Academic Sciences International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 4, Issue 2, 2012

Research Article

# ELUCIDATION OF A CORE-SHELL MODEL FOR *LAUHA BHASMA* THROUGH PHYSICO-CHEMICAL CHARACTERIZATION

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*Received: 21 Dec 2011, Revised and Accepted: 1 Mar 2012*

**ABSTRACT**

*Lauha bhasma*, an iron-based herbo-metallic preparation, is an Ayurvedic medicine prescribed for the treatment of ailments due to iron deficiency. The preparation of *Lauha bhasma* involves a rigorous procedure meant to convert the metal in to a fine, non-toxic and bio-available form. The physico-chemical properties of a commercially available *Lauha bhasma* have been studied based on classical parameters and through the use of modern analytical techniques. *Lauha bhasma* sample was found to conform to few of AYUSH specifications, while deviations were found in other. A core-shell structure has been proposed for chemical nature of *Lauha bhasma* using information obtained from various complimentary characterization tools.

**Keywords:** *Lauha bhasma*, Core – shell model, Fe-complex, floatability, X-ray diffraction, Elemental analysis.

## INTRODUCTION

*Ayurveda* is one of the alternative medicines, originally conceptualized by the sages of ancient India, as the *veda* (science) of *Ayush* (life)[1]. *Ayurveda* may also be defined as a medical science, aimed at helping the human body to stay fit by providing cures and remedies for various ailments, with drugs obtained from indigenous plants, animal products and minerals[1]. *Rasasastra* (Alchemy) is one of the branches of *Ayurveda* dealing with the alchemical and pharmaceutical process of metals and minerals of aquatic and soil origin[2]. When such metals and minerals undergo purification and calcination processes, fine powders called *bhasma* (ash) are obtained[2]. *Bhasmas* are also referred as herbo-metallic preparations as they contain both metallic and herbal ingredients. *Ayurvedic* formulations, including *bhasmas* have been reported to contain heavy metals such as mercury, lead, cadmium, arsenic etc[3,4,5,6]. However, the results in the literature do not indicate the state of the metals in the *bhasma*. The ancient literature lays emphasis on the purification processes involved during the preparation of *bhasmas*. *Ayurvedic* practitioners believe that the purification processes are vital in removing the toxicity of metals.

In developing countries, anemia is one of the major health problems and in India, *Lauha bhasma*, an iron-based herbo-metallic preparation, is prescribed for the treatment[7,8]. The process for preparation of *Lauha bhasma* is complex, which involves several steps aimed at converting metal into a de-toxified, biocompatible form that can be easily absorbed and assimilated. The first stage in the preparation of *Lauha bhasma* involves a normal purification step by subjecting the raw material (iron) to heat treatments in various plant extracts and dairy products[9]. This is followed by treating the purified material with herbal ingredients, in a special purification process, meant to de-toxify the metal and incorporate specific therapeutic properties. Subsequently, calcination is performed to transform the material into the form of a fine powder that facilitates easy absorption.

Several physical and chemical parameters have been prescribed for ascertaining the quality of *Lauha bhasma*[9]. Physical parameters include color, luster, floatability, fineness, taste, etc., while chemical parameters include test of *Lauha bhasma* for irreversibility to metallic state[9].Most of these parameters are qualitative and the methods for their determination are simple. Kumar et al.[10] observed the presence of essential elements like Na, K, Ca etc. at µg/g level in *bhasmas* and hypothesized them to be in chelated form with organic ligands from herbs[9]. Rajendraprasad et al.[11] prepared and analyzed *Lauha bhasma*, from which the presence of $Fe_2O_3$ was observed through chemical characterization[11]. Singh et al.[12]characterized *Lauha bhasma* through X-ray diffraction and vibrating sample magnetometry and concluded *Lauha bhasma* to be consisting of $Fe_2O_3$ and $Fe_3O_4$[12]. Recently, Singh et al.[13]have reported the particle size and elemental composition of *Lauha bhasma* that was prepared according to methods provided in the classical texts of *Ayurveda*[13]. The presence of particles in nano-dimension and that of trace elements in the *bhasma* was identified[13]. The state of iron and its interaction with organic moieties added is still unclear and needs to be investigated since the toxicity of metallic iron is well established[14-15]. More insight about the nature of *Lauha bhasma*, its efficacy and quality can be obtained by systematic characterization of the samples using complementary analytical techniques that lead to its morphological, structural, elemental and chemical characterization.

