Research Article

A PHARMACEUTICAL APPROACH OF PEARL AS A BIOSAFE NANOMEDICINE

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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 as a modified infusion method so as to preserve its essential volatile oils. Mukta Pishti (MP) was prepared by triturating purified Pearl with Rose water per day 6 hours for 21 Days. To assure the quality of MP, Rasa-shastra quality control tests like Nishchandravta, Rekhapurnavta and Varitaravta were used. After MP was found to be in compliance with these tests, it was further analyzed using X-ray diffraction (XRD) analysis. Analysis of raw Pearl and MP revealed that both of them contain CaCO₃ in Aragonite form. Determination of particle size was done by Dynamic light scattering (DLS) and Nanoparticle tracking analysis (NTA) for raw Pearl and MP respectively. The particle size was reduced greatly from 14.019 µm to 62 nm. It may be concluded that raw Pearl and MP have no change in the form which can be attributed to the fact that Pishti preserves properties of Ratna. Also thorough trituration can reduce particle size up to nanometers. Thus it can validate the sukshmatva of Pishti and can be correlated with concept of nanomedicine, essential for its quick and cellular activity.

Keywords: Mukta Pishti, X-ray Diffraction, Dynamic light scattering, Nanoparticle tracking analysis.

INTRODUCTION

MP, a unique herbo-mineral preparation has been used in large proportion in diseases like Tuberculosis, Diabetes, Asthma, cough and various diseases due to vitiated Vata¹ owing to its miraculous results; Although method of preparation of MP is mentioned in Unani therapeutics but also used in practice by Ayurvedic physicians. MP is prepared by triturating pearl powder with rose water for 21 days. In which the particle size of pearl is greatly reduced. 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 phases². Ayurvedic concept of Mardan (Trituration) and Bhavana (Levigation) to reduce particle size is an ultimate result of these processes³. 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. Pishti is also considered as sukshma (fine) as Bhasma. Till date no valid data is available which can confirm its physico-chemical parameters and activities mentioned in various Ayurvedic as well as Unani literature. So an attempt was made to assess structural form as well as particle size to confirm its properties and to validate it as nano medicine. The Analytical presentation Pishti 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 raw Pearl and MP by ancient and modern parameters such as microscopic examination, XRD and particle size by DLS and NTA.

MATERIALS AND METHODS

Pearl Processing

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

Raw Pearl Procurement

The raw Pearl was procured from the local gem dealer, Mumbai, India. Lemon and Rose were procured from local market. Leaves of Jayanti were collected from Keshav srushti, Bhayander, Mumbai, India. These were subjected to ancient and modern selection criteria. The raw herbal drugs were authenticated in Pharmacognosy Department of Nicolas and Piramal, Mumbai, India. Their identification is summarized in [Table 1]

Instruments

Mortar and pestle of Motiya stone, steel vessels, gas stove, thermometer

Simple Purification of Pearl

Simple Purification was done by swedan in Lemon juice for 3 hours⁴. 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 hours⁵. 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 Pishti.

Preparation of Rose Water

Rose water was prepared as a modified infusion method⁶. The dried rose petals were soaked in four times water and kept in an air tight vessel with lid. The joint between the two was sealed with mud and cotton cloth and dried. The entire arrangement was subjected to water bath at the temperature of 100°C for 1 hour. It was then strained to obtain Rose water.

Preparation of MP

MP prepared by triturating purified Pearl with Rose water per day 6 hours for 21 Days⁷. MP was then dried in shed and filled in an air tight glass jar. MP obtained from above process was subjected to analytical tests.

Analysis using parameters described in Ayurveda texts

In one of Rasashastra text, MP is mentioned as Anagni Bhasma⁸ (bhasma prepared without using heat). So the parameters mentioned for quality control of bhasma in ancient texts were used for analyzing MP and results found are as follows:

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

Analysis using modern parameters

The MP 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, India.

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 colour⁹. Results are summarized in [Table 2].

X-ray Diffraction

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

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 calculated¹⁰. Results are summarized in [Figures 1 and 2] as well as [Table 3].

