ISSN: 2322 - 0902 (P)
ISSN: 2322 - 0910 (O)

International Journal of Ayurveda and Pharma Research

Research Article

PHARMACEUTICAL AND ANALYTICAL EVALUATION ON *GANDHAKA MARITHA LOHA BHASMA*

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Article info

Article History:
Received: 19-02-2023
Revised: 14-03-2023
Accepted: 25-03-2023

KEYWORDS:
Loha bahsma, Gandhaka, XRD, Particle size analysis.

ABSTRACT

Rasasastra treatises have mentioned various methods for *Loha bhasma* preparation. The analysis on *Bhasma* is carried to show that the raw metals used for preparations lose their metallic characteristics and turn into mineral complex after processing. Physico chemical evaluation helps to ascertain the difference in chemical composition, elemental analysis and external morphology of *Bhasmas.* Thereby we can assure the quality and safety of *Bhasma*. Here *Loha bhasmas* was prepared with *Loha, Gandhaka* and *Kumari swarasa* thereafter its physico chemical properties are evaluated. The raw material used was 100 mesh size iron powder. It was first subjected to *Sodhana* and thereafter grinded with equal part of *Gandhaka* in *Kumari swarasa* and subjected to *Marana* under 600°C temperature with muffle furnace. From third *Puta* onwards amount of sulphur reduced to half subsequently. Final products were subjected to *Bhasmapareeksha* as per PLIM and with XRD, ICP-OES, XRF and Particle Size Analysis. XRD analysis revealed the body centered cubic lattice of raw iron powder changed into crystalline nature of alpha ferric oxides in final product. Elemental analysis shows reduction in silica and other toxic elements after *Bhasmeekarana*. The particle size reduction of *Bhasma* in micro meter was confirmed through dynamic light scattering. This data also will reveal the role of *Gandhaka* for quick formation of *Lohabhasma*.

INTRODUCTION

Variation in methodology adopted to formulate a drug is chief causative agents for non-uniform quality aspect of Ayurvedic formulation. Preparations are still done by traditional methods. Physico chemical evaluation is essential to ascertain the chemical composition, elemental analysis and external morphology of the metallic medicines. Analysis will ensure safety and purity of formulations. *Loha bhasma* is extensively used herbo mineral preparation in Ayurveda for *Pandu, Rajayekshma* etc. Over the past 10 years major advances have been made in identification of new proteins related with iron metabolism.[1] Various methods for *Loha bhasma* preparation have mentioned on Rasasastra treatises. Here *Gandhaka maritha lohabhasma* preparation is selected from Rasaratna samuchaya.

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https://doi.org/10.47070/ijapr.v11i3.2737

MATERIALS AND METHODS

The synthesis of *Loha bhasma* involves 2 major steps

Step 1. Ayurvedic purification of iron (*Shudhikarana*)

Step 2. Destruction of metallic structure or character (*Marana*) and transformation into the *Bhasma* state (*Bhasmeekarana*).

Step 1

2kg of Iron metal powder (100 Mesh size) was selected for preparing *Bhasma*. Iron powder can help to increase the contact of *Drava dravya* at the time of *Nirvapa*. The results from ICP-OES of raw iron powder shows 58% of iron and 16% of carbon which can probably equalize it with steel variety of iron.

1750 gram of Iron powder was heated in an iron pan until it become red hot and then immediately plunged into one litre of *Taila* taken in a bronze vessel. After self-cooling, the *Loha* is taken out and washed with hot water. This was repeated for seven times. For each time fresh *Taila* was taken. The same procedure was carried out in *Takra, Gomutra, Aranala* and *Kulathakashaya* successively for seven times.[2] *Aranala* was prepared with 2kg coarsely powdered wheat and 6 liter of water put into mud pot and properly sealed[3]. Then kept for seven days. For

Visesha sodhana Triphala kashaya was to be prepared with 2kg each of *Hareethaki, Vibheetaki, Amalaki* boiled in 48 liter of water and reduced to 12 liter. [4]

