IJPSR (2019), Volume 10, Issue 10 (Research Article)

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 17 January 2019; received in revised form, 20 April 2019; accepted, 13 June 2019; published 01 October 2019

PHARMACEUTICAL VALIDATION & PROCESS CONCEPTUALISATION OF ANCIENT INDIAN CALCIUM PREPARATION: SHANKHA BHASMA

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Keywords:

Shankha Bhasma, Swedana, Bhavana, Ayurveda, Marana, Quantum of heat

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ABSTRACT: In the present study, the pharmaceutical and therapeutic dimensions of Shankha Bhasma (Classical Ayurvedic marine medicine) is explored as a substitute for calcium supplements available in the market. Shankha Bhasma was prepared & standardized by Ayurvedic pharmaceutical procedure accordingly in Rasa Tarangini (a classical 20th-century book on Ayurvedic pharmaceutical medicine). Conch shell (Shankha) was subjected to bio-fomentation (Swedana) in a precise heating container (Dolayantra) containing lemon juice for 12 h. Further, triturated with *Aloe vera* juice (Kumari Swarasa) for bio-impregnation (Bhavana) and subjected to the controlled quantum of heat (Marana) in the sealed earthen saucer at 700 °C for 2 h, till all the classical and contemporary quality parameters about bio-metallic medicinal powder (Bhasma) was assured. On completion, average weight loss was 17-21%. The final product obtained was calcite form of $CaCO_3$ and calcium oxide hydrate ($CaO.H_2O$). This paper explores scientific evidence of ancient Ayurvedic pharmaceutical procedures in terms of bio-fomentation, bio-impregnation, transfer mechanism of quantum of heat along with the principles of fluid mechanics & thermodynamics.

INTRODUCTION: Minerals (mica, arsenic, calcium compounds, chalcopyrite, *etc*.) and metals (mercury, iron, copper, lead, zinc, *etc*.) are the vital constituents of the advance Ayurvedic pharmaceutical procedure used in the form of bio-metallic medicinal powder (Bhasmas). Classical textbook of Rasa Shastra delineated various pharmaceutical processes of Shankha Bhasma, such as Shodhana, Marana, *etc*. This classical procedure of repeated detoxification and incineration enriches it with biochemical constituents.

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.10(10).4724-30

The article can be accessed online on www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.10(10).4724-30

Specific redox reaction with the insertion of herbal assimilates causing substitutive addition of many trace elements, converting the metals into a suitable form for internal use, making it more absorbable to the human body. Ancient Ayurveda classics depict the therapeutic uses of suitably processed Bhasmas, including their deleterious effects if not prepared properly [1].

Standardization of Ayurvedic Bhasmas is need of the hour, but due to the absence of standard parameters, it's a difficult task. Standardization of crude drugs, pharmaceutical processes, and the finished product is to be validated by setting a standard manufacturing procedure (SMP) for every step of drug manufacture. Standardization comprises of classical and contemporary physicochemical parameters [2].

Conch shell (Shankha) is the hard calcareous shell of large predatory sea snails species. According to Rasatarangini, two types of Shankha are mentioned: Dextrorotated (Dakshinavarta) and Levorotated (Vamavarta). Vamavarta is very common and hence is therapeutically useful for preparing Ayurvedic medicines [3]. Conch shell (Shankha) is known since the Vedic period (1500 B.C.-500 B.C.), but its internal application as a medicine has been recognized from Charak Samhita period (500B.C. - 6thcentury AD). Bhasmas of Rasa Shastra has proved its high therapeutic efficacy in comparison to herbal medicines and earned an overwhelming acceptance in the mainstream of Ayurveda.

Bhasmas are required in a smaller dose and are quicker in action with targeted drug delivery. It has been demonstrated with high therapeutic efficacy due to their nano-carrier nature and was very portable with a much-enhanced shelf life. These factors propelled Bhasmas as popular dosage form and established their demand in clinics of Ayurveda. Shankha Bhasma is used either as a single drug or in formulation with other medicines. It is indicated for various disorders, especially chronic diarrhea, colic pain, acidity with biliousness, and heart disease. It removes looseness of stools, cures white blot in eyes and is a tonic, also used in pimples on the face of young peoples [4].

Shankha Bhasma had been documented to be prescribed in indigestion (Ajirna), decreased enzymatic secretion (Agnimandhya), irritable bowel syndrome (Grahani), sour eructation/acidity (Amlapitta), duodenal ulcer (Parinamashula), hepatosplenomegaly (Yakrittaplihavriddhi) and toxins (Visha) [5]. It has been known to us that Calcium is vital to many enzymatic reactions, involved in neuro-hormonal transmission and maintains cellular integrity. Approximately, half of the total serum calcium is ionized and active and the rest is bound primarily to albumin [6].

