

# In Vitro Bioaccessibility Assessment of Shankha Bhasma Prepared Using Two Different Herbal Media

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## Abstract

Shankha bhasma, a classical Ayurvedic formulation prepared from conch shell, is traditionally used for gastrointestinal and metabolic disorders. The therapeutic efficacy of such mineral preparations depends not only on elemental content but also on the fraction that becomes bioaccessible during digestion<sup>(1)</sup>. This study aimed to evaluate the in vitro bioaccessibility of calcium, magnesium, and potassium in four samples of Shankha bhasma prepared using two different herbal media. The samples were subjected to a standardized two-step simulated gastrointestinal digestion, followed by FTIR and ICPOES analyses. Results indicated that ShB4 (prepared with Nimbukamla for shodhana and Kumari swarasa for marana) exhibited the highest bioaccessible calcium (11.23% gastric, 12.44% gastrointestinal), with magnesium and potassium also showing significant release. The findings suggest that the choice of herbal media critically influences elemental release, providing scientific validation for traditional preparation methods and supporting the use of Shankha bhasma in gastrointestinal disorders.

**Keywords:** Shankha Bhasma, Bioaccessibility, Calcium, Magnesium, Potassium, Ayurvedic Minerals, In Vitro Digestion

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## 1. Introduction

Shankha bhasma, derived from conch shell (*Turbinella pyrum*), has been employed in Ayurveda for centuries to treat gastrointestinal disturbances such as Amlapitta, Parīṇamashoola, Grahani, and Atisar. Its therapeutic efficacy is attributed primarily to its mineral content, particularly calcium, magnesium, and potassium. However, the extent to which these minerals are bioaccessible during digestion determines their actual therapeutic potential.

Bioaccessibility is defined as the fraction of a compound released from its matrix within the gastrointestinal tract, rendering it available for absorption. Unlike bioavailability, which considers absorption, metabolism, and systemic circulation, bioaccessibility serves as a preliminary measure that can be assessed using in vitro models, offering a reproducible, cost-effective, and ethically sound alternative to in vivo studies.

Previous studies on Abhrak, Naga, and Tamra bhasma demonstrated variable elemental release depending on the preparation method and the digestive phase, emphasizing the importance of traditional processing media in modulating pharmacokinetic behavior.

Similarly, investigations into calcium bioaccessibility in  $\alpha$ -lactalbumin and  $\beta$ -lactoglobulin highlighted the role of protein interactions in enhancing solubility during intestinal digestion. Despite these studies, no reports on the bioaccessibility of Shankha bhasma are available, making this study novel in its approach.

## 2. Materials and Methods

### 2.1 Preparation of Shankha Bhasma

Four distinct samples of *Shankha bhasma* were prepared following the classical Ayurvedic procedures of *Shodhana* (purification), *Marana* (incineration), and *Bhavana* (trituration with herbal media), as outlined in the *Rasa Shastra* texts. Each sample was formulated by varying the *Shodhana* and *Marana* media to assess their impact on the final physicochemical characteristics and bioaccessibility (Table 1). The *Shodhana* process was performed to remove physical and chemical impurities, reduce toxicity, and render the raw *Shankha* (conch shell) suitable for medicinal use. This was followed by *Marana*, a controlled incineration process involving repeated calcination cycles with selected herbal juices, which transformed the purified material into a fine,

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bioabsorbable *Bhasma*. The *Bhavana* process ensured homogenization through repeated trituration with specific herbal media, enhancing the physicochemical interactions between the mineral and organic constituents. Each step was meticulously executed under standardized laboratory conditions to ensure reproducibility, uniformity, and compliance with classical guidelines. The variation in processing media—such as *Nimbu Swarasa*, *Kumari Swarasa*, and *Nimbukamla*—was intentionally introduced to evaluate their influence on elemental transformation, stability, and eventual bioaccessibility of calcium, magnesium, and potassium in the prepared *Shankha bhasma* samples.

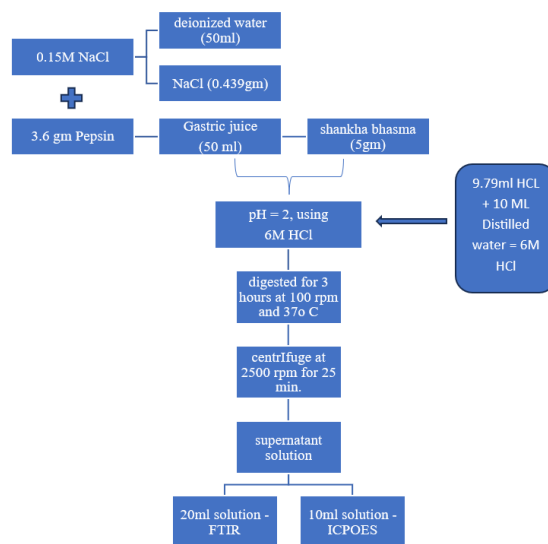
**Table 1. Preparation media for Shankha bhasma samples**