Step 2

Gandhaka sodhana was done as per *Kurma puta* method[5]. 500g *Gandhaka* was powdered in fine. Three liters of cow's milk was filled in mud pot. The mouth was covered with a kora cloth and tied properly. Powdered *Gandhaka* was spread over this cloth and closed with earthen *Sharava. Sandhibandhana* was done at the joint using multani mitti smeared cloth for seven times. Then the pot was buried in *Bhoodhara yantra*. 42 equal sized pieces of outer fibrous covering of coconut shell was arranged on above said brim of the vessel and set on fire. On the next day *Gandhaka* was seen collected in the milk washed and dried. 250 gram of purified *Loha choorna* and equal quantity of purified *Gandhaka* was added and grinded with 250ml *Kumari svarasa*[6]*. Chakrikas* were made out of it and dried. It was then kept in *Sharava* and covered with another *Sharava.* The joint was sealed with kora cloth smeared with multani mitti for seven times. It was then kept in furnace and incinerated at 600 degree temperature for one hour. The procedure was repeated for nine times. From the third *Puta* onwards the quantity of sulphur was reduced to half in quantity and last *Puta* was done without adding *Gandhaka.*

Gandhaka sodhana

Observations of *Gandhaka marana* of *Loha choorna*

No: of *Puta*	Weight of *Gandhaka maritha loha bhasma* (in gram)		Max: Temp	Weight loss (in gram)
	Before *Puta*	After *Puta*		
1.	515	306	600°	209
2.	675	310	600°	365
3.	400	272	600°	128
4.	354	308	600°	46
5.	428	295	600°	133
6.	330	246	600°	84
7.	300	232	600°	68
8.	254	203	600°	51
9.	236	189	600°	47

No: of *Puta*	Quantity of *Gandhaka* taken (in gram)	Quantity of *Kumari svarasa* taken (in ml)	Quantity of *Bhasma* taken for analysis	Duration of grinding (in hour)
1.	250	250	10	2
2.	296	250	10	2
3.	150	200	10	1
4.	75	150	10	1
5.	37.5	150	10	1
6.	18.75	200	5	1
7.	9.3	140	10	1
8.	4.5	100	20	1
9.	Not added	90		1

No: of *Puta*	Before *Puta paka*	At the time of *Putapaka*	After *Puta paka*
1.	Initially cement colour was observed while grinding. The colour changed to blackish after one day soaking in *Kumari svarasa*. Slight heat is emitted at the time of drying of pellets. The pellets before *Putapaka* were brownish black with shining appearance	Suffocating Yellowish smoke emitted from furnace after 15 minutes and the smoke persist for 2 hour	Scarlet red coloured particles were seen as a layer in top of pellet arrangement and blackish mixture was seen in below
2.	Cement colour was seen while grinding	Suffocating whitish smoke was noticed after 15 minutes and the smoke persist for 2 hour	Clay red coloured particles were seen as a layer in the upper layer of pellet arrangement and blackish mixture was seen in below
3.	Cement colour was seen while grinding	Fumes emitted after 45 minutes from furnace. Emission of fumes was with less in intensity	Bluish black coloured particles seen over the top layer Majority of pellets was in Blue color and some in blackish and some other in reddish on appearance
4.	Greyish black colour was while grinding	Fumes appeared after 45 minutes with less intensity	Clay red coloured particles seen on the upper layer with bluish black mixture in below

5.	Greyish black color was while grinding	Fumes appeared after 45 minutes with less intensity	Brick red particles are seen over the upper layer and autumn green coloured pellets was seen in below
6.	Brownish black color was while grinding	Slight fumes noted after 45 minutes	Orange red coloured particles seen over the top layer and blackish mixture below it in slight amount
7.	Brownish color was while grinding	Suffocating smell in less intensity noted after 45 minutes	Orange red coloured pellets was seen
8.	Reddish color was while grinding	Smoke was not observed	Dark reddish pellets was seen
9.	Reddish color was while grinding	Smoke was not observed	Reddish coloured powder was obtained

Physical analysis of *Gandhaka maritha Loha bhasma*

No: of *Puta*	Color	Odour	Taste	Touch	Luster
1.	Blackish brown	Pungent	Metallic taste	Little rough	Lusterless
2.	Black	Pungent	Metallic taste	Little rough	Lusterless
3.	Bluish black	Pungent	Metallic taste	Little rough	Lusterless
4.	Brownish black	Pungent	Metallic taste reduced	Roughness reduced	Lusterless
5.	Dark brown	Pungent	Metallic taste reduced	Little smooth	Lusterless
6.	Brownish red	Pungent	Sourness	Smooth	Lusterless
7.	Light brick	Pungent smell reduced	Sourness reduced	Smooth	Lusterless
8.	Light Brownish red	Absent	Tasteless	Smooth	Lusterless
9.	Dark brownish red	Absent	Tasteless	Smooth	Lusterless

