

Physical properties of an Indian Ayurvedic medicine (*Shankh Bhasma*) as nano materials for its application

Rakesh Kumar Singh*^{1,+}, Sanjay Kumar¹, Abhay Kr. Aman¹, Sunil Kr², Manoranjan Kar², Shankar Kumar³, & S.K.Tripathi⁴,

¹Aryabhata Centre for Nano science & Technology, School of Engineering & Technology, Aryabhata Knowledge University, Patna, 800001, India

²Indian Institute of Technology (IIT) Patna, Bihta, Patna 801 103, India

³Dept. of Physics, Patna University, Science College, Bihar 800 005, India

⁴Art of living Foundation, Bangalore 600 082, India.

E-mail: ⁺rakeshsinghpu@gmail.com

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The aim of present study is to describe Physical properties of an Indian Ayurvedic medicine (*Shankh Bhasma*) as nano materials for its applications. *Shankh Bhasma* has been synthesized by using method mentioned in an Ayurvedic text using conch shell and characterized by the modern scientific tools such as; X-ray diffractometer (XRD), Fourier Transform spectroscopy (FTIR), Scanning Electron Microscopy (SEM) and Vibrating Sample Magnetometer (VSM). When we treated shankh bhasma on *C. elegans* and *E. coli*. *C. elegans* produces more egg than normal calcium and there is no effect on *E. coli*. X-ray diffraction pattern and SEM analysis revealed that this *Bhasma* is in agglomerated nano crystalline materials and be considered as nanomedicine. An FTIR measurement reveals the bond stretching during the incineration process and found peaks of carboxylic group, Carbon with chloride and fluoride. Magnetization-Hysteresis (MH) loop study by the Vibrating sample magnetometer reveals that the di-magnetic nature of the *Bhasma*. Investigation of *Shankh bhasma* nanoparticles on *C. elegans* shows slightly increasing in egg production with compare to normal calcium oxide. There is not bacterial static effect was found on *E.coli*.

Keywords: *Shankh Bhasma*, Nanomaterials, Traditional medicine, Modern equipment, Egg production

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Bhasmas may be classified under the biologically produced nanomedicine of ancient India and are being used as an effective Indian based traditional medicine¹⁻⁵. It is the need of the moment for Ayurveda to conduct the research to extend the use of our ancient nanomedicine bhasma in various aspects like detection of chemical contained and other physical properties to make them more effective in serving the society. Preparation methods of bhasma are in tune of nanotechnology of contemporary era and proved advancement of Rasa-Sashtra. Rasa-Shastra (Vedic- chemistry) is one of the parts of Ayurveda which deals with herbomineral/minerals/nonmetals preparation called Bhasma⁶. Bhasma is a Ayurvedic metallic/mineral treated with herbal juice and exposed for certain quantum of heat as per puta system of Ayurveda,

which itself is well known in Indian subcontinent since Vedic lore^{7,10}. Shankh (Conch or Sea Shell) Bhasma basically contains inorganic substances mainly Calcium carbonate or oxide. Some amount of Mg, Mn, Fe, P, Na are also found due to treatment of herbal extract. Classically, *Shankh Bhasma* is alkaline in nature; it is used for indigestion, flatulence, abdominal pain, vomiting belching, diarrhea, belching and gastritis. Researchers have been explained the importance of the Bhasma as nanomaterials by employing the modern scientific tools and other scientific evidences^{11,14}. The soil nematode *C. elegans* represents an excellent model to investigate the effect of different types of nano materials on aging at the molecule, cell and whole organism level. Nano materials give rise to unique biological reactivity that needs to be thoroughly investigated.

In this context, the use of simple *in vivo* models, such as; *Caenorhabditis elegans* (*C. elegans*)

*Corresponding author

response to nano particles is currently increasing demand as it offers low cost and informative. However, it needs to standardize the preparation method as well as its uses in disease treatment. Also, further research is required to standardize the use in technological applications as natural nanomaterial products. Hence, the Shankh Bhasma has been prepared by the method described in the Indian Ayurvedic literature. Physical properties of the Shankh Bhasma have been explored by employing modern scientific tools. The present study opens a window not only for better way to use the Shankh Bhasma but also, one can use this Bhasma as nano material in technological, medicinal and pharmaceutical applications.

