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Comparative characterization study of *Loha Bhasma* and *Triphala* derived Iron Nano Particles

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ABSTRACT

Character of a particle varies as size changes from macro to nano scale. Size of the particle is of great value in the field of medicine. Size has the influence on dosage, delivery and penetration of the medicine. Rasashastra is the branch which deals with the use of minerals and ores for health benefit. Bhasma is the unique preparation which is very minute in nature intended to penetrate deep into the tissues with minimal dosage. Nano medicine is the recent develop in the field of medicine where nano particles are used to reach the target cells. Nano particles are synthesised by different methods. Green synthesis is the method where herbal extracts were used to synthesize the nano particle. This method is similar to preparation of *Bhasma* where different herbs are used. In this study Iron nano particles were synthesized using *Triphala* extract and it is compared with *Loha Bhasma. Bhasma Pareeksha* and Characterization studies were done. This study confirms the nano nature of *Bhasma* and similarity in the structure between *Bhasma* and Nano particles.

Key words: *Iron Nano particles, Loha Bhasma, Bhasma Pareeksha, Characterization Study.*

INTRODUCTION

Minerals and metals were not been used for internal administration in the yester years as there was no proper methodology to convert them into fine particles and to get themselves enable for the absorption in the body. *Charaka Samhitha* and *Sushrutha Samhitha*, oldest Ayurveda treatises mention use of metals like Iron in the form of *Ayaskriti*, where thin leaves of iron was heated to red hot and dipped in liquid media. Properties of iron were thought to be passed in the prepared medicine.[1] However after development of *Rasashastra* various concepts got emerged and the concept of *Marana* and otherwise called as *Bhasmikarana* took a lead role in the therapeutic application. The metals and minerals were subjected to incinerate in a classical method called *Marana* and *Bhasma* was obtained. They were subjected to different tests like *Varitara, Unmana, Rekhapurnatwa, Niruttha* and *Apunarbhava* before it was used for the body.[2] The needed qualities like minuteness, deep penetration into the tissue, less adverse effects, high efficacy in minimum dosage were thus achieved. *Bhasma* are herbomineral preparations of the size of nano scale.[3]

Character of a particle varies as size changes from macro to nano scale. Size of the particle is of great value in the field of medicine. Size has the influence on dosage, delivery and penetration of the medicine. Nano technology is widely used in the field of medicine as nano medicine. Green synthesis of the nano particles gained lot of attention owing to the instinctive features such as usage of natural resources, rapidness, eco friendliness and benignancy. These appealing features are essential in the medical application. The other advantage of green synthesis include well defined and controlled size of the nano particles. The various bio molecules present in the plant extract such

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as enzymes, proteins flavonoids, terpenoids and co factors act as both reducing and capping agents. The plant mediated synthesis of nano particles is relatively fast as there is no need of maintaining specific media and culture conditions unlike microbial synthesis.[4]

Herbs are essential for the preparation of *Bhasma* and Nano particles. Studies have been carried on nano features of *Bhasma*.[5] There is need to prepare the nano particles by green synthesis using herbs explained under preparation of particular *Bhasma* and study characteristics of *Bhasma* and nano particles. Iron is one of the frequently used metal in medicine. *Triphala* is the herb used in the preparation of *Loha Bhasma*.[6] In this study Iron nano particles were prepared using *Triphala* extract and its characteristics were studied in comparison with *Loha Bhasma.* Anti-microbial and anti-cancer activity of *Loha Bhasma* and Iron nano particles were also studied to understand their clinical utility.

Preparation of Nano particles

Iron oxide nanoparticles were prepared by green synthesis using *Triphala* extract (*Triphala*: Dried Fruits of *Emblica officinalis, Terminalia bellirica* and *Terminalia chebula*).

Extract preparation: 0.1g of *Triphala* fine powder was added to 100 ml of distilled water and heated at 80 C for 1 h. The solution is cooled, filtered, and stored in the refrigerator for further use.

Iron oxide nanoparticles: 0.1M of iron sulphate heptahydrate is mixed with *Triphala Choorna* extract in a ratio of 1:1, the pH is adjusted >10 with 1M NaOH and heated in a water bath for an hour at 80°C. The formed black turbid solution is let to settle, washed with water until neutral, and dried to obtain iron oxide nanoparticles.