Analysis of Physical test of *Gandhaka maritha Loha bhasma*

S. no	No of *Puta*	*Varitaratva*	*Uttama*	*Rekhapoornatva*
1.	After first *Puta*	Absent	Absent	-------
2.	After second *Puta*	Absent	Absent	-------
3.	After third *Puta*	Some particles seen floating	Absent	*Bhasma* filled the lines of the fingers but fell down after sometime
4.	After fourth *Puta*	Some more particles seen floating	Absent	*Bhasma* filled the lines of the fingers but fell down after shaking
5.	After fifth *Puta*	Particles forms as a continuous layer on floating	Absent	*Bhasma* filled the lines of the fingers but fell down after shaking
6.	After sixth *Puta*	Particles forms as a continuous layer on floating	Absent	Attained
7.	After seventh *Puta*	Particles forms as a continuous layer on floating	Absent	Attained
8.	After eighth *Puta*	Particles forms as a uniform layer on floating	Absent	Attained
9.	After ninth *Puta*	Some particles forms as a uniform layer on floating	Absent	Attained

Magnetism

10 gram of *Bhasma* after each *Puta* was taken in a plate. A magnet on paper is placed over *Bhasma* for a few second. The attracted *Bhasma* is then weighed and calculated. Five gram was attracted out of 10 gram for the first three *Puta*, thereafter the amount of *Bhasma* attracted is increased up to eight gram after seventh *Puta*. After eighth and ninth *Puta,* nonmagnetic property was observed. Pyrite forms and alpha ferric oxides of iron compounds are nonmagnetic.

Organoleptic characters

Sample	Color	Odour	Taste	Touch
Raw Iron	Grey	Pungent	Metallic	Rough, powdery
Sodhitha Loha	Black	Pungent	Metallic	Rough, powdery
Gandhaka maritha Loha bhasma	Dark Red	Nil	Nil	Smooth

***Varitharatva Rekhapurnatha* Final product**



Total ash

Before *Sodhana*	100%
After *Sodhana*	100%
Gandhaka maritha loha bhasma	99.013%

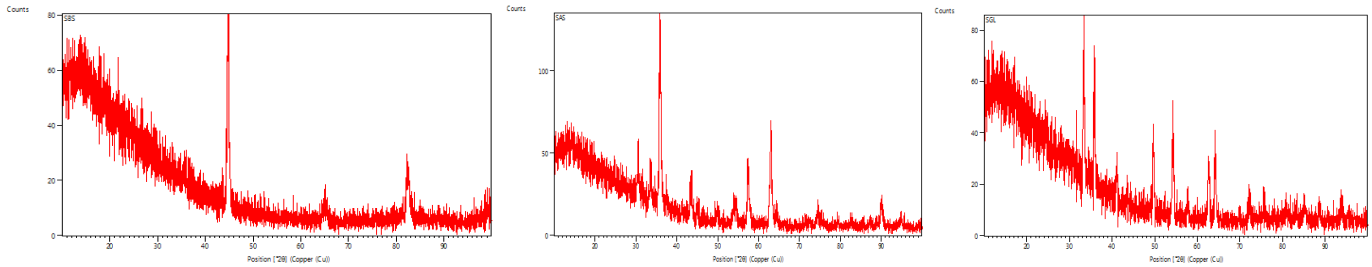
Acid insoluble ash

After *Sodhana*	73.61%
Gandhaka maritha loha bhasma	84.05%

pH

Before *Sodhana*	5.12
After *Sodhana*	4.85
Gandhaka maritha lohabhasma	3.99

XRD Graph 1 **Graph 2** **Graph 3**



Raw Iron

XRD of Sample before *Sodhana* gave characteristic peaks at 2θ values at 44.7748, 65.1540 and 82.2369. The Fwhm is very small indicative of the crystallinity of the iron powder. The data reveals that it is bcc lattice structure.