MATERIALS AND METHODS:

Procurement and Authentication of Raw Material: The best variety of Shankha was procured from Gola Deenanath, local market of Varanasi, Uttar Pradesh. Fresh lemon (Nimbu) was purchased from the vegetable market in Varanasi, and *Aloe vera* (Kumari) was obtained from Ayurvedic Pharmacy, Banaras Hindu University. A sample of raw Shankha was authenticated from Department of Rasa Shastra & Bhaishajya Kalpana, Faculty of Ayurveda, Institute Medical Sciences, Banaras Hindu University, Varanasi.

Detoxo - purification (Shodhana): Bio - fomentation (Swedana) of Shankha: The process of Shodhana of Shankha was followed cautiously about Rasa Tarangini [7]. This was performed in three steps namely Purva Karma (pre-purificatory pharmaceutical procedure) which includes preparation of the liquid media to perform Swedana Samskara (bio-fomentation) of Shankha (conch shell) followed by Pradhan Karma (chief purificatory pharmaceutical procedure), in the process of Swedana an indirect heat through membrane is applied and finally the Paschat Karma (post purificatory pharmaceutical procedure) which is washing and cleaning of Shodhita (detoxo-purified) Shankha. The principle to extract nimbu swarasa is as per reference in Sharangdhar Samhita, which involves Nishpidana (expression).

Nimbu was washed properly under tap water along with the removal of the outer layer and apex of nimbu by the knife. The internal pulpy part was placed in between the extractor and pressure was applied. The extracted organic juice was collected in stainless steel vessels and sieved through a cotton cloth, finally, organic juice was measured. This measured liquid was used in shodhana process. In chief pharmaceutical procedure raw Shankha (conch shell) was crushed into small pieces, kept in a clean cotton cloth and pottali was prepared. Then it was hanged into dolayantra with the help of iron rod (Container containing nimbu swarasa), and it was boiled with the help of heating device continuously for 4 yama (12 h) on mild heat.

On completion of 12 h, the heater was put off, and pottali was allowed to self cool. Later the pieces of Shankha were separated from cloth and washed under lukewarm water along with the precautious cleaning of the Shankha pieces, and eventually it was dried and weighed the whole process showing in **Fig. 1**. Remaining two batches were prepared following the procedure mentioned above.

Transfer Mechanism of Quantum of Heat (Marana) of Shankha: Marana can be probably

correlated to oxidato-reductive incineration process of Shankha which was performed in the following steps. Marana of Shankha was done as per the reference in Rasa Tarangini [7].

Procedure:

1st Puta: Initially the pieces of Shodhita Shankha were arranged in a Sharava (earthen container) and closed by another Sharava. Joint was sealed by using cloth smeared with clay and allowed to dry. Total seven coatings was required to Samputa (sealing), and each coating was done after drying the previous coating. On completion of sealing procedure, Sharava Samputa (Sealed earthen container) was subjected to heat in an electric muffle furnace (EMF) and the temperature was maintained at 700 °C for 2 h. After that, the furnace was switched off and allowed for self-cooling. Next day samples were taken out of the self-cooled Sharava Samputa and pieces were powdered in mortar-pestle.

2nd Puta: In the second Puta, for Bhavana Kumari Swarasa was extracted by Nishpidana (expression) followed by trituration. On the appearance of Subhavita Lakshan (chief desired character after Bhavana) Chakrikas (pellets) were prepared, and for this procedure, a small amount of levigated doughy mass was made into round, flat pellets. Diameter, thickness, and weight of one Chakrika were 2.0-2.5 cm, 0.5-0.7 cm and 5-7 g respectively. After proper drying of pellets, it was placed in Sharava and covered with another Sharava. For sealing the Sharava clay smeared cloth was used with seven coatings which were given successively after drying the previous coating.

The prepared smeared Samputa was subjected to heat in EMF at temperature 700 °C maintained for 2 h duration. After completion of self-cooling, the Samputa was removed from the furnace and opened; Bhasma was weighed and then subjected to Bhasma Pariksha (analytical completion test for quality control of bio metallic, medicinal powder, *i.e.* Bhasma); eventually, these Bhasmas did not pass all parameters of Bhasma Pariksha. So, to pass all the parameters of Bhasma, it was subjected to 3rd Puta.