Sample	Shodhana Dravya	Marana Dravya
ShB1	Nimbu swarasa	Nimbu swarasa
ShB2	Nimbu swarasa	Kumari swarasa
ShB3	Nimbukamla	Nimbu swarasa
ShB4	Nimbukamla	Kumari swarasa

### 2.2 In Vitro Bioaccessibility Study

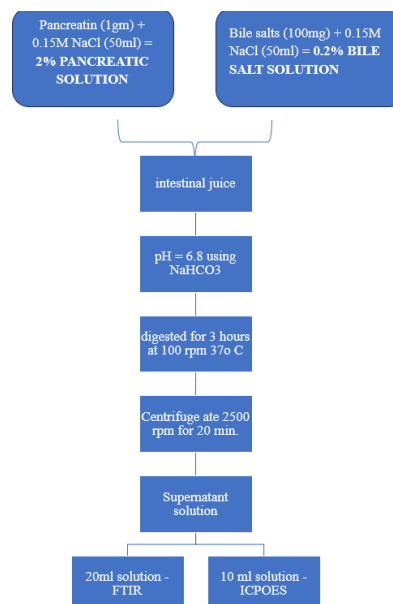
A standardized two-step in vitro digestion protocol was employed to simulate human gastrointestinal conditions and assess the bioaccessibility of calcium, magnesium, and potassium in the four Shankha bhasma samples. Initially, for the gastric phase, each sample was suspended in distilled water and acidified to pH 2.0 using hydrochloric acid, replicating the acidic environment of the adult human stomach. Pepsin, derived from porcine stomach, was then added to the samples to facilitate proteolytic activity, ensuring the breakdown of the bhasma matrix and associated organic residues. Maintaining the acidic pH is critical, as pepsin exhibits optimal enzymatic activity at  $\text{pH} \leq 2-3$ , and its activity diminishes significantly at higher pH values. The samples were incubated under controlled temperature and agitation to mimic gastric motility, allowing sufficient time for mineral release into the soluble fraction. (Fig. 1)

Fig. 1 – In vitro gastric digestion of the four samples of *Shankha bhasma*



Following gastric digestion, the samples were gradually neutralized to pH 5.5–6.0 in preparation for the intestinal phase. A 2% solution of Pancreatin, was added along with a 0.2% solution of bile salts, which act as emulsifiers to enhance solubilization of fat-soluble components. The pH was then adjusted to 6.5–7.0 to replicate the intestinal environment. This phase was conducted under continuous gentle agitation to simulate intestinal peristalsis, allowing further release and solubilization of minerals. The combination of sequential enzymatic activity, pH adjustment, and incubation provides a physiologically relevant in vitro model to estimate the fraction of elements that would potentially be bioaccessible for absorption in vivo. (Fig.2)

Fig. 2 – In vitro gastrointestinal digestion of the four samples of *Shankha bhasma*



After digestion, the supernatant was separated by centrifugation, filtered, and analyzed for calcium,

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magnesium, and potassium using ICPOES, while FTIR was employed to evaluate associated bio-elements and functional groups. The percentage of bioaccessibility for each element was calculated as the ratio of the element released during digestion to the total content in the bhasma, expressed as a percentage.

### 2.3 Analytical Techniques

The following analytical tests were employed to evaluate the four samples of *Shankha bhasma* after in vitro gastric and gastrointestinal digestion.

**FTIR (Fourier Transform Infrared Spectroscopy)** was used to identify and compare the functional groups present in each sample, providing insights into the molecular interactions, chemical bonds, and stability of bio-elements and associated organic compounds. The presence or absence of specific peaks helped in understanding changes in functional groups such as carbonate, hydroxyl, and phenolic moieties that may occur during digestion, reflecting the structural transformation and bio-interaction potential of the samples.

**ICP-OES (Inductively Coupled Plasma Optical Emission Spectroscopy)** was employed for precise quantification of elemental concentrations specifically calcium, magnesium, and potassium both before and after digestion. This allowed the estimation of the percentage of each element available in the bioaccessible form, thus providing a quantitative measure of elemental release and potential physiological availability following gastrointestinal processing. Together, these techniques ensured a comprehensive physicochemical and elemental characterization of *Shankha bhasma* to correlate traditional preparation methods with modern scientific validation.