In the present study, physico-chemical properties of commercially available *Lauha bhasma* was evaluated as specified in the ancient literature, in addition to characterizing the same using modern analytical tools. Also, parameters specified by the Department of AYUSH (Ayurveda, Yoga, Unani, Siddha and Homeopathy), Government of India such as color, appearance, solubility, total ash, acid insoluble & water soluble ash, loss on drying have been determined[16]. This study attempts to understand the chemical nature of *Lauha bhasma* through the information obtained from appropriate spectroscopic and microscopic techniques.

## MATERIALS AND METHODS

### Materials

*Lauha bhasma* was procured from manufacturer 'X'. Hydrochloric acid, nitric acid and sodium chloride were procured from Merck, Mumbai. Phosphate buffered saline solution was purchased from Gibco, USA.

### Methods

#### Estimation of Total Ash, Acid Insoluble Ash and Water Soluble Ash

One gram of the *Lauha bhasma* was weighed accurately and taken in a silica crucible. The sample was spread uniformly on the bottom of the crucible and incinerated at 450 ºC for 3 hours and allowed to cool naturally to room temperature. The residue was weighed and total ash was estimated[16].

The residue from total ash estimation was boiled with 25 mL of dilute hydrochloric acid for 5 minutes. The insoluble matter was washed with hot water, transferred to a crucible and dried. The mass of the residue was determined to estimate the acid insoluble ash[16].

The residue from total ash estimation was boiled with 25 mL of distilled water for 5 minutes. The insoluble matter was washed with hot water, transferred to a crucible, dried and weighed. The water soluble ash was determined by subtracting the mass of insoluble matter from the total ash[16].

**Determination of Loss on Drying (LOD)**

One gram of *Lauha bhasma* was taken in a crucible and dried in an oven at 105°C for about 5 hours. The sample was allowed to cool and the dry mass was determined. The difference in mass was used to determine the loss on drying and expressed in percentage.

**Determination of Bulk Density, Tapped Density and Hausner ratio**

About 5 g of *Lauha bhasma* was carefully poured into a long measuring jar and the volume corresponding to top level of the sample was noted. The bulk density was calculated as the ratio of mass to volume of the sample.

About 5 g of *Lauha bhasma* was carefully poured into a long measuring jar and subjected to 100 tappings, as per United States Pharmacopoeia –II (USP–II) in a tap density tester (TD1025, Lab India, India). The volume corresponding to top level of the sample was noted and the tapped density was calculated as the ratio of mass to volume of the sample. Hausner ratio was calculated as the ratio of tapped density to bulk density. The flow property has been qualitatively characterized as good, passable, poor, very poor, very very poor etc. based on Hausner ratio[17].

**Varitara (Floatability test)**

A pinch of the *bhasma* was sprinkled on water taken in a beaker and the floating tendency of the *bhasma* was observed.

***Niruttha* (Test for irreversibility)**

A known amount of silver was heated in a muffle furnace for 15 minutes with the *Lauha bhasma* at various temperatures in a silica crucible. The silica crucible was cooled to room temperature and the mass of silver was recorded. The change in the mass of silver is an indication of improper calcination during the preparation of *bhasma*.

**Sub–Sieve Particle Size Distribution & Surface Area Analysis**

Lauha bhasma was dispersed in water and sonicated for 5 mintues. The sub–sieve particle size distribution was determined using laser diffraction technique (Bluewave Microtrac, Nikkiso, Japan). The surface area, pore volume and the adsorption isotherms were using BET technique (ASAP 2020, Micromeritics, USA).

