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Development and Evaluation of a Chocolate Based Dosage Form Containing Shankha Bhasma

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ABSTRACT

Osteoporosis is a rising threat for wide-reaching inhabitants, to treat this calcium supplements are suggested but available option of calcium supplements are not satisfying the need of the therapies, nor the patients' acceptability. To explore traditional system of medicine in osteoporosis, concept of bhasma was opted for the study. Preparing bhasma with traditional method is a stepwise and complex process, hence to check any alteration is possible or not; preparing bhasma in electrical muffle furnace is tried out. Prepared bhasmas were evaluated by different parameters (*varna, varitara, rekhapurana, nischandra*) mentioned in Ayurveda literature and few modern parameters (*ash value, pH, LOD, NPST, XRD, particle size, calcium content*). These evaluation parameters give a scientific background to bhasma as nanomedicine that could be better absorbed in the body. To overcome the problem related to organoleptic properties (*taste, appearance*), delivering bhasma in chocolate base was attempted and this chocolate dosage form were evaluated by for its patients' acceptability. Findings of the study reveals that chocolate could serve as a patient friendly dosage form to deliver bhasma as a source of calcium.

Keywords: Shankha bhasma, osteoporosis, calcium supplement, nanomedicine, chocolate.

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INTRODUCTION

Osteoporosis has been defined as "a systemic skeletal disease characterized by low bone mass and micro-architectural deterioration of bone tissue, with a consequent increase in bone weakness and susceptibility to fracture risk". This is clearly demonstrated in Figure 1, with the diagram on the left showing normal bone with thick extensive plates of trabeculae, while on the right these plates are notably thinner with the central one in the process of fracturing. When osteoporotic bone is inadequately strong to withstand normal day-to-day activities, the structure fails, and the resulting fracture is called an 'insufficiency' fracture.

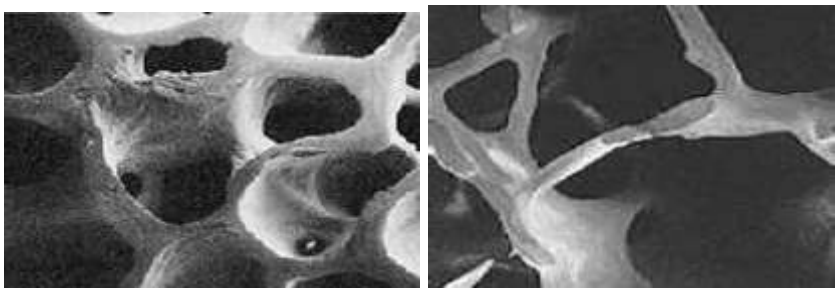


Figure 1: Normal bone (a) and osteoporotic bone (b)

Osteoporosis has been identified as an orthopedic 'epidemic' because of the increased numbers of fractures of the hip and wrist which have been occurring since the 1970s . There is, however, now evidence to suggest that the incidence of fractures of the hip may be leveling off. The insidious onset of osteoporosis usually lies undetected until it presents as a fracture after minor trauma and the condition is now seen both in the India and worldwide as a major problem in health care. [1]

As a combination therapy, calcium and vitamin D is the accepted baseline treatment for osteoporosis. In a three year clinical study, supplementation with calcium and vitamin D3 reduced the risk of hip fractures and other non-vertebral fractures among elderly women and also a significant benefit was seen after 18 months. For elderly patients treatment with Vitamin D may have additional benefits, because vitamin D therapy increases muscle strength and thus may reduce the possibility of fractures. The problem of calcium deficiency in senile osteoporotic patients can be corrected by administration of Vitamin D, which facilitates calcium absorption, but such treatment alone may increase risk of hypercalcemia and hypercalciuria. It has been reported earlier in many studies that bisphosphonates may cause hypocalcemia. Different mechanisms of Vitamin D and bisphosphonates suggest that a combination therapy of these agents may be very effective in improving calcium homeostasis and bone mass in patients with osteoporosis. [2]

Conventional therapy for calcium supplement has limitation that it does not have any taste so patient incompliance may occur. Moreover body needs vitamin D to absorb calcium; without enough vitamin D, one cannot form enough of the hormone calcitriol (known as the "active vitamin D"). This in turn leads to insufficient calcium absorption from the diet. In this situation, the body must take calcium from its stores in the skeleton, which weakens existing bone and prevents the formation of strong, new bone.