After *Sodhana*

The d values 2.66, 2.499, 2.074, 1.8277, 1.693, 1.607, 1.474 and 1.444 perfectly match with the XRD peaks of rhombohedral alpha Fe_2O_3. This crystal formation has begun during the time of high heating. These peaks are obtained at d spacing value (A°) at 2.929, 2.499, 2.074, 1.6929, 1.607, 1.475 corresponds to cubic phase of maghemit gama Fe_2O_3. Since sample after *Sodhana* is blackish in color and also highest intense peak to be found at 35.92 the magnetism of sample was checked. Apart from αFe_2O_3 and γFe_2O_3 it can also be inferred that the sizable proportion of the material is consisting of the magnetic mixed oxides Fe_3O_4 also. It's may be due to the combination of ferrous oxides during the *Sodhana* process.

Loha bhasma

The final XRD of sample of *Gandhaka maritha Loha bhasma* typically equates that of α Fe_2 O_3. The major peak being 2 θ at 33.41. Many peaks correspond to Fe_3 O_4 and γ Fe2O3 are also found to be absent in this case.

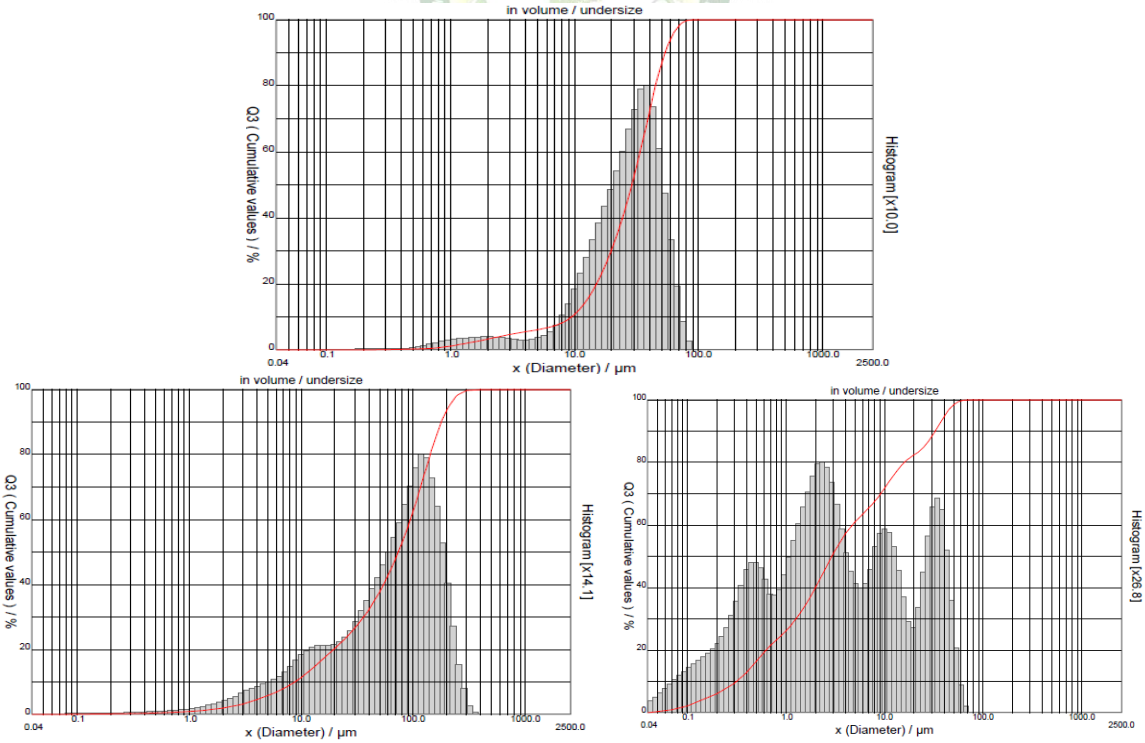
ICP-OES – Raw iron powder

Iron	58%
carbon	16%
Silica	12%
Alumina	6%
Calcium	4%
Magnesium	2%

XRF

After *Sodhana*		***Loha bhasma***	
Fe_2O_3	94.70%	Fe_2O_3	94.53%
SiO_2	1.96%	SO_3	1.50%
Al_2O_3	0.69%	SiO_2	1.17%
K_2O	0.62%	K_2O	0.56%
P_2O_5	0.51%	Al_2O_3	0.42%
MgO	0.42%	CaO	0.32%
CaO	0.40%	P_2O_5	0.16%
SO_3	0.22%	MgO	0.14%
Na_2O	0.17%	MnO	0.06%
CuO	0.03%	ZnO	ND
ZnO	0.02%	CuO	ND
MnO	0.06%	Na_2O	ND

Particle size analysis of 1. Raw iron, 2. *Sodhitha loha* and 3. *Loha bhasma*



Raw Iron

In the raw iron powder all particles have more or less the same particle size diameter. The main diameter calculated is 83.59 micro meter. It can be seen that there are virtually no particles lesser than 10 micro meter.