3rd Puta: In this Puta, the procedure mentioned in previous Puta was followed here. After self-cooling, Bhasma Pariksha was done and after third Puta, the obtained Bhasma passed all the completion test as mentioned in the classics and finally its quality was assured. The final product was powdered and sieved through 120 # mesh to obtain fine Bhasma. At last, it was weighed and collected in an airtight container. The whole process of Shankha Bhasma preparation, showing in **Fig. 1**.

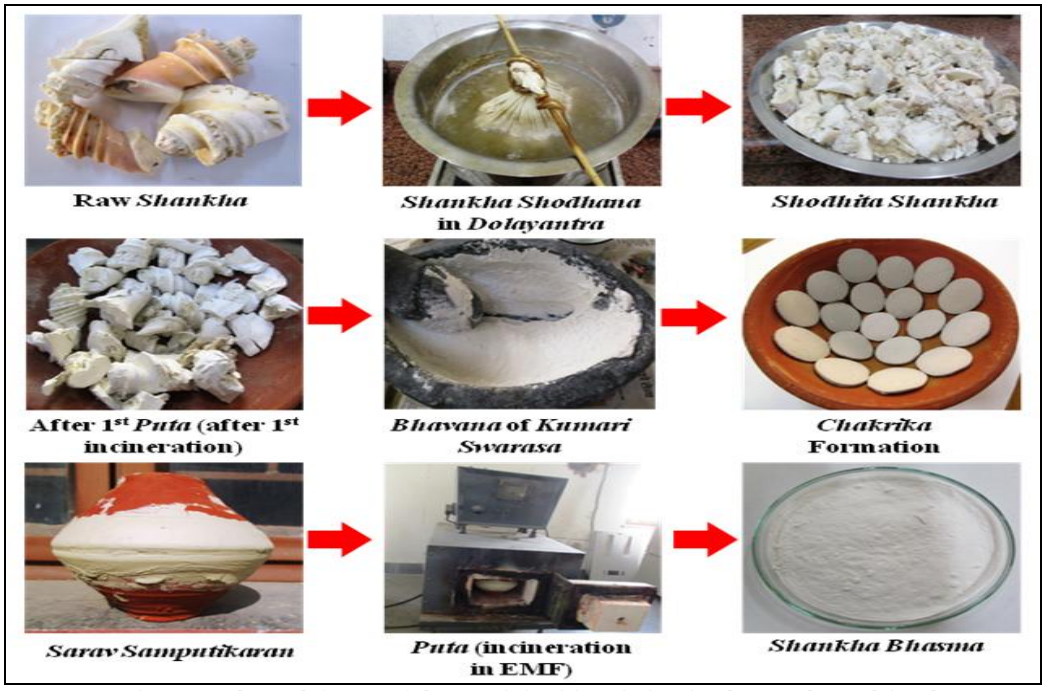

FIG. 1: PHARMACEUTICAL PROCESSING OF SHANKHA BHASMA

RESULTS AND DISCUSSION: Three batches of 1kg Shankha were taken for the Shodhana procedure. 6 litres of nimbu swarasa was utilized in each batch for the Swedana of Shankha, and this process took 12 h. Loss in weight was 3.45%, 3.0% and 3.3% respectively in all three samples after Shodhana. The loss was due to the removal of impurities. The summary of Shodhana of three batches of Shankha has been mention in **Table 1**.

TABLE 1: SUMMARY OF SHODHANA OF SHANKHA

Batch	Name of media	Quantity of media	Duration of Swedana	Wt. of Shankha before Shodhana (gm)	Wt. of Shankha after Shodhana (gm)	% loss
I	Nimbu Swarasa	6 litres	12 h	1000	965.50	3.45
II	Nimbu Swarasa	6 litres	12 h	1000	970.00	3.00
III	Nimbu Swarasa	6 litres	12 h	1000	967.00	3.30

During Shodhana procedure, it was observed that fumes appeared on heating in Dolayantra for 45 min and on completion of the process color of Shankha became whitish, unsoiled, brittle and lustreless. The color of nimbu swarasa was light yellow initially, but after completion of the process, it changed to slightly greyish. The temperature of the media was maintained at 100 °C – 110 °C throughout the procedure. A whitish powder-like substance was deposited at the bottom of the Dolayantra, suggesting the sedimentation of impurities, these are due to the removal of impurities that dissolved and escaped through the pores of pottali during Shodhana procedure.

During the process of Swedana in Shankha Shodhana, a probable hypothesis can be postulated that an indirect heat applied to Shankha, which is covered in pottali. The convection current generated causes active randomized movement of the principle ingredients of lemon juice. This movement facilitates the entry of the components of lemon juice inside the pottali, the cloth of pottali acts as a selectively permeable membrane which filters the impurities. The citric acid is the chief constituent of lemon juice which acts as heavy metal mobilizer due to its chelating property [8]. There is some sort of electrostatic attraction or the formation of surface Ca-citrate complexes in the ongoing process.