In *Ayurveda* it has been defined as a substance obtained by calcination. More than 3000 years ago, humankind has recognized that the nature could be effective therapeutic entities for the treatment of human illness. Countries like India with ancient civilization have a traditional system of medicine and using the ingredients from marine source has cured several diseases which are challenging the allopathic medicine till date. The Ayurveda and Siddha had proven themselves the most successful medical systems in alternative system of medicine. Bhasma is potent in small dose, without specific taste and quick acting. [3]

Conch shell ash is used for earache, ulcers, eye troubles, sore throat, cough and asthma. Shankha is also a good antacid and as such is used in gastritis and peptic ulcer in Siddha medicine. Conch shell is also useful in treating itching of eyelids and chronic conjunctivitis with mucous discharge, especially in children. Also gave in treatment calcium deficiency diseases. [4,5]

Chocolate is characteristically sweet, often brown, food preparation of Theobroma cacao seeds, roasted and ground, frequently flavored as with vanilla. It is made in the form of a liquid, paste or in a block or used as a flavoring ingredient in other sweet foods. The earliest evidence of utilize traces to the Mokaya (Mexico and Guatemala), with evidence of chocolate beverages dating back to 1900 BC. In fact, the majority of Mesoamerican people made chocolate beverages, including the Maya and Aztecs who made it into a drink a Nahuatl word meaning "bitter water".

Chocolate is an extremely sophisticated and flexible food that is combined to generate completely different taste and texture sensations. Chocolate is also an anhydrous medium and is as a result resistant to microbial growth and to hydrolysis of water-sensitive active agents. Chocolate is well-matched as a vehicle for delivering active agents in numerous aspects. For example, the organoleptic characteristics of chocolate are brilliant for masking disagreeable flavors linked with some active agents and giving a even and soft texture to compositions of active agents that are or else undesirably gritty. [6]

Delivering Shankha bhasma through chocolate based dosage form will be an interesting approach to overcome the limitation associated with conventional therapies and also it will

bridge the gap of traditional and modern system of medicine. Bhasmas are traditionally acclaimed effective but there are no or less evidence available for that, hence to give a scientific background to all traditional claim, this study can be a supportive adds on. As such bhasma preparations are not that much palatable to patients so to make it more palatable or patient friendly, development of the dosage form which can be widely acceptable by patients of all age groups.

MATERIALS AND METHOD

Powder of a substance obtained by calcination is called Bhasma. In this section, it is applied to the metals and minerals and animal products which are, by special processes, calcined in closed crucibles in pits and with cow dung cakes (Puta).

Bhasmas are prepared from purified minerals, metals and marine and animal products. In Ayurveda, the process of purification is called shodhana. Chemical purification is different from medicinal purification. In chemical purification it is only elimination of foreign matters. In medicinal purification the objects aimed are

(a) Elimination of harmful matter from the drug.

(b) Modification of undesirable physical properties of the drug.

(c) Conversion of some of the characteristics of the drugs.

(d) The enhancement of the therapeutic action, thereby potentizing the drug.

The second stage is the preparation of Bhasma. The purified drug is put into a khalva (stone mortar and pestle) and ground with juices of the specified plants or Kasayas of drugs mentioned for a particular mineral or metal. It is ground for the specified period of time. Then small cakes (chakrikas) are made. The size and thickness of the cakes depend on the heaviness of the drug, the heavier the drug, the thinner are the cakes. These cakes are dried well under sunlight and placed in one single layer in a shallow earthen plate (sarava) and closed with another plate. The edge is sealed with clay-smeared cloth in seven consecutive layers and dried.