After *Sodhana*

Some particles have diameter higher than 100 micro meters. Comparatively higher fraction of particles have diameter lesser than 100 micrometer ranging upto 1 micrometer.

Gandhaka maritha lohabhasma

Shows a unique distribution pattern of particle size. Maximum percentage of particle size of less than 5 micrometers. The mean diameter is 9.56 only.

Mean diameter in micro meter

Before *Sodhana*	83.59
After *Sodhana*	85.16
Gandhaka maritha loha bhasma	9.56

DISCUSSION

Sodhana includes seven time nirvapa in different media. *Taila* is a non-aqueous solution, so there is less chance to form an oxides or hydroxides of Iron. After *Taila nirvapa* metallic lusture was lost and iron seen as blackish in colour because of presence of carbon compounds. The increased viscosity of *Taila* after *Nirvapa* suggests that most of the hydro phobic impurities of iron is removed through *Taila nirvapa*. *Takra* is an aqueous solution, here may be a chance to form oxides of iron which can be seen as some reddish coloured iron particles after *Takra nirvapa*. *Gomutra* which is an alkaline media reduced most of the silica present in *Loha choorna*. Phytic acid present in *Aranala* works as a good chelating agent. The studies reveals that gallic acid content of *Kulatha kashaya* reduces toxic trivalent Iron. *Triphala kashaya* convert *Loha choorna* into a more bio compatable form. The reaction between iron and sulphur is exothermic. So there is a chance of easy formation of *Loha bhasma*. For the first two puta a layer of reddish particles seen as a surface layer and a blackish mixture was observed below. After third *Puta* a blue coloured mixture and after fourth *Puta* a mixture of red, blue and green was observed. After fifth *Puta* greenish colour was in majority mixed with reddish pellets. After sixth *Puta* a brick red coloured *Bhasma* and after seventh and eighth *Puta* reddish coloured bhasma was seen.Colour indicates various compounds of iron and sulphur. Atlast a dark reddish coloured bhasma with 80% *Varitharatva* was obtained without any smell and taste. *Gandhaka* has a specific role in the quick formation of *Loha bhasma*. Oxidation of iron sulphide is highly exothermic

ICP-OES for raw iron powder and XRF for *Sodhitha loha* and *Loha bhasma* was done for elemental analysis. XRD analysis shows body centered cubic lattice structure of raw iron. After *Sodhana* it is converted into a mixture of γFe_2O_3, αFe_2O_3 and Fe_3O_4. *Loha bhasma* was identified only with αFe_2O_3. The product obtained showed non-magnetic property. Elemental analysis of raw iron powder and *Sodhitha loha* implies that after *Sodhana* the percentage of silica which was 12% for raw iron was reduced to 1.96 after sodhana. Alumina also seen reduced. Particle size analysis by using dynamic light scattering shows that after *Bhasma* formation particles are there below 0.5 micrometer.

CONCLUSION

The form and state of chemical compounds in a final product of a *Bhasma* depends upon the intial ingredients added, given temperature and the method of preparation. The presence of alpha ferric oxide and very less sulphur content in the final product clearly reveals the role of *Gandhaka* in *Bhasmeekarana* of iron. Particle size analysis on samples by using dynamic light scattering shows that range of particle size was seen reduced from 100 micrometer to 1 micrometer after *Sodhana* and below 1 micrometer after *Bhasmeekarana*. Further analysis with higher techniques and clinical studies are essential to find out the activity of *Bhasma*.

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Cite this article as:
Suja.S, Anand.S, Jacob.M.Titus. Pharmaceutical and Analytical Evaluation on Gandhaka Maritha Loha Bhasma. International Journal of Ayurveda and Pharma Research. 2023;11(3):43-49.
https://doi.org/10.47070/ijapr.v11i3.2737
Source of support: Nil, Conflict of interest: None Declared

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