Bioaccessibility (%) was calculated as:

$$\text{Bioaccessibility \%} = \frac{\text{Concentration of element released during gastric or gastrointestinal phase}}{\text{Total concentration of element}} \times 100$$

## 3. Results

### 3.1 Bioaccessibility of Calcium, Magnesium, and Potassium

The bioaccessibility of calcium, magnesium, and potassium in the four samples of *Shankha bhasma* (ShB1–ShB4) was assessed during both the gastric and gastrointestinal digestion phases, and the results are presented in Table 2. Distinct variations were observed among the samples, reflecting the influence

of different *shodhana* and *maarana* media on elemental release during simulated digestion.

**Table 2. Bioaccessibility of elements (%) in Shankha bhasma samples**

Element	Sample	Gastric Phase (%)	Gastrointestinal Phase (%)
Calcium	ShB1	9.02	0.78
	ShB2	2.07	0.05
	ShB3	7.28	6.45
	ShB4	11.83	13.11
Magnesium	ShB1	9.02	0.78
	ShB2	2.07	0.05
	ShB3	7.28	6.45
	ShB4	11.83	13.11
Potassium	ShB1	55.55	124.35
	ShB2	25.12	37.44
	ShB3	18.90	28.21
	ShB4	121.30	115.58

The analysis revealed that calcium bioaccessibility varied considerably among the four samples. ShB4, prepared using *Nimbukamla* for *shodhana* and *Kumari swarasa* for *maarana*, exhibited the highest calcium bioaccessibility in both gastric (11.83%) and gastrointestinal (13.11%) phases. This indicates that the combination of acidic (*Nimbukamla*) and mucilaginous (*Kumari swarasa*) media synergistically enhances the solubilization and subsequent release of calcium under digestive conditions. ShB3, prepared using *Nimbukamla* and *Nimbu swarasa*, also demonstrated relatively stable calcium release across both phases (7.28% and 6.45%), suggesting the acidic medium's favorable influence on elemental liberation. In contrast, ShB1 and ShB2 showed markedly lower calcium release, particularly in the gastrointestinal phase, which may be attributed to weaker complex formation or reduced solubility of calcium salts in neutral pH conditions.

The trend for magnesium bioaccessibility closely followed that of calcium, showing comparable percentages across samples and digestion phases. Notably, ShB3 and ShB4 again exhibited the highest magnesium bioaccessibility (6.45%–13.11%), emphasizing that the same herbal processing conditions facilitating calcium release may also support the solubilization of magnesium. Although magnesium was detected in smaller quantities compared to calcium, its consistent presence across all samples underscores its physiological significance, as magnesium plays a crucial role in calcium metabolism, neuromuscular function, and enzymatic regulation.

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Potassium displayed a distinctly different behavior from calcium and magnesium, showing exceptionally high bioaccessibility values in certain samples. ShB1 and ShB4 exhibited the most significant release, reaching 124.35% and 115.58% during the gastrointestinal phase, respectively. Such high percentages suggest that potassium, being more soluble and less prone to precipitation, is actively released or mobilized from the *bhasma* matrix under the combined influence of bile salts and pancreatic enzymes during digestion. The elevated potassium bioaccessibility aligns with its known role in maintaining electrolyte balance, smooth muscle functioning, and gastric mucosal protection—properties that resonate with *Shankha bhasma*'s classical indications in *Amlapitta*, *Parinamashoola*, and *Grahani*.

Overall, the findings highlight that *Shankha bhasma* prepared using *Nimbukamla* and *Kumari swarasa* (ShB4) demonstrated the highest and most balanced bioaccessibility of all three key elements—calcium, magnesium, and potassium. This indicates that the physicochemical environment created by these media facilitates optimal digestion-phase solubility, possibly through enhanced ion exchange and organic acid chelation. Such results reinforce the Ayurvedic understanding that *bhavana dravya* and *shodhanadravya* play decisive roles in determining the therapeutic potency of *bhasma*.

### 4. Discussion

The present study underscores the pivotal role of herbal processing media in determining the bioaccessibility and therapeutic potential of *Shankha bhasma*. The comparative evaluation of four samples (ShB1–ShB4) demonstrated that the elemental release of calcium, magnesium, and potassium during in vitro digestion was strongly influenced by the choice of *shodhana* and *maranadravyas*. Among the samples, ShB4, prepared using *Nimbukamla* for *shodhana* and *Kumari swarasa* for *marana*, exhibited the highest bioaccessibility of all three elements. This finding not only validates the traditional Ayurvedic methodology but also provides a scientific rationale for the classical selection of media in *bhasma nirmāna*.

From the Ayurvedic standpoint, **Shankha bhasma** is known to pacify *Vata* and *Kapha* doshas, act as a *Kṣhāra* (alkalizer), and promote *āmapachana*, the digestion and elimination of metabolic toxins. It is traditionally prescribed for gastrointestinal ailments such as *Amlapitta* (acid-peptic disorders), *Pariṇāmasūla* (gastric ulcer-like pain), and *Grahani*

(malabsorption syndrome). The present findings support these classical indications, demonstrating that the processed mineral undergoes effective solubilization and ion release under simulated digestive conditions, which could contribute to its therapeutic efficacy.