**Electron Microscopy**

The surface morphology of *Lauha bhasma* was qualitatively assessed using a cold field emission scanning electron microscope (JSM 6701F, JEOL, Japan). The sample was mounted on a brass stub and sputter coated with platinum and introduced into the specimen chamber. Imaging was carried out at an acceleration voltage of 3 kV.

**Spectroscopic Analysis**

*Lauha bhasma* was pelletized using a 25-ton hydraulic press to prepare thin discs of 34 mm diameter. The elemental composition of *Lauha bhasma* was determined using an X-ray fluorescence spectrometer (S8 Tiger, Bruker AXS, Germany) using a 4 kW Rhodium anode X-ray tube. The percentage of carbon was determined using a CHNS/O analyser (Series II 2400, Perkin Elmer, USA).

*Lauha bhasma* was mixed with KBr and pelletised using a hydraulic press. The Fourier Transform Infra Red (FTIR) spectra were recorded between 4000–400 $cm^{-1}$ in a FTIR spectrometer (Spectrum 100, Perkin Elmer, USA).

**XRD Analysis**

The crystallinity of Lauha bhasma was analyzed using an X–ray diffractometer (D8 Focus, Bruker, Germany), by irradiating with Cu–Kα radiation. The analysis was performed from 10° to 60° (2θ) with a step size of 0.01°.

**Thermal Analysis**

The thermal analyses of the samples were carried out using thermogravimetry (SDT Q600, TA Instruments, USA). 5 mg of the sample was introduced in an alumina cup and heated gradually in a nitrogen atmosphere at the rate of 10°C/minute.

**Hemolysis Assay**

Hemolysis assay was performed to assess the membrane stability of erythrocytes in the presence of *Lauha bhasma*. A specific quantity of *Lauha bhasma* was dispersed in phosphate buffered saline and incubated for 60 minutes with blood sample containing 0.2 % NaCl solution. The effect of *Lauha bhasma* on the membrane stability was quantitatively estimated by measuring the absorbance of supernatant at 560 nm.

**RESULTS AND DISCUSSION**

Table 1 shows the total ash, acid insoluble ash, water soluble ash and moisture content of *Lauha bhasma*. Total ash value is useful in determining the purity of *bhasma* since higher total ash content indicates the absence of free organic moieties. During the preparation of *Lauha bhasma*, herbal ingredients are added resulting in the formation of complexes between the herbal constituents and the metal. The formation of coordination compounds will be precluded however, if the *bhasmas* are not prepared properly, resulting in lower total ash content.

Acid-insoluble ash is an indicator of quantity of acid non-digestible mass in the sample[18]. Hence, lower acid-insoluble ash indicates higher bioavailability of the drug[18]. Lower value of loss on drying indicates the absence of moisture in the drug.

The *bhasma* was found to be a brown powder without any metallic luster (Table 2), conforming to the specifications of Ayurvedic Formulary of India[9]. The bulk density and tapped density of bhasma were found to be 0.69 $g/cm^3$ and 1.05 $g/cm^3$ respectively. The true density or particle density is greater than the tapped & bulk density and determines the position of a particle in a static liquid. For particles with density lower than that of water, floating is observed (buoyant force greater than the gravity) while denser particles sink. The position of the particle in a liquid is not influenced by the sequence of events i.e. independent of whether powder is added to liquid or liquid is added to the powder. However the floatability test for *bhasma* is to observe the floatability of a powder sprinkled on the surface of water and this is expected to involve interfacial forces that act at the three interfaces (gas-liquid, liquid-solid and gas-solid).