***Loha Bhasma*:** Market sample of *Loha Bhasma* manufactured by Shree Doothapapeswar limited, (SDS monograph no. 020008, License number AYU 150, batch no. P200800182, manufactured date August/2020, Reference Bharatha Baishajya Ratnakara 4/6416) was purchased for the study.

Bhasma Pareeksha

Bhasma Pareeksha was conducted to confirm the properly formed *Bhasma*. Tests conducted were, *Varitara* means *Bhasma* should float on water, *Unmana Pareeksha* where a rice grain was kept over the floating *Bhasma* it should not sink. *Rekhapoornatva*, When *Bhasma* is taken in between the thumb and index finger and rubbed, *Bhasma* should fill in the furrows of the fingers. *Nirdhooma* means when little quantity of the *Bhasma* is sprinkled over the burning coal it should not produce smoke. As both *Loha Bhasma* and Iton nano particles are derived from iron their Magnetism was tested.

Colour of *Loha Bhasma* was black and that of Iron nano particle was brown. *Loha Bhasma* showed the *Varitara* property and also it did not wet but most of the Iron nano particles sink in water. (Photo 1). In *Unmana*, Rice grain floated on *Loha Bhasma* but it sinked when kept over Iron nano particles. (Photo 2). Both *Loha Bhasma* and Iron nano particles show *Rekhapoornatv*a but *Loha Bhasma* was unctuous in nature and Iron nanoparticles were rough to touch (Photo3). *Loha Bhasma* and Iron nano particles did not produce smoke on dropping over red hot crucible. (Photo4). *Loha Bhasma* and Iron nano particles attracted by the magnet. (Photo 5)

Characterization of *Loha Bhasma* and Iron nano particles:

Zeta potential was measured using Brookhaven model ZetaPALS to assess the particle size of the *Loha Bhasma* and Iron nano particle. FTIR Spectroscopy was carried out to find out the functional groups on the surface of *Loha Bhasma* and nano particles. FTIR spectrum was obtained with Perkinelmers, Frontier spectrometer analysed in ATR mode (4000-650cm-1). X ray diffraction was performed to examine the crystallographic structure of the purified *Bhasma* and nano particles. XRD pattern was recorded using Rigaku, Smartlab X-Ray Diffractometer with standard mode.

RESULTS

Particle size was estimated using zeta potential. Size of *Loha Bhasma* was 591nm and that of Iron nano particles was 417 nm. XRD pattern of *Loha Bhasma*

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showed Magnetite (Fe2O3) and Haematite (Fe3O4) as the major components in *Loha Bhasma*. (Fig 1) XRD pattern of Iron nano particles showed Magnetite (Fe3O4) and Goethite (FeO) as the major components (Fig 2). FTIR analysis was performed with the intention of identifying several functional groups responsible for the synthesis of *Loha Bhasma* and Iron nano particles. FTIR spectrum of *Loha Bhasma* revealed prominent peaks at 450 and 600 range corresponding to the important forms of Iron oxide or Fe-O bonding. The small band at 2900 was due to the –CH stretching of the alkanes (Fig 3). FTIR spectra for Iron nano particles showed prominent peaks at 3400, 1600, 1400, 1200, 1000, 900 800, 600 and 400. Appearance of Peaks at 450 and 550 range confirms the presence of Iron Oxygen which confirms the synthesised nano particles have Iron Oxide. Peaks positioned at 1600 and 3400 corresponds to O-H stretching due to absorbed water. Small stretching at 1400, 1200 and 1000 connoted the C-O stretching of esters an C-O stretching of amines present in the extract. FTIR spectra details shows the presence of various biomolecules in the *Triphala* extract used in the preparation of Iron nano particles (Fig 4).