Citric acid reduces the positive charge of calcium compounds, thus indicating the adsorption of the citrate on the positively charged sites [9]. The exchange diffusion process occurring during the intermediary procedures leads to the bio-impregnation of the active principles of media in Shankha. Lemon juice's high acidity means that it has a high concentration of hydrogen ions and its corrosive properties make it a useful ingredient in cleaning products, because it softens the minerals in hard water, allowing the cleaning agents to work more effectively [10]. During the process, temperature increases and the molecules of the liquid gain energy, causing an increase in the molecular motion leading to overcoming the force of attraction. Probably this results in encircling (agitated motion) around the pottali with high kinetic movements the media (acidic) neutralizes the alkaline impurities of Shankha and also detoxify the toxic impurities in it, making the drug qualitatively available for further processing.

For the Marana process, 600 gm of Shankha was taken for each batch, the temperature was set at 700 °C and was maintained for 2 h. On completion of first Puta, Chakrikas was grayish white from superficially with hard consistency, but on breaking internally, it was dull white. In this Puta, loss was observed at 15.33%. After 1st Puta, Bhavana of Kumari Swarasa was given for 3 h, and during the whole process of Bhavana 450 ml of Kumari Swarasa was utilized. Levigation was done properly with uniform and sufficient pressure to make the materials fine. The pellets were made uniform in shape and size for proper heat exposure. After drying, the average weight of one Chakrika was observed in the range of 5 to 7g; the average diameter was 2.0 to 2.5 cm, and the thickness was 0.5 to 0.7 cm.

After proper drying Sharava samputikarana was done as per the previous procedure. The prepared smeared Samputa was subjected to heat in EMF at temperature 700 °C maintained for 2 h duration after 2nd Puta weight loss was observed 2.16%. Color changed from grayish white to white, pellets consistency changed from hard to soft.

Rekhapurnata was seen after 2nd Puta, 40% Varitara was observed, but the all quality control parameter did not pass after 2nd Puta. Therefore, one more Puta was given on the same temperature and completion of the 3rd Puta 2.21% weight loss was observed, 80% Varitara and Niswadatva, Unnam test was positive after 3rd Puta. The total weight loss in the first batch was 19.7%, and total of three Putas was required to prepare standard Shankha Bhasma. The final product obtained was calcite form of $CaCO_3$ and calcium oxide hydrate ($CaO.H_2O$). The details of three batches were given in **Tables 2**, **3,** and **4**.

TABLE 2: SUMMARY OF MARANA OF SHANKHA (BATCH I)

Puta	Amount of Liquid Media	TT	ST	Reaching duration on ST	Maintaining time on ST	Initial wt. of Shankha (gm)	Final wt. of Shankha (gm)	% loss
1st	-	-	700 °C	55 min	2 h	600	508	15.33
2nd	450 ml	3 h	700 °C	60 min	2 h	508	497	02.16
3rd	460 ml	3 h	700 °C	60 min	2 h	497	486	02.21

TT- Triturating time, ST- Set temperature

TABLE 3: SUMMARY OF MARANA OF SHANKHA (BATCH II)

Puta	Amount of Liquid Media	TT	ST	Reaching duration on ST	Maintaining time on ST	Initial wt. of Shankha (gm)	Final wt. of Shankha (gm)	% loss
1st	-	-	700 °C	55 min	2 h	600 g	523 g	12.83
2nd	470 ml	3 h	700 °C	55 min	2 h	523 g	507 g	03.05
3rd	470 ml	3 h	700 °C	60 min	2 h	507 g	497 g	01.97

TT- Triturating time, ST- Set temperature

TABLE 4: SUMMARY OF MARANA OF SHANKHA (BATCH III)

Puta	Amount of Liquid Media	TT	ST	Reaching duration on ST	Maintaining time on ST	Initial wt. of Shankha (gm)	Final wt. of Shankha (gm)	% loss
1st	-	-	700 °C	60 min	2 h	600 g	502 g	16.33
2nd	450 ml	3 h	700 °C	55 min	2 h	502 g	489 g	02.58
3rd	460 ml	3 h	700 °C	60 min	2 h	489 g	471 g	03.68

TT- Triturating time, ST- Set temperature

In the pharmaceutical procedure, Bhavana plays a very significant role, according to Ayurvedic classics Bhavana Samskara alters Guna and Karmas (pharmacokinetics & pharmacodynamics) of a drug at macroscopic as well as at microscopic level. It is wet trituration which facilitates particle size reduction, homogenization, form chelates, and also there is the formation of some coordination complex among the ingredients leading to a modification of properties of the end product [11]. The following parameters were analyzed for the Bhavana dravya, *i.e.* proximal composition, water activity, pH, acidity, non-enzymatic browning, surface color, vitamin content, mineral content, and antioxidant capacity, minor alterations in the physicochemical and nutritional properties at drying temperatures of 60–70 °C [12]. It also plays the role of a buffering agent by maintaining a specific pH.