Materials and Methods

Shankh Bhasma Synthesis

In the present work for the preparation of Shankh Bhasma, the small pieces of conche shells used and prepared by the principle of putta (incineration) in the muffle furnace (EMF). Take a heavy no cavity sinister Conches (Shanka in Sanskrit) boiled with water and lemon juice for one hour and wash with hot water to clean. Small pieces of conch shells mix with Aloe vera pulp and kept in muffle furnish for 4 hours. After that, conch shell was crushed to fine powder. Conch shell powder mix with lemon juice and kept in muffle furnish for 2 hours. Process repeated for 4 times. Finally, we obtained shankh bhasma powder. The detail method of preparation is explained in previous literatures^{6,9,10,15-16}.

E. coli culture and growth

C. elegans is usually grown monoxenically in the laboratory using *E. coli* strain OP50 as a food source¹⁷⁻¹⁹. *E. coli* OP50 is a uracil auxotroph whose growth is limited on nematode growth media (NGM) plates.

C. elegans strains maintainance and assay

C. elegans wild type strains were maintained at 20°C on nematode growth media (NGM) agar plates seeded with *E. coli* strain OP50. For mean survival assay, L4 stage animals are treated with known concentration of iron nanoparticles, nano iron particles; and analyzed for dead animals till 8 days for live animals. For body bends per minute, Treated worms were counted manually using a dissecting microscope for body bends for 10 second at the age of 5 days of adult hood and then expressed as the mean

number of body bends per minute. For spontaneous movements, 10 days adult animals were analysed for its movement activity in response to touch.

Treatment of Shankh Bhasma on *C. elegans*

For the study on development of egg, *C. elegans* nematodes were taken in three different stages:

Stage 1

At this stage, *C. elegans* as a control animal after incubation at 37°C for 72 hours. with feeding of normal food *E. coli* strain, the number of eggs hatched by *C. elegans* is 8000 thousands every culture plates and its percentage is approx. 95%.

Stage 2

At this stage, *C. elegans* as experimental animal was put normal calcium in culture plate and incubates at temperature 37°C for 72 hr. After incubation period ends, the number of eggs hatched 8100 thousand per plates and percentage of eggs is approximately 96%.

Stage 3

At this stage, *C. elegans* as experimental animal was put Shankh Bhasma (Nano calcium) in culture plate and incubates at 37°C for 72 hours. After incubation period ends the number of eggs hatched 8100 thousand per plates and percentage of eggs is approximately 96%.

Results

X-ray diffraction Analysis

The crystallographic phase analysis of prepared Shankh Bhasma materials was carried out by using the XRD (Rigaku TTRX-III X-ray diffractometer, Japan) with CuK α radiation source ($\lambda = 1.5418 \text{ \AA}$). The XRD pattern of Shankh Bhasma is shown in Fig. 1. All the intense peaks in the XRD pattern of Shankh Bhasma was fully matched with the PDXL database. These XRD database (ICDD card number)

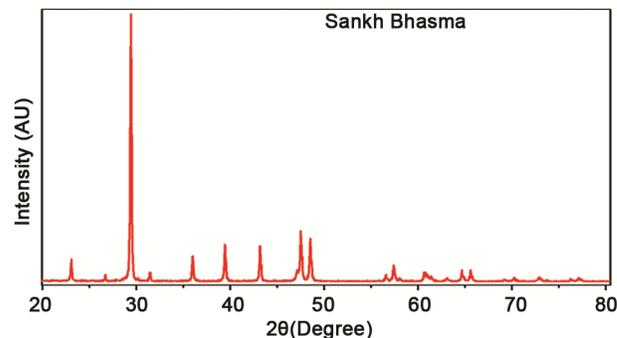


Fig. 1 — X-ray diffraction pattern of nanocrystalline Shankh Bhasma

and corresponding XRD intense peaks are enlisted in the Table I. The peaks of XRD pattern are matched with the different compound available in the Shankh Bhasma and corresponding chemical (compound) formulae are enlisted in Table I.

Morphology of shankh Bhasma

Morphology of the prepared “Shankh Bhasma” has been carried out by the SEM (Scanning Electron microscopy) technique (Evo 18 research, Zeiss UK). The tungsten electron source was used for obtaining the SEM micrograph, which are depicted in Fig. 2.