Discussion

*Sookshmatw*a or minuteness is an important quality required in a drug for proper absorption and penetration into cells. Journey from macro molecule to nano particle was not new in India. *Rasashastra* in Ayurveda concerned about preparation of medicines using metal and minerals. *Bhasma* is a calcined herbomineral preparation of Nano size which is safe for internal administration. Development of Nano medicine has revolutionized the field of modern drug delivery and dosage. Both *Bhasma* and Nano particles have many similarities in their method of preparation and usage. Different herbs are used during *Shodhana* and *Marana* of the metals to get pure *Bhasma* form. Metal will undergo different physical and chemical changes during these process. Green synthesis of nano particles use different herbal extract for the preparation of Nano particles. In green synthesis of nano particles, it is not subjected to high temperature as *Bhasma* preparation. Application of high temperature or calcination will make *Bhasma* different form Nano particle. In this study *Loha Bhasma* was selected and it was compared with Iron nano particles. *Triphala* are the major herbs used in different stages in the preparation of *Loha Bhasma*.[7] Iron nanoparticles were prepared by green synthesis method using *Triphala* extract. When both particles were subjected to *Bhasma Pareeksha* of Ayurveda, Colour of the *Loha Bhasma* was black, this may be because of the method of preparation of *Loha Bhasma* where *Hingula* and *Triphala Kashaya* were used.[8] *Loha Bhasma* showed *Varitara* and *Unmana* property whereas most of the Iron nano particles sink in water. When a powder of higher density like *Loha Bhasma* is sprinkled on surface of water its ability to float on the surface depends on the surface energy of the powder. When the adhesive forces between powder and a liquid is lower than the cohesive forces between the molecules of liquid, the powder surface is not wetted by the liquid. Hence particle with lower surface energies are associated with increased contact angle with water, implying non wetting character. For such non wetting solids there exists a critical contact angle for the surface above which the material floats. This happens when the weight of the solid is overcome by the surface tension forces. As weight of the particle decreases with particle size the critical contact angle also decreases and reduction in surface free energy.[9] That may be the reason for the *Varitara* property of *Bhasma*. In nano particles the adhesive force between the nano particles and contact angle may be less making nano particles to sink. Nano particles where coarse and rough when compare to *Bhasma. Rekha Poornatw*a confirms their fineness while *Nirdhuma Pareeksha* was positive for both confirm absence of moisture or *Bhasma* transformed to complete ash.

Characterization studies like Zeta potential revealed the size of the *Bhasma* and nano particles at nano scale with *Loha Bhasma* at 591 nm and size of Iron nano particles were 417nm.XRD studies revealed that *Loha Bhasma* contains mainly Iron oxides like Magnetite Fe3O4 and Hematite (Fe2O3). Previous studies also confirmed the presence of iron oxide in *Loha Bhasma*.[10] Major component of Iron nano particles

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were Magnetite (Fe3O4) and Goethite (FeO). FTIR studies of *Loha Bhasma* confirmed the presence of Iron oxides. This result agree with the similar finding of studies on *Loha Bhasma*.[11] FTIR of nano particles showed extra peaks which indicate the presence biomolecules present in *Triphala* extract which were responsible for the reduction of Iron nano particles. These finding matches with other characterization studies on green synthesis of Iron nano particles.[12] As *Loha Bhasma* was produced by calcination it was only showing oxide form. Based on characterization studies core shell model of *Loha Bhasma* has been suggested with Fe2O3 as core and Iron complexes with organic moieties as shell.[13]

Further study is required to understand the rationality behind the use of different herbs and metals in the process of preparation of *Bhasma* and changes taking place during different stages of preparation.

Conclusion

Characterization studies showed similarity in structure of *Loha Bhasma* and Iron nano particles. This study confirms the nano characteristic features of *Bhasma* and opens new ideas in green synthesis of Nano particles using herbs mentioned in the synthesis of *Bhasma*.