Pit is dug in an open place. The diameter and the depth of the pit depend on the metal or mineral that is to be calcined. Half of the pit is filled with cow dung cakes. The sealed earthen container is placed in it and the remaining space is filled with more cow dung cakes. Fire is put on all four sides and in the middle of the pit. When the burning is over, it is allowed to cool itself completely. The earthen container is removed, the seal is opened and the contents are taken out. The medicine is ground into a fine powder in a khalva. This process of triturating with the juice, making cakrikas and giving Putas, is repeated as many times as prescribed in the texts or till the

proper fineness and quality are obtained. The Putas are described under different names to indicate the size of the pit and the number of cow dung cakes to be used, details of which are given in the Paribhasa. They also indicate the amount of heat required and the period of burning. The follwing Putas are commonly used in the preparation of Bhasmas:-

1. Maha Puta
2. Gaja Puta
3. Varaha Puta
4. Kukkuta Puta
5. Kapota Puta
6. Bhanda Puta [7,8,9]

MATERIALS AND METHOD

Marana (Purification) of Conch shells-:

Marana (Purification) is procedure used in Ayurvedic system of medicine for purification of raw materials. Purification helps in getting good results of prepared formulation.

In earthen pot lemon juice was placed in such way that all the conch pieces were dip in that lemon juice. After that earthen pot was placed on heating mentle for 2-3 hours at temperature about 100-110^{0}c. After 2-3 hours conches were removed from pot and washed with distilled water for 3-4 times to remove all remaining impurities and lemon juice form conch pieces. After drying of conch pieces again smaller pieces were made by triturating them in iron mortar and pestle.

Putapak (Incineration)

A. INCINERATION BY TRADITIONAL METHOD (in a pit)

Ingredients: Conch powder -150gm, Lemon juice.

Equipments: mortar and pestle, multani mati (fuller's earth), earthen pots, cotton cloth.

Conch powder obtained after triturating mixed with lemon juice in mortar and pestle till pellets were formed. Thin pellets known as chakrika were formed. Pellets were dried and kept in earthen pots. These were covered by another earthen pot. The junction was sealed by cloth which was dipped in Multani mati (fuller's earth). This arrangement is known as sharav samputa. These were dried in sunlight.

Pit was constructed which measures about 2*2*2 feet. 150 number of cow dung cakes were used for one incineration. Half of the cow dung cakes were kept at bottom. Sealed sharav samputas were kept over then. Again half of the cow dung cakes were kept above them till the top of pit.

Then cow dung cakes were ignited from the four sides and from the top. Complete burning was allowed and the arrangement was allowed to cool. After complete cooling, sharav samputa (earthen pots with pellets) were taken out. Pellets was observed and taken in mortar pestle. These were triturated again and another cycle of incineration was carried out. [10]

B. INCINERATION BY USING ELECTRIC MUFFLE FURNACE.

Ingredients- Conch powder - 50gm, Lemon juice

All procedure according to traditional method and incineration was carried out in electric muffle furnace. Sharavsamputas (earthen pots with pellets) were kept in electric muffle furnace. Temperature of furnace was set at 130-150^{o}c. after temperature is achieved; it was maintained for one hour. It was allowed to cool and then earthen pots were taken out. Pellets were triturated again and incinerated again. [11]

Evaluation of ***Shankha bhasma*** prepared by both methods was done by following parameters.

Evaluation by classical parameters:-

Varna (color):

Color was observed of prepared bhasma.

Sparsha (touch)

Bhasma was touched by hands to feel any coarse material or gritty feel.

Rasa (taste) and Gandha

Prepared bhasma was tasted to check any characteristic taste and smell.

Varitara(floating on water)

Water was taken in a beaker. Pinch of bhasma was kept slowly by fingers on surface of water. The bhasma was observed from close distance.

Rekha purnatva (furrow filing)

Little amount of bhasma was taken and rubbed between thumb and index finger. Ability of bhasma to fill furrows of finger was observed.