FTIR Study

The FTIR measurement shown in Fig. 3. In case of Shankh bhasma, a broad and deep absorption peak is detected at 1409 cm⁻¹. This peak due to the carbon oxygen bond. It is also the signature of carboxylic group. A second intense peak is at 873cm⁻¹, which is the signature of strong bond of carbon with chloride and fluoride, shown in Fig. 3. The results are well matched with the earlier reports^{21,22}.

Magnetic Measurement

The M-H hysteresis curves are shown in the Fig. 4, which were measured by the Vibrating sample magnetometer (7410, Lakeshore, USA) at room temperature. The maximum magnetization of this material is found to be 0.07 emu/g at 2T. This material shows the di-magnetic properties at room temperature.

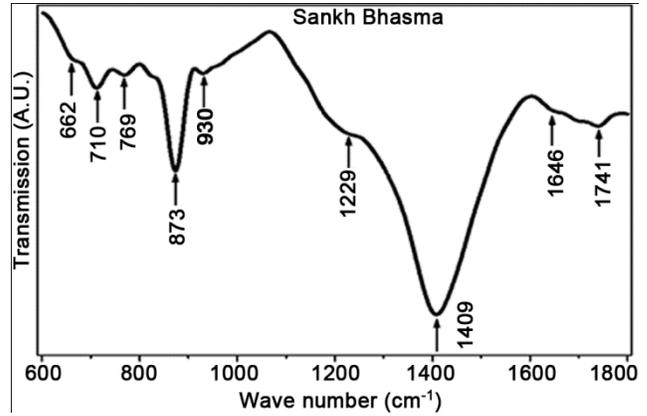


Fig. 3 — FTIR spectrum of Shankh Bhasma Nanoparticles

Table 1 — Different search peaks list of Shankh Bhasma nanoparticles

2θ°	Chemical formula	ICDD card number
23.07(12)	CaCO ₃ , P ₂ O ₅	00-005-0586, 01-083-0602
26.69(8)	P ₂ O ₅	01-083-0602
29.43(3)	CaCO ₃ , P ₂ O ₅	00-005-0586, 01-083-0602
35.96(5)	Fe O, CaCO ₃	01-089-7100, 00-005-0586
39.43(8)	CaCO ₃ , P ₂ O ₅ , Na O	00-005-0586, 01-083-0602, 03-065-1996
43.18(9)	MgFe ₂ O ₄ , CaCO ₃ , P ₂ O ₅	01-073-1960, 00-005-0586, 01-083-0602
47.15(2)	CaCO ₃ , P ₂ O ₅	00-005-0586, 01-083-0602
48.53(9)	CaCO ₃	00-005-0586
57.38(2)	CaCO ₃ , P ₂ O ₅	00-005-0586, 01-083-0602
60.70(3)	Fe O, CaCO ₃ , P ₂ O ₅	01-089-7100, 00-005-0586, 01-083-0602
65.60(11)	CaCO ₃ , P ₂ O ₅	00-005-0586, 01-083-0602
72.59(5)	Fe O	01-089-7100