**Table 1: Total ash, acid insoluble ash, water soluble ash & loss on drying of *Lauha bhasma***

| Total Ash (%) | Acid Insoluble Ash (%) | Water Soluble Ash (%) | Loss on Drying (%) |
|---|---|---|---|
| 92.48± 2.50 | 62.09±1.77 | 33.87±1.70 | 0.32±0.23 |

**Table 2: Appearance, floatability on water, bulk density, tapped density, Hausner ratio and flow property of *Lauha bhasma***

| Appearance | Luster | Bulk Density ($g/cm^3$) | Tapped Density ($g/cm^3$) | Hausner Ratio | Flow Property | Floatability on Water |
|---|---|---|---|---|---|---|
| Brown powder | No | 0.69 | 1.05 | 1.52 | Very poor | Partially |

When a powder of higher true density like *Lauha 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 force between the 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, the particles with lower surface energies are associated with increased contact angle with water, implying hydrophobicity and non-wetting character. For such non-wetting solids, there exists a critical contact angle for the surface, above which the material floats[19]. This happens when the weight of the solid is overcome by the surface tension forces[20]. As the weight of the particle decreases with particle size, the critical contact angle also decreases with particle size[19]. Also, the reduction in surface free energy with decrease in particle size has been demonstrated[21,22]. Hence, for a properly prepared *bhasma* the contact angle with water would be greater than the critical contact angle owing to extremely smaller size of *bhasma* particles, making them float on water. The partial floatability of *Lauha bhasma* in the present case may be attributed to the sinking of coarse particles present in the *bhasma*. Our floatability data reveal that the commercial sample analyzed does not conform to the specifications of an ideal *bhasma*[9].

The results of *Niruttha* test indicate that there was no weight reduction upon heating the *bhasma* with silver till 550 °C. A weight loss of 1.43% was observed when heated to a temperature of 600 °C. The decrease in the weight of silver after heating may be attributed to the presence of free metal (iron) in the *bhasma* which forms an alloy with silver thereby reducing the melting point of the latter, as per the silver-iron phase diagram[23].

Sieve analysis of *bhasma* indicates that 30 % (by mass) of the particles were smaller than 45 µm. The sub-sieve size distribution ranged between 1.7 and 10.4 µm. Hausner ratio was found to be 1.52 indicating very poor flow property of the *bhasma*[17]. The BET surface area and pore volume were found to be 6.46 $m^2/g$ and 0.026 $cm^3/g$ respectively. The adsorption isotherm (Fig. 1) shows the formation of additional layers of physically adsorbed gas molecules, indicating weak van der Waals interaction between the layers[24].

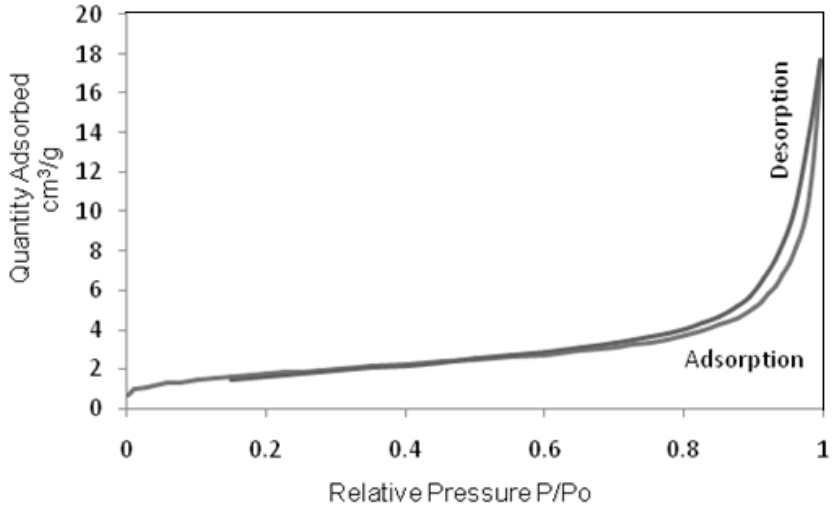


**Fig. 1: Nitrogen adsorption–desorption profiles of commercial *Lauha bhasma* sample**

Figures 2a and 2b show the scanning electron micrographs of *Lauha bhasma* at magnifications of 10000 and 50000. Irregular shaped aggregates (distorted spheres) of nano–dimensional particles (~ 28 nm) are observed. The aggregates are decorated with nano-structures on the surface. The role of nanostructured materials as therapeutic agents has been reasonably established [25,26]and we believe that the efficacy of Lauha bhamsa may be attributed to the presence of nanostructures.