Unama (grain kept on floating bhasma)

Water was taken in a beaker. Bhasma was kept on surface of water. Grain was kept on surface of bhasma. It should remain on surface. This test was observed.

Nischandratva (lusterlessness).

Bhasma was observed in sunlight to check any metallic shine.

Apurnabhava (incomplete preparation).

Shankha bhasma was mixed with mitra-panchaka and chakrika's were made. It was incinerated. After self cooling, observation was done if any lustrous particle is present.[12]

Namburi phased spot test.

Namburi phased spot test is known as circular paper chromatography. When a drop of clear solution of a substance that is under examination is put on one of the chemical reacting papers, a spot with series of changes in color and pattern will appear. It is the study of this spot and color at three successive phases spreading over three different time interval is known as "phased spot test".

In this test whatman filter paper cut used. Paper cut in circular shape and wet by using curcuma launga decoction and allowed to dry. After that solution of bhasma placed on it and then observed color change for several minutes to 24 hours. [13]

Evaluation by modern analytical parameters:-

Loss on drying [14]

Petri plate was taken and weighed.1 gm of bhasma was taken and placed in Petri plate. It was kept in oven at 105^{o}c for five hours. It was allowed to cool and weighed. Loss on drying can be calculated by formula,

% LOD= 100 (initial weight-final weight)/ initial weight

Total ash value

Silica crucibles were taken and weighed. 1gm of shankha bhasma was added in crucibles. Crucibles were weighed again and kept in muffle furnace at 450^{0}c for three hours. Total ash value was calculated by following formula,

Weight of empty crucible = x

Weight of the drug taken = y

Weight of crucible + Ash (after complete incineration) = z

Weight of the ash = (z-x) g of ash

'y' g of the crude drug gives (z-x) g of ash

$$\text{Total Ash content} = \frac{100\,(z-x)}{y}\,\%$$

Acid insoluble ash

After determination of total ash content this ash was diluted with 25ml diluted Hydrochloric acid. Then this mixture was boiled for 5 minutes on burner and filtered through ash less filter paper; washed the residue twice with hot water. Put filter paper and residue together into crucible; heated gently until vapors cease to be evolved and then heated more strongly until carbon was removed. Weighed the residue and calculated acid- insoluble ash of drug by using a given formula as follow:

Weight of the residue = a

Weight of air dried drug = y

Acid insoluble ash = $\frac{a.100}{y}$ %

Water soluble ash [15]

Boiled the obtained total ash content with 25 ml of water for 5 minutes, collected the insoluble matter on an ash less filter paper and washed with hot water and ignited for 15 minutes at a temperature not exciding 450°C for 3 hours. Subtracted the weight of insoluble matter from the weight of insoluble matter from the weight the ash, the difference in weight represented the water insoluble ash. The percentage water-soluble ash with respect to the air-dried drug was calculated.

pH

1 gm of sample was mixed with 10 ml of distill water. It was measured by pH meter. [16]

Particle size analysis [17]

Particle size of powered drug was determined by simple microscopy method using compound microscope. Powdered drug was spread on glass slide and observed under microscope. About 500 particles were analyzed.

Determination of % Calcium Content [18]

Shankha Bhasma was first analyzed for calcium content. For the determination of calcium content the sample (0.1gm) was first dissolved in 3 ml of dilute HCl. It was then further diluted with 10 ml of distilled water. The mixture was then boiled for 10- 15 minutes, cooled and diluted to 50ml with distilled water. The mixture was then back titrated with 0.05M disodium edetate using eriochrome black as indicator.

Sugar Bloom Test[19]

Each sample was subjected to treatment cycles comprised (1) 30°C for 11 hours, (2)

Temperature shifting for 1 hour, (3) 18°C for 11 hours, and (4) temperature shifting for 1 hour. A test chocolate formulation observed, after the step at 18°C for 11 hours, whether or not blooming has taken place.

Fat Bloom Test

When a thin layer of fat crystals forms on the surface of the chocolate formulation. This will cause the chocolate to lose its gloss and a soft white layer will appear, giving the finished article an unappetizing look. Fat bloom is caused by the recrystallization of the fats and/or a migration

of a filling fat to the chocolate layer. Storage at a constant temperature will delay the appearance of fat bloom.