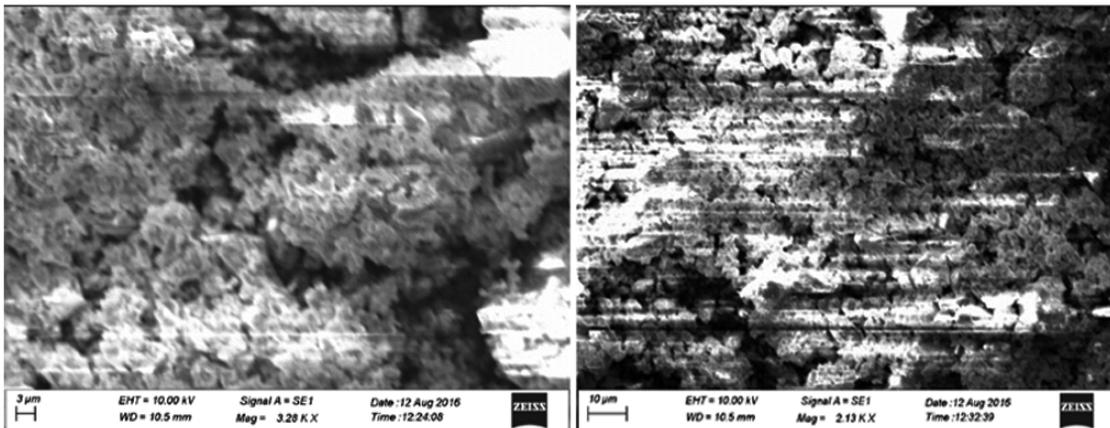


Fig. 2 — SEM micrographs of Shankh bhasma at different magnifications

Effects of Shankh Bhasma on *C. elegans*

C. elegans as experimental animal we put *Shankh Bhasma* in culture plate and incubates at 37°C for 72 hours. After incubation period ends, the number of eggs hatched 8100 thousand per plates and percentage of eggs is approximately 96%, and results are shown in Fig. 5^{A-B}

Effect of Shankhbhasma on *E.coli*

The details of effect of Shankh Bhasma on *E.coli* are shown in Table 2 and their results are shown in Fig. 6. The method of mechanism is explained in Materials and Method section.

Discussion

From the XRD patterns shown in Fig. 1, it is clear that the *Shankh Bhasma* powder is crystalline in nature. The measure sharp peak (29.4°) shows good

crystallinity, it could be due to the presence of calcium carbonate in the Shankh Bhasma (Table I). The average crystallite size is found to be ~ 68 nm by employing the Debye-Scherrer's equation²⁰ for Shankh Bhasma, which is the average of all the peaks analysis. Most of the compounds are oxides along with calcium carbonate. The crystallite size calculated by the XRD analysis of Shankh Bhasma in the order of nanometer size, whereas the particle size obtained from the SEM micrograph are in micro-meter range of nano crystalline agglomerated particles (Fig. 2). It reveals that the sample is in polycrystalline nature. In a single particle (Fig. 2), there may be numerous crystallographic grain boundaries. Also it is worthy to point out that the XRD technique has limitation to calculate the particle size above 50 nm. The FTIR spectrum shown in Fig. 3 shows the presence of Caboxyl group along with chloride and fluoride of