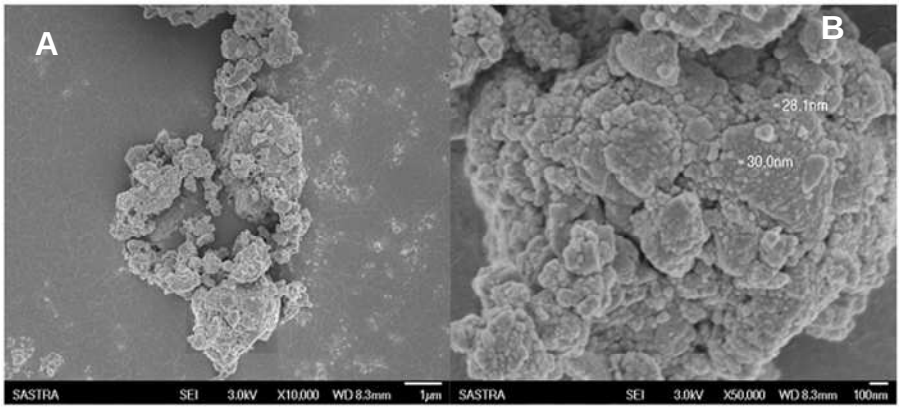


**Fig. 2: Scanning Electron Micrographs of commercial *Lauha bhasma* sample**

The thermogravimetric analysis of *Lauha bhasma* (Fig. 3) shows negligible loss in weight when subjected to programmed temperature change in nitrogen atmosphere. This indicates the absence of unreacted organic species in the *bhasma*. The elemental analysis of *Lauha bhasma* (Table 3) indicates the major elements to be iron (>60%) and oxygen (>30%). Other elements like Ca, K, Na, Cl from the herbal ingredients are present at >0.1% may be involved in pharmacological activities of the *bhasma*.

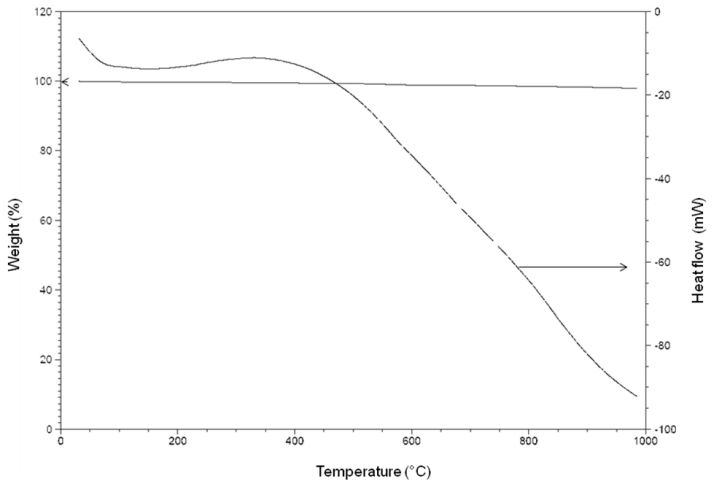


**Fig. 3: Thermogram of commercial *Lauha bhama* sample**

**Table 3: Elemental analysis of commercial *Lauha bhasma***

| Elements | Mass % |
|---|---|
| Iron | 66.63 |
| Oxygen | 30.39 |
| Calcium | 0.87 |
| Potassium | 0.32 |
| Sodium | 0.15 |
| Chlorine | 0.26 |
| Other elements | 1.38 |

The FTIR spectrum of *Lauha bhasma* (Fig. 4) shows a broad band between 3400 and 3500 $cm^{-1}$, characteristic of $\upsilon_{O-H}$ (stretching vibrations of O-H bond). The broad band in the region 1700-1650 $cm^{-1}$ is assigned to $\upsilon_{C=O}$ of organic constituents. The absorption band near 1620 $cm^{-1}$ may be attributed to the presence of aromatic ring, while the sharp band at 560 $cm^{-1}$ may be due to the Fe-O bond. All the above FTIR data suggest that the *Lauha bhasma* may contain a complex with organic moieties present in the treating agents used in the various stages of its preparation. Presence of metal-complex in *Lauha bhasma* has been reported earlier by Kumar et al.[10].