Acknowledgement

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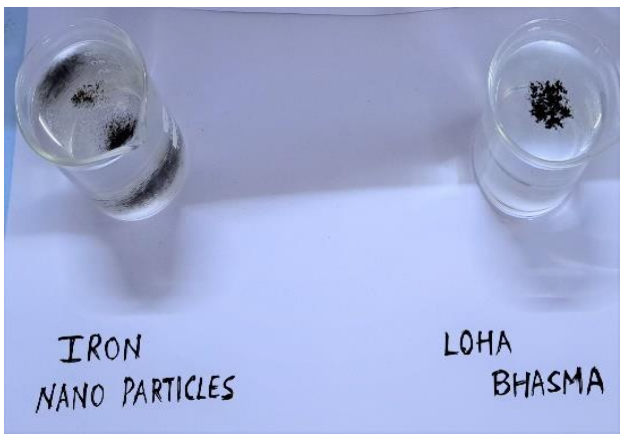

Photo 1: ***Bhasma Pareeksha : Varitara Pareeksha***

Photo 2: ***Bhasma Pareeksha : Unmantwa***

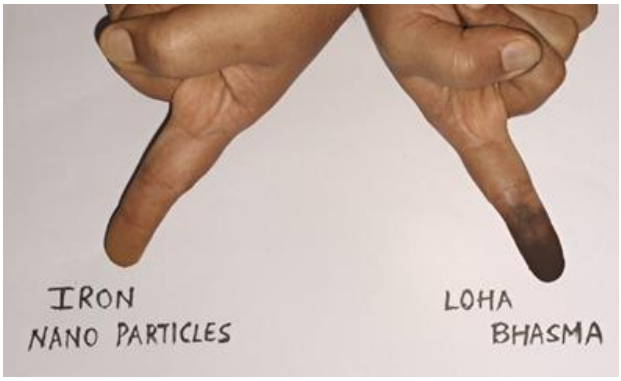

Photo 3: ***Bhasma Pareeksha : Rekha Poornatwa***

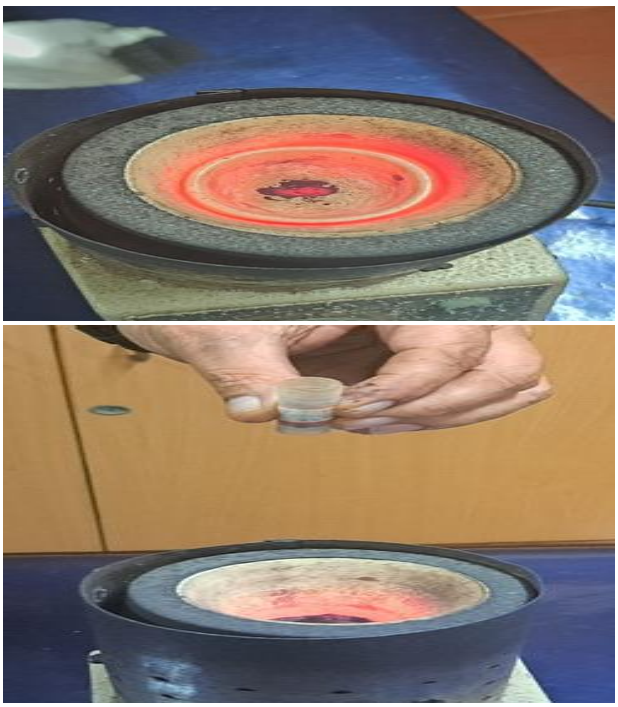

Photo 4: ***Bhasma Pareeksha : Nirdhuma***

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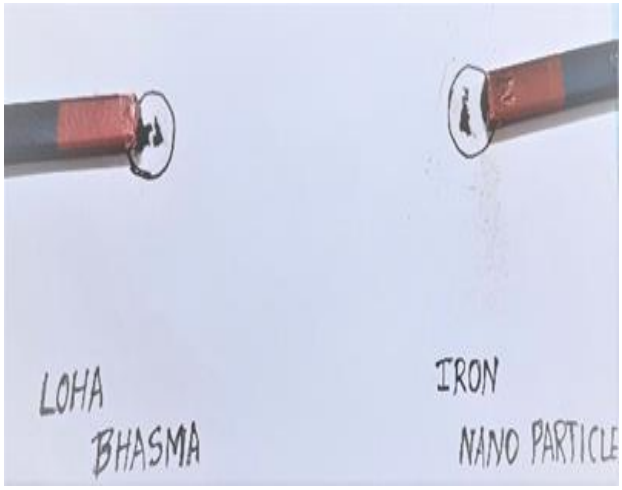