The strongest peak identified in the raw material was CaCO₃, Aragonite which was similar in final product (CaCO₃, Aragonite).

Particle size by DLS

DLS studies were performed at Shraddha Analytical Services, Ghatkopar, Mumbai, India 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 experiment¹¹. [Figure 3 and Table 4]

Particle size by NTA

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

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

The powdered sample was dissolved in 1 cc 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 possible¹². The result is summarized in [Figure 4 and table 5]

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 MP particles was 62 nm.

Table 1: Identification of Raw Drugs

S. No.	Raw drug	Part used	Latin name
1.	Lemon	Fruit juice	<i>Citrus acida</i> Linn
2.	Jayanti	Leaves	<i>Sesbania sesban</i> (Jacq.) W. Wight
3.	Rose	Flower (petals)	<i>Rosa centifolia</i> Linn

Table 2: Microscopic study of Pearl

S. 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

Table 3: XRD of the raw Pearl

Compound	Raw Pearl		Mukta Pishti	
	Intensity counts: 2- Theta values	d (Å)	Intensity counts: 2- Theta values	d (Å)
Aragonite	26.180	3.401	26.240	3.393
	27.140	3.283	27.240	3.271
	31.040	2.879	31.120	2.871
	33.060	2.707	33.140	2.701
	36.040	2.490	36.140	2.483
	37.820	2.377	37.340	2.406
	38.520	2.335	37.900	2.372
	42.840	2.109	38.521	2.335
	45.761	1.981	41.240	2.187
	48.320	1.882	42.940	2.104
	50.199	1.816	45.880	1.976
	52.400	1.745	48.401	1.879
	52.940	1.728	50.220	1.815
			52.480	1.742
			53.001	1.726
			59.240	1.558

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

Table 5: Particle size of MP by NTA

S. No.	Sample	Average Particle size (in nm)
1.	MP	62

Figure 1: XRD of raw Pearl

Figure 2: XRD Analysis of Mukta Pishti

Figure 3: DLS of raw Pearl

Figure 4: Particle size of MP

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. Pishti though mentioned in Unani medicine, has long been in use by many Ayurvedic physicians. Valuable gems have to be converted into Pishti form prior to its internal administration. The shelf life of Pishti is longer and rejuvenation is achieved after internal administration for a longer duration¹³. 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 MP. 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 genuinely. Smooth nacreous layers with uneven growth pattern reveal that it is a cultured pearl⁹. Rose contains essential volatile oils¹⁴. To prevent its loss, Rose water is prepared as a modified infusion where direct heat is avoided. Further phase identification by diffract gram using X- ray diffraction method was carried out. In raw Pearl major peaks were of CaCO_3 in Aragonite form. Even in MP similar peaks 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 Meta stable condition¹⁵. No change in form can be attributed to the fact that preparation of Pishti is devoid of heat and thus preserves innate properties of prepared drug. Present study was carried to assess the research drug with respect to nano medicine. Thus particle size was assessed for raw Pearl and MP. Average size of raw Pearl is $8.7 \times 5.4 \times 4.4$ mm. After coarse powdering, particle size of 10 % particles was below $114.019 \mu\text{m}$. MP achieved particle size as 62 nm only by rigorous trituration for 21 Days. Thus MP can be considered as a nano medicine. It is this significant reduction of size and that allows the phenomenon of Rekhapurnatva and Varitartva to develop. Generally Pishti is prepared to stabilise the inherent properties of drug with immense reduction in its particle size allowing penetration of that drug at cell level. It was confirmed by presence of CaCO_3 in both samples as Aragonite and presence of nano particles in MP. 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 functions¹⁶. This can be attributed to the role of MP 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 MP plays a significant role in preserving the Aragonite form of raw Pearl in the final product, MP. This could be important biomarker for MP prepared using this particular method. As a result of different stages of processing techniques like Simple purification, Special purification and Trituration 6 hours daily for 21 Days, the particle size reduces significantly. This may facilitate easy absorption and assimilation of the drug into the body system. MP can be considered as nanomedicine owing to its particle size of 62 nm. This facilitates its intracellular activities, cell penetration and cell alteration. The particle size of the final Pishti 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 MP.

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