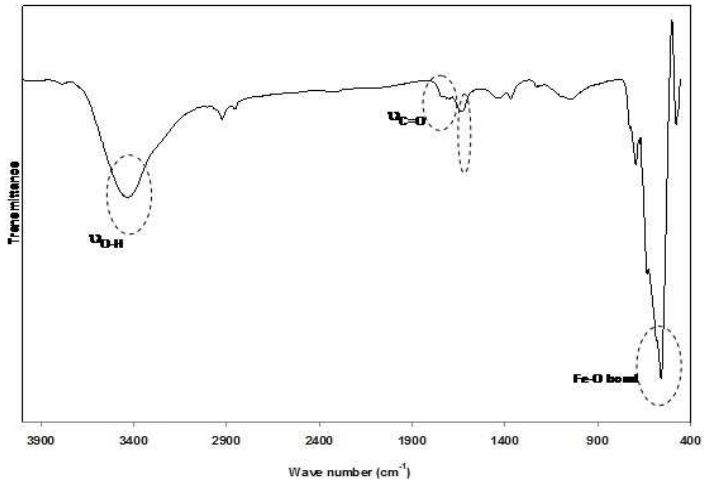


**Fig. 4: FTIR spectrum of commercial *Lauha bhasma* sample**

The X-ray diffractogram of *Lauha bhasma* (Fig. 5) shows intense peak at 36º indicating the presence of Fe(III), which conforms to $Fe_2O_3$ (Hematite) as per the PC PDFWIN data. This confirms the presence of $Fe_2O_3$ in *Lauha bhasma*. In the absence of any prominent peak at 45º, it may be inferred that the amount of free iron is insignificant. Rajendraprasad et al.[11] and Singh et al.[12] have also reported the presence of $Fe_2O_3$ in *Lauha bhasma*[11,12]. Nevertheless, FTIR results discussed above indicate the formation of iron complexes which are expected to be amorphous[27,28]. The intense peaks observed in X-ray diffractogram, despite the presence of significant bands (characteristic of the complexes) in the FTIR spectra suggest that the *bhasma* is composed of both $Fe_2O_3$ and iron complexes. When these results are analyzed with reference to the preparation procedure, it could be inferred that iron oxide on the surface forms complexes with organic moieties in the herbal extracts. Repeated trituration is performed to reduce the size and expose metallic ingredients to herbal constituents, as fluid-solid interactions are facilitated by smaller particle size and higher surface area[29,30]. Hence the surface may represent a shell containing organic moieties while $Fe_2O_3$ may still be present in the core (Fig. 6). The presence of organic moieties is also confirmed from the results of percentage of carbon in *Lauha bhasma* (0.3%) and in the residue obtained after estimation of total ash (0.19%).