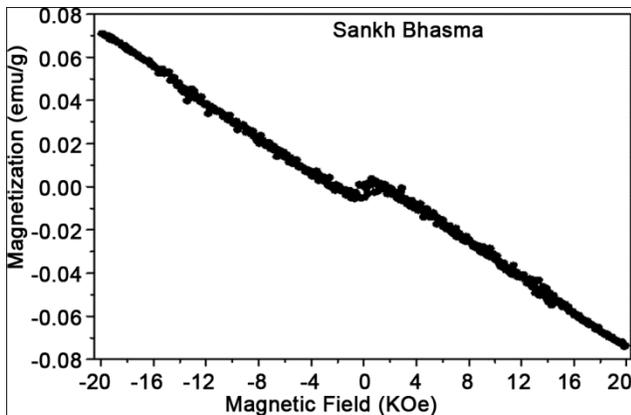


Fig. 4 — M-H hysteresis curves for Shankh Bhasma nanoparticles

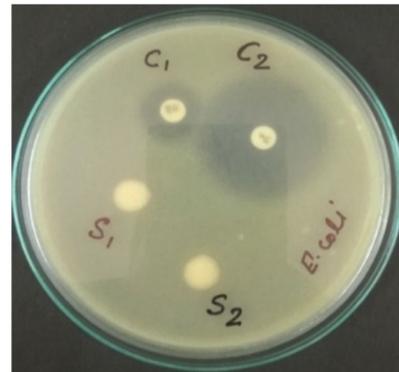


Fig. 6 — Shankh Bhasma on *E.coli*

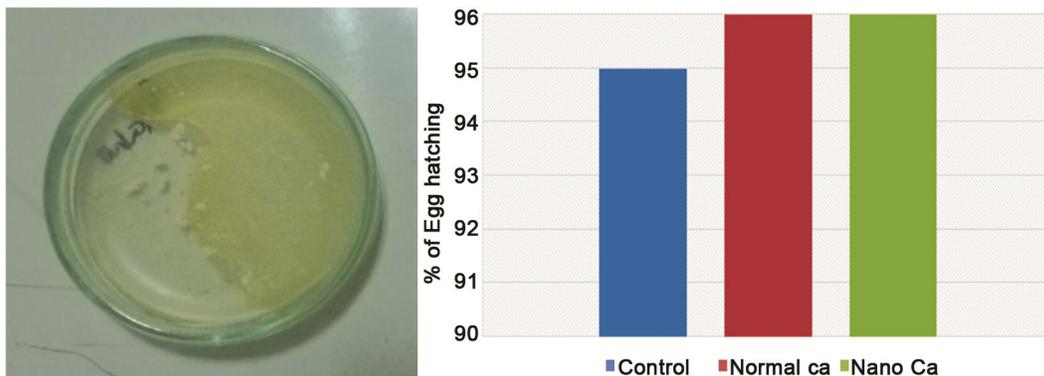


Fig. 5 — (A) Culture plate of *C. elegans* with nanometric shankh and (B). Developmental study egg of *C. elegans*

Table 2 — Effect of Shankh Bhasma on pure bacterial strain of *E.coli*

Name of bacteria	Name of bhasma	Inhibition zone of Bhasma	Control antibiotic (I)	Zone of inhibition	Control antibiotic (II)	Zone of inhibition
<i>E.coli</i>	Shankh	00mm	AK	13mm	LE	28 mm
	Shankh with acetone	00mm	AK	13mm	LE	28 mm

carbon. Thus the FTIR and XRD result support the presence of herbal ingredient along with majority of Calcium based oxides in the Shankh Bhasma.

The M-H hysteresis loop as shown in the Fig. 4 reveals the diamagnetic behavior of the Shankh Bhasma. Although, there is Iron oxide in the Shankh Bhasma. However, its amount is very less to contribute in magnetization. Thus the present finding reveals that calcium based Shankh Bhasma ayurvedic medicine exhibits diamagnetic nature. It is interesting to note that, the self magnetic behavior of medicines can be utilized for targeted drug delivery, molecular imaging etc.²³.

Medicinal effect of Shankh Bhasma is shown in Fig. 5^{A-B}. It has been tested on *C. elegans* and it showed that there is no any defect cause by Shankh Bhasma in development of eggs. Over all percentage of eggs slightly increased in case of *E.coli* (food material of *C. elegans*) with normal calcium and nano calcium added plates. Therefore it is pointed that, there are no any toxicity effects of Shankh Bhasma on *C. elegans*. This result reveals that in case egg development, nanocrystalline Shankh Bhasma may be useful in addition to normal bulk size calcium based compound. Further detail study on different crystalline size of this Bhasma on *C.elegans* can be studied. This is our future research plan. Action of Bhasma on *C. elegans* was also reported by some research group¹².

Acetone treated and without acetone treated shankh bhasma (sample dilution 0.5gm/ mL) and its results are shown in Fig. 6 and Table 2. There is no any zone of inhibition shown after 18 hours incubation at 37°C were as levofloxacin and amikacin have shown zone of inhibition as control antibiotics, so we can say Shankh Bhasma have not found any antimicrobial properties. Consequently it can be pointed that there is no effect of Shankh Bhasma on colitis and wound related disease. But, in this present work, Shankh Bhasma shows positive effect on *C.elegans* in egg lying. However, some Bhasma show effective on bacterial infection disease, reported by other research group^{13, 24-26}

Conclusion

The X-ray diffraction, scanning electron microscope measurements reveal that prepared Shankh Bhasma as mentioned in ancient Ayurvedic text are agglomerated nanocrystalline materials. In addition to Ca based compound, iron and magnesium

based oxides were found in the Shankh Bhasma. FTIR and XRD data support the presence of herbal ingredient in the Shankh Bhasma. The magnetization of this material is found to be 0.07 emu/g at 2T magnetic field and it is diamagnetic nature. Antimicrobial studies and effect of *C. elegans* studies show that there is no effect of Shankh Bhasma on *E. coli* but shows positive effect on *C. elegans* in egg lying. Thus the present study reveals that an Ayurvedic Shankh Bhasma are nanocrystalline materials and may be used calcium deficient disease.

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