XRD

XRD study was carried out from Shivaji University. XRD was done to identify the crystalline or amorphous nature of powder.

RESULTS AND DISCUSSION

ON THE BASIS OF CLASSICAL PARAMETERS:

Organoleptic properties:

These tests are done by visual inspection of prepared bhasma. These tests give exact idea about bhasma color, odour, taste and how bhasma feel on touch.

Table 1: Organoleptic properties of Shankha Bhasma.

Sr.No.	Test	Traditionally prepared Shankha Bhasma	EMF prepared Shankha Bhasma	Desired properties
1	Varna (color)	White	White	White
2	Sparsha (touch)	Smooth	Gritty	Smooth
3	Rasa (taste)	Sour	Sour	Sour
4	Gandha(odour)	Odourless	Odourless	Odourless

Classical test for ideal bhasma:

These are traditional method for evaluation of bhasma. Classical test gives an idea about complete preparation of bhasma.

Table 2: Classical Evaluation Parameters

Sr. No	Test	Traditionally prepared Shankha bhasma	EMF prepared Shankha Bhasma
1	Varitara	Floating	Partial floating
2	Unama	Positive	Positive
3	Rekha purnatva	Positive	Partial filing
4	Nischandratva	Positive	Positive
5	Apurnabhav	Positive	Positive

MODERN ANALYTICAL PARAMETERS:

Loss on drying:

Lower value denotes the absence of moisture in the sample. It is important parameter as moisture may lead to microbial growth.

Ash value:

High ash value denotes the purity of sample. High ash value means the absence of free organic contents in the sample.

Acid insoluble ash:

It denotes the acid Non digestible mass so lower the acid-insoluble ash, higher will be the bioavailability of sample.

Water soluble ash:

It gives the water soluble extractives.

Table 3: Total ash, acid insoluble ash, water soluble ash and loss on drying of sample

Sr.No	Parameter	Traditional Shankha bhasma	EMF prepared Shankha bhasma	Standard
1	Loss on drying	0.15%	0.22%	0.12-0.24%
2	Total ash	97.7%	96.2%	96.8-99.7
3	Acid insoluble ash	9.2%	10.8%	0.101-2.803
4	Water soluble ash	0.13%	0.17%	NA

pH:

pH was checked for knowing its better absorption in gastro intestinal tract.

Table 4: pH of Bhasma

Sr.No	Test	Traditional Shankha bhasma	EMF prepared Shankha bhasma
1	pH	6.4	6.9

Particle size analysis

Particle size was decreased in subsequent formation of shankha bhasma.finer the particle, more will be the bioavailability. Particle size was calculated using compound microscope. Particle shape was observed under optical microscope.

Particle size was done for measurement of particle size this was to know about particles of prepared bhasma were in micro to nano size**.**

Table 5: Particle size of samples

Particle size	Traditional shankha bhasma	EMF prepared Shankha Bhasma
	10-40(15 µm)	10-40(20.19 µm)

Namburi phased spot test.

Namburi phased spot test is known as circular paper chromatography. When a drop of clear solution of a substance that is under examination is put on one of the chemical reacting papers, a spot with series of changes in colour and pattern will appear. It is the study of this spot and colour at three successive phases spreading over three different time interval is known as "phased spot test".

This technique is very useful for quality assessment of bhasmas as per the standards of Rasashastra. The study of differential identification of various bhasmas is made possible by N.P.S.T. single extended spot proves fineness of bhasma.