**Calcium** bioaccessibility was the highest in ShB4, with 11.83% in the gastric and 13.11% in the gastrointestinal phases, compared to lower values observed in ShB1 and ShB2 (9.02% and 2.07% gastric; 0.78% and 0.05% intestinal, respectively). The enhanced calcium solubilization in ShB4 may be attributed to the synergistic action of the acidic medium (*Nimbukamla*), which promotes calcium salt dissolution, and the mucilaginous, enzymatically active properties of *Kumari*,<sup>(17)</sup> which stabilize liberated ions and prevent their precipitation. This combination effectively mimics a bio-friendly environment conducive to ion exchange and absorption. The findings emphasize that *Nimbukamla-Kumari* pairing enhances both the breakdown and assimilation potential of mineral components in *Shankha bhasma*.

**Magnesium** bioaccessibility followed a similar trend, with ShB3 and ShB4 demonstrating relatively higher release values, reaching up to 9.17% during the intestinal phase. Although magnesium was detected in smaller amounts than calcium, its consistent presence across samples is significant, as magnesium supports calcium homeostasis, enzymatic activation, and smooth muscle regulation. In the context of gastrointestinal physiology, magnesium's ability to modulate smooth muscle tone and neutralize acidity further complements the classical *Kṣhāra* property of *Shankha bhasma*. The combination of bioaccessible calcium and magnesium aligns with both Ayurvedic and modern views, where these minerals act synergistically to relieve hyperacidity and muscular discomfort.

**Potassium** bioaccessibility was remarkably high, particularly in ShB1 (55.55% gastric; 124.35% intestinal) and ShB4 (121.30% gastric; 115.58% intestinal). Such elevated values indicate active release or mobilization of potassium ions during digestion, likely due to interactions with bile salts and pancreatic enzymes that facilitate ion solubilization. Potassium's high bioaccessibility is of considerable physiological importance, it maintains electrolyte balance, supports neuromuscular transmission, sustains gastric mucosal integrity, and prevents epithelial damage under acidic stress. These findings directly correlate with *Shankha bhasma*'s traditional

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use in *Amlapitta* and *Pariṇāmasūla*, where maintenance of mucosal protection and electrolyte equilibrium is vital for recovery.

The superior performance of ShB4 demonstrates the scientific soundness of classical Ayurvedic processing principles. The acidic, corrosive, and solubilizing nature of *Nimbukamla*, combined with the mucilaginous, enzymatic, and absorptive qualities of *Kumari swarasa*, appears to enhance the breakdown of the *Shankha* matrix and promote the release of elemental ions. This reflects the Ayurvedic doctrine that *Bhāvana* and *Māraṇadravyas* directly govern the *Samskāra* (transformative refinement) and *Veerya* (potency) of a *bhasma*. By modifying the physicochemical environment of the raw material, these herbal media facilitate both detoxification and bioactivation, ensuring that the final preparation is therapeutically potent yet physiologically compatible. From a modern pharmacological perspective, the observed bioaccessible elements correlate with well-established biochemical roles. Calcium helps in maintaining bone stability, neuromuscular coordination, cardiac function, and intracellular signaling. Magnesium, an essential cofactor, catalyzes over 300 enzymatic reactions, stabilizes ATP, and regulates both muscle and nerve functions. Potassium is crucial for maintaining membrane potential, regulating muscle contractions, facilitating nerve impulse transmission, and protecting the gastric mucosa.<sup>(18)</sup>

The concurrent bioaccessibility of these elements reinforces the understanding that the therapeutic efficacy of Shankha bhasma arises from the synergistic interplay of multiple bio-elements rather than a single constituent. The optimal combination of preparation media and mineral composition in ShB4 therefore represents a scientifically and traditionally validated form of *Shankha bhasma*, suitable for further pharmacological and clinical investigation.

### 5. Conclusion

A standard operating procedure was developed for the preparation of simulated gastric and gastrointestinal juices and for conducting the in vitro digestion of four Shankha bhasma samples. The percentage of bioaccessible Calcium, Magnesium, and Potassium was determined using the ICP-OES technique. The in vitro bioaccessibility results revealed that ShB4, the sample prepared using *Nimbukamla* as the *Shodhanadravya* and *Kumari swarasa* as the *Marana dravya*, demonstrated the highest release of Calcium, Magnesium, and Potassium compared to the other three samples. These findings highlight the crucial

role of selected herbal media in enhancing elemental bioaccessibility and validate the classical Ayurvedic principles that emphasize the significance of processing methods in determining the therapeutic potency of a *Bhasma*.

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