Photo 5: Both *Loha Bhasma* and Iron nano particles attracted towards Magnet

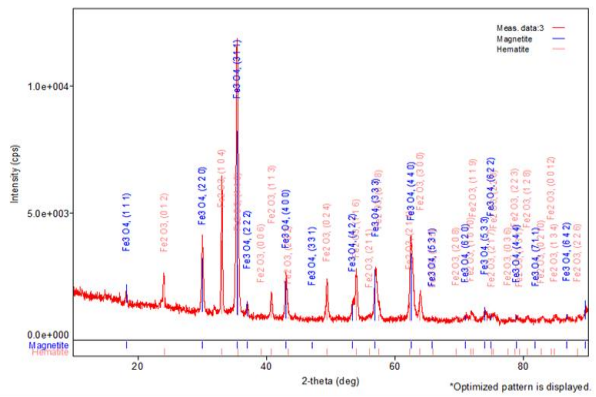

Figure 1: XRD pattern of *Loha Bhasma*

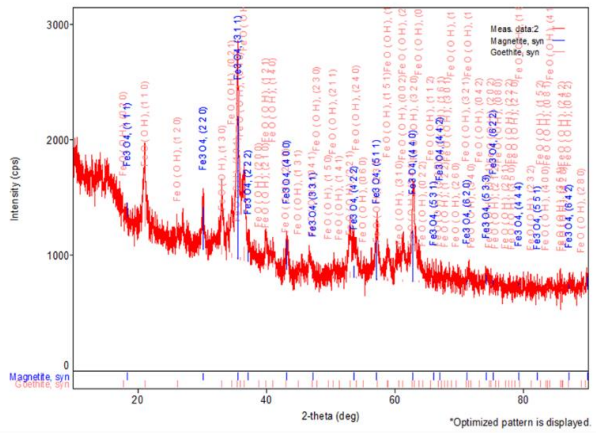

Figure 2: XRD pattern of Iron Nano particles

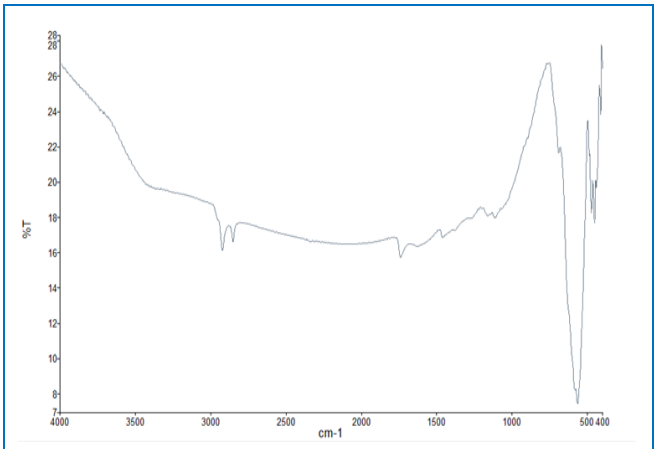

Figure 3: FTIR spectrum of *Loha Bhasma*

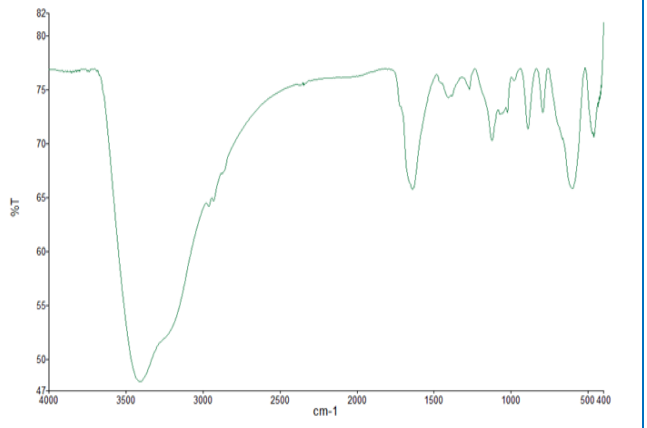

Figure 4: FTIR spectrum of Iron Nano particles

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