of the final bhasma could be specified as the criterion for the final product confirming to all the traditional parameters under Bhasma Pariksha. Thus modern techniques would definitely form the guideline for the authentication of raw Pearl and standardization of MB.

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