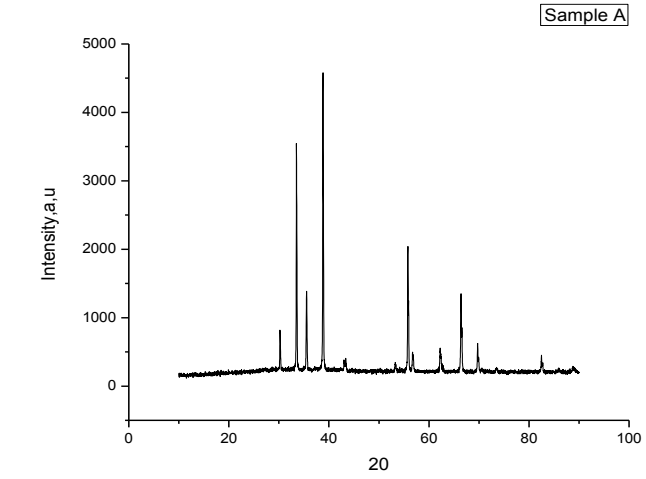
Table 6: N.P.S.T test on samples

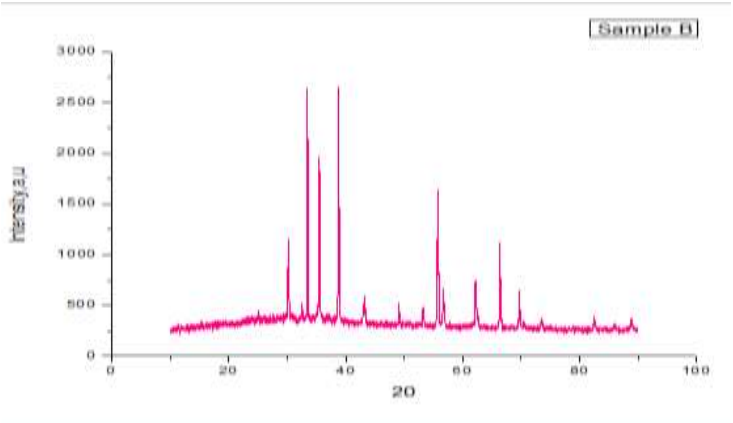
Phase	Traditional Shankha bhasma	EMF prepared Shankha Bhasma
Phase I (0-5 minutes)	Central pink spot	Central pink spot
Phase II (5-20 minutes)	Extended pink spot	Extended pink spot
Phase III(20 mins – 48 hours)	No other changes	No other changes

Namburi phase spot testing has been done for checking purity of prepared bhasma. It is simple and cost effective method for checking purity of bhasma.

XRD ANALYSIS

X – Ray diffraction is based on the scattering of X-ray through the crystals, used to detect the crystal structure of compounds.





Sample A-Bhasma prepared by traditional method

Sample B-bhasma prepared by EMF

XRD analysis is done to identify the crystalline or amorphous nature of the drug.

Identified peak is – Calcium chloride.

DARK CHOCOLATE AS A DRUG DOSAGE FORM

Why chocolate dosage form for Novel formulation of Shankha bhasma?

Chocolate is having the property of amorphous drug stabilization. Shankha bhasma is quite amorphous in nature. Shankha bhasma when given as single drug, it should be taken with adjuvant like lemon juice. Patient compliance is poor due to difficulty in administration. So we recommend chocolate formulation. Shankha bhasma with chocolate is combined to make completely different taste and texture sensations. Calcium deficiency anemia is very common in India, especially in women. So chocolate formulation may increase their desire to take calcium chocolates.

FORMULATION OF MEDICATED CHOCOLATE

1. Chocolate squares containing drug in appropriate quantity is known as medicated chocolate.
2. It was made by double boiler method. Bhasma was added in the melted chocolate base and stirred completely.
3. Normal shankha bhasma dose is 500 mg. we took 500 mg dose for each square. Each square is of 5.5 gm of chocolate. So calculations were done according to the total number of squares with bhasma.

EVALUATION OF PREPARED CHOCOLATES WITH SHANKHA BHASMA (CALCIUM CHOCOLATES)

General appearance-

General appearance is evaluated as it is important parameter for acceptance and desire to take medication.

- **Moisture content –**

It was carried out at the Nisarga biotech, Satara. It was found to be 0.486%

- **Blooming test-**

Fat bloom and sugar bloom was observed after samples were incubated for 12 hours at 30^0c.