It can be probably understood that the possible interactions between the adsorbates and active adsorption sites of the metalo-organic frameworks (MOF) are explained with the following mechanisms: (1) adsorption onto a coordinatively unsaturated site, (2) adsorption *via* acid-base interaction, (3) adsorption *via* π-complex formation, (4) adsorption via hydrogen bonding, (5) adsorption *via* electrostatic interaction, and (6) adsorption based on the breathing properties of some MOFs and so on [13].

Flat disc-like structure (Chakrika) of specific size and shape, helps in the uniform distribution of the thermal energy along with the maximum surface area of exposure for heat transfer. Microscopic collisions of particles and movement of electrons within a body are occurring on heat exposure.

Conduction of heat through Chakrika can be interpreted by Fourier's law. According to this law, the rate of heat flow through a uniform material is proportional to the area, and the temperature drops and inversely proportional to the length of the path of flow. So, the pellets must be flat in shape rather than spherical mass, and thickness of pellets must be as less as possible to facilitate easy flow of heat. Heat transfer occurs by wave-like motion a type of

quantum conduction and heat takes the place of pressure in normal sound waves [14].

Sandhibandhana is required to disconnect the exposure of air from the external environment during reactions. The exchange of heat from the Puta to the material inside Sarava Samputa can be explained by Hess's law of thermodynamics and Zeroth law of thermodynamics which directly focused on the idea of conduction of heat and proper isolation of the system [15]. Puta is defined as an exact quantum of heat required for pharmaco-therapeutic transformation to prepare superior quality medicine. The heat transfer at an interface is considered a transient heat flow. To analyze this problem, the Biot number is important to understand how the system behaves. If the system has a Biot number of less than 0.1, the material behaves according to Newtonian cooling, *i.e.*, with negligible temperature gradient within the body. If the Biot number is greater than 0.1, the system behaves as a series solution [16].

Puta is closed system excluded from the universe in which quantum of heat is delivered to cause oxidative addition, reductive elimination and insertion among the ingredients present inside the system where the reaction takes place at a specific temperature, pressure, volume, etc. required for the formation of the final product [17]. The process causes biochemical modification and the biological media act as selective donor ligand. Changes can be depicted in terms of reduction in particle size, conjugation of trace elements, the formation of desirable compounds, and medicinal potentiation of therapeutic ingredients.

Thermodynamic entropy is central in chemical thermodynamics, which is needed to be quantified in terms of Clausius equation and the Gibbs free energy equation for reactants and products. The second law of thermodynamics states that entropy in an isolated system increases during all spontaneous chemical and physical processes [18]. On the basis of the above point, an attempt was made to understand the basic concepts during Ayurvedic pharmaceutical procedure, which may further open the doors for future researches in this field. $CaCO_3$ of aragonite form was converted into a mixture of calcite form of $CaCO_3$ and calcium oxide hydrate ($CaO.H_2O$) after the final processing.

CONCLUSION: Based on factors discussed, we may conclude that the pharmaceutical procedures mentioned in preparation of Shankha Bhasma viz. detoxo-purification (Shodhana), bio-fomentation (Swedana), bio-impregnation (Bhavana), transfer mechanism of quantum of heat (Marana) of Shankha well collaborate with contemporary scientific laws. We suggest in developing technology regarding Ayurvedic pharmaceutics, applying all this core scientific principle involved in the process of making Bhasma, to ensure its quality, efficacy, and safety. This work has resulted in a better understanding of the pharmaceutical preparation of Shankha Bhasma as per Ayurvedic protocol.

ACKNOWLEDGEMENT: Nil

CONFLICT OF INTEREST: None

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How to cite this article:
Meena V, Bhushan S and Chaudhary A: Pharmaceutical validation & process conceptualisation of ancient Indian calcium preparation: Shankha Bhasma. Int J Pharm Sci & Res 2019; 10(10): 4724-30. doi: 10.13040/IJPSR.0975-8232.10(10).4724-30.