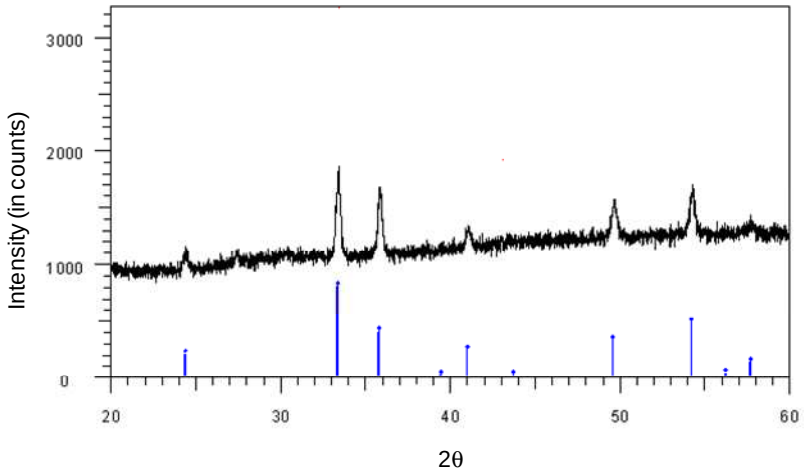


**Fig. 5: X-ray diffractogram of commercial *Lauha bhasma* sample**

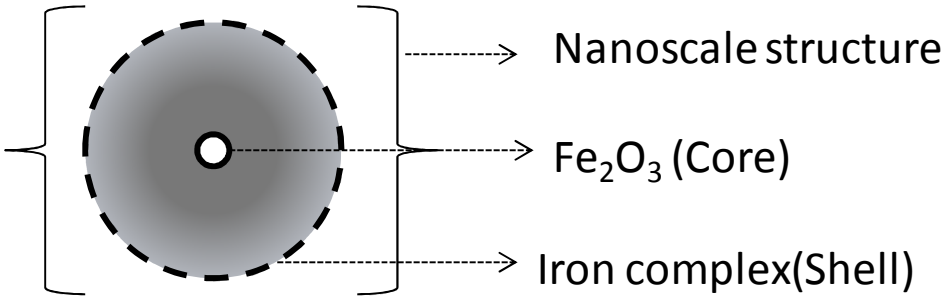


**Fig. 6: Proposed core-shell model for commercial *lauha bhasma.***

In order to evaluate the toxicity of this hybrid structure, hemolysis assay was carried out. Figure 7 shows the influence of *Lauha bhasma* dosage on the membrane stability of the erythrocytes under hypotonic stress. The data reveal that the membrane stability of erythrocytes is not compromised up to a *Lauha bhasma* concentration of 20 µg/mL

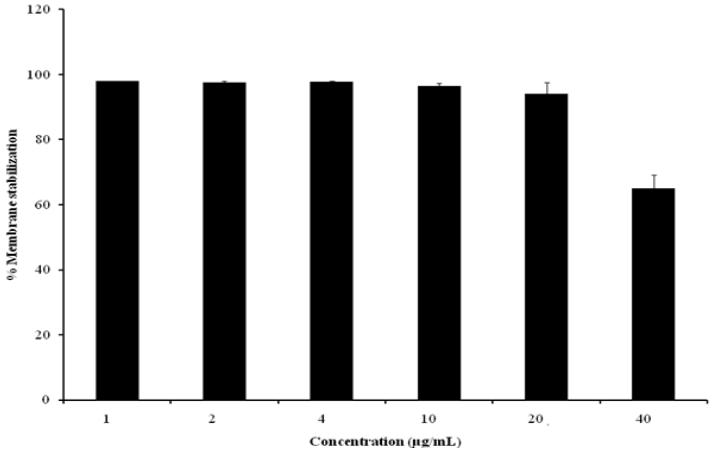


**Fig. 7: Influence of *Lauha bhasma* dosage on membrane stabilization**

## CONCLUSION

In the present study, we have comprehensively evaluated the physico-chemical properties of commercially available *Lauha bhasma*, based on AYUSH parameters as well as using modern analytical tools. Deviations from the AYUSH specifications in certain properties of *Lauha bhasma* were revealed in the study, though the appearance and luster of *bhasma* matched the AYUSH specifications. More specifically, there was change in mass during *niruttha* test, apart from settling of some particles in the floatability test. This indicates improper calcination.

The FTIR spectrum demonstrates that iron complexes are formed during the preparation, while X-ray diffractogram indicates the presence of $Fe_2O_3$. Hence, we propose a core-shell structure with $Fe_2O_3$ core and iron complex shell for the commercial *Lauha bhasma*.

## ACKNOWLEDGEMENT

The authors gratefully acknowledge the funding provided by the Drugs and Pharmaceutical Research (VI-D&P/267/08/09/TDT), Department of Science & Technology (DST), India and SASTRA University for this work. We also acknowledge the funding from

Nano Mission Council (SR/S5/NM-07/2006 and SR/NM/PG-16/2007), DST, India for SEM and XRD.

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