When a thin layer of fat crystals forms on the surface of the chocolate, it causes loss of gloss and gives unappetizing look. Fat bloom is caused by the recrystallization of the chocolate layer. Thus fat bloom was evaluated.

Sugar bloom is caused by condensation. This gives a rough and irregular layer on top of chocolate formulation. So sugar bloom was evaluated.

Table 7: Bloom tests for chocolates

Sr.No	Characteristic	Result
1	Fat bloom	No bloom
2	Sugar bloom	No bloom

- **Calcium content-**

Calcium content was determined by the back titration method using disodium edetate and eriochrome black as indicator.

Calcium content was found to be **63.18 %(Traditional Method)**

Calcium content was found to be **59.45 % (Modern Method)**

- **Dimensions-**

Height- 12 mm, width- 10mm, lenght-30mm.

Table 8: General Properties of chocolates

Sr No	Characteristic	Result
1	Colour	Dark brown
2	Odour	Pleasant
3	Taste	Sweet
4	Texture	Smooth and glossy

Stability study

Samples were kept at room temperature for three month to evaluate the formulation.

Stability studies was done to check stability of formulation i.e. bhasma containing chocolates for knowing effect of various temperature and moisture conditions on storage for longer period.

Table 9: Stability studies on Shankha bhasma chocolates (3 Months)

Sr.No	Characteristic	Observation	Result
1	Colour	Dark brown	No change
2	Odour	Pleasant	No change
3	Taste	Sweet	No change
4	Texture	Smooth and glossy	Change
5	Moisture content	0.428%	Increases
6	Fat bloom	No bloom	No change
7	Sugar bloom	No bloom	No change
8	Calcium content	62.03%	Decreases by 1.15%

CONCLUSION

Convenience of administration and patient compliance are gaining significant importance in the design of dosage forms. Recently, more emphasis is laid down on the development of organoleptically elegant and patient friendly drug delivery system for pediatric and geriatric patients. Osteoporosis is a rising threat for wide-reaching inhabitants, to treat this calcium supplements are suggested but available option of calcium are not satisfying the need of the

therapies, nor the patients acceptability. To explore traditional system of medicine in osteoporosis, concept of bhasma was opted for the study. But the problem is again with the evidence that will prove this therapy is effective is lacking; additionally the patient acceptability for bhasma is poor due to its unpleasant organoleptic properties. So overall complications/ limitations like less effective therapy with modern concept and poor patient compliance are the key factors for opting this study of chocolate based shankha bhasma preparations.

Purification techniques are important step in preparing bhasma. Raw conches are purified by classical techniques like shodhana and marana. Raw conch pieces are placed in lemon juice and heated for 2-3 hours and then washed. After washing powder was made of conch pieces and that powder was further used for preparation of bhasma. Preparing bhasma with traditional method is a stepwise and complex process, hence to check any alteration is possible or not; preparing bhasma in electrical muffle furnace is tried out. Prepared bhasmas were evaluated by different parameters (varna, varitara, rekhapurntva, nischandra) mentioned in Ayurveda literature and few modern parameters (ash value, pH, LOD, NPST, XRD, particle size, calcium content). Results of the findings reveal that bhasma prepared by both methods shows comparable results, and out of that bhasma prepared by traditional method was quite better compared to other one. These evaluation parameters give a scientific background to bhasma as nanomedicine that could be better absorbed in the body.

To overcome the problem related to organoleptic properties (taste, appearance), delivering bhasma in chocolate base was attempted and this dosage form were evaluated by parameters like color, odour, taste, texture and its shows good results and fat bloom test and sugar bloom tests were carried out and it shows no fat bloom and no sugar bloom.

Findings of the study reveals that chocolate could serve as a patient friendly dosage form to deliver bhasma as a source of calcium.

Prepared dosage form shows problems related to stability issues, so more studies are required in this direction. Also to prove its effectiveness in patients, clinical studies could be planned and that could give evidence to this traditionally claim medicine.

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