Iron deficiency anaemia is the commonest nutritional deficiency disorder present throughout the world. The metal iron is vital for red cell production, as it forms the haem molecule in haemoglobin. Its scarcity leads to iron deficiency anaemia. Though Annabhedhi is an iron based bhasma to treat anaemia, no physicochemical characterization has been reported in the recent past as compared to the enormous works of Lauha Bhasma and Mandura Bhasma which are commonly used for the preparations of incinerated iron. It is therapeutically used for both externally and internally, for the treatment of diseases like Anaemia, Leucoderma, Prolapse of rectum and uterus, splenic disorders. It also possesses hematonic and anti-ulcer properties. Several vitamins, namely vitamin c (ascorbic acid) play an indirect role by facilitating the iron turnover in the body. The main objective of the present work is to characterize the traditional drug to understand the chemistry behind the traditional preparation conditions and its role in the human body.

The main objective of the present work was to carry out the structural characterization of traditional Ayurvedic drug and compare these results with those of raw material used for the synthesis. The preparation method of ‘Annabhedhi’ involved repeated calcination cycles, thus, facilitating agglomeration and hence bigger crystallites. These results together with PXRD, FTIR, Particle size, SEM, ICP – AES and BET surface area analysis showed that the ‘Annabhedhi’ of Ayurvedic origin consisted of mainly iron oxide however, this form was significantly different from raw material due to the modifications during the preparation by addition of ingredients and the strict post and pre preparation conditions followed. We report here for the first time in the literature the effect and importance of strict preparation conditions followed in the preparation of an iron based traditional drug Annabhedhi. Based on the results of the characterization of the Annabhedhi, we explained the formation of iron oxide, the role of lime in the production of ascorbic acid to form iron complexes with organic moieties and incineration by traditional method ‘putam’ in determining the particle size, crystalline nature, surface energy and medicinal activity.

MATERIAL AND METHODS

Traditional Method of Preparation

Ferrous sulphate is preferred as the source of pure iron procured from local manufacturers for the preparation of Annabhedhi. As per the traditional Ayurvedic method of

**Keywords:** Annabhedhi Sindooram, Bhasma, Iron oxides, Organo-metal complexes, Particle size, Medicinal activity

**INTRODUCTION**

Metal based drugs known as ‘bhasma’ plays an important role in Ayurveda; the traditional medicinal system practiced in India. The most important aspect of the preparation of bhasma involves the conversion of a metal into its mixed oxides which directly eliminates the toxicity, thereby increases the bioavailability and improves the medicinal properties of the materials. Minerals when treated with herbal juices leads to reduction (trituration) in the particle size that facilitates increased effectiveness. The scientific basis or a materialistic approach behind the conversion of the valent state of the metal into higher oxidation state is one of the less frequently reported area based on the literature of traditional medicines. To synthesis and standardize these traditional drugs in large scale with high selectivity and activity, it is highly desirable that these drugs should be characterized with modern spectroscopic and non-spectroscopic techniques to understand the basic scientific aspect of various steps involved in the preparation protocol.

**ABSTRACT**

Iron based traditional Ayurvedic drug Annabhedhi Sindooram is used therapeutically for the treatment of diseases like Anaemia, Leucoderma, Prolapse of rectum and uterus, Spleenic disorders. The preparation method of iron based Indian traditional drug Annabhedhi Sindooram involves conversion of a pure metal into its mixed oxide by drying and incineration. Commercially available ferrous sulphate is used as the source of iron for the preparation of Annabhedhi. The structural and textural properties of the starting materials and the prepared drug were characterized systematically by different characterization techniques like PXRD, Zeta Potential Analysis, particle analysis, FTIR, ICP – AES, SEM and BET surface area analysis. The results obtained by characterization of the samples clearly explain the formation of Fe₂O₃, reduction in particle size, modification of surface energy and formation of metal complex with organic moieties. The strict post and pre preparation conditions followed play an important role in the morphology and medicinal activity of the drug Annabhedhi Sindooram.

**MATERIAL AND METHODS**

**Traditional Method of Preparation**

Ferrous sulphate is preferred as the source of pure iron procured from local manufacturers for the preparation of Annabhedhi. As per the traditional Ayurvedic method of
preparation\(^8\), ferrous sulphate was finely powdered in mortar and pestle and soaked in lime juice and it is exposed to sunlight for 2-3 days, till the samples are completely dried. This process of soaking in lime and drying is repeated three times. The traditional calcination process ‘putam’ was carried out to prepare the required drug. This involves the incineration of the dried sample in earthen crucibles by using cow dung cakes. After overnight incineration the samples were made into fine powders by mortar and pestle and stored in air tight containers. Incineration leads to higher conversion to metal oxide and increases the stability of particle that reduces the agglomeration\(^7\).

The raw material and the final drug was characterized systematically by the following methods, The powder X-ray diffraction analysis of the samples was carried out in the range of 2\(\theta = 2 - 70^\circ\) using Xpert Pro Philips diffractometer equipped with a Ni filtered Cu-K\(\alpha\) radiation with \(\lambda = 1.5418\) A\(^\circ\) using a graphite crystal monochromator. Zeta Potential Analysis and Particle analysis was performed using Model NPA152-31A Zetatrac Supplied by Microtrac, USA. The Light scattering particle size technology measurement range size 0.8 to 65 microns, measurement angle 180 degrees Zeta Potential range from -125 to +125 mV. The Fourier Transform Infra Red spectra (FTIR) of the samples were recorded using Nicolet IR 200 instrument by the KBr pellet technique over a range of 400 – 4000cm\(^{-1}\). The determination of specific surface area was carried out by BET (Brunner–Emmett–Teller) \(N_2\) adsorption using NOVA 1000 Quanta chrome high speed gas sorption analyzer (ver3.7) instrument.

**RESULTS**

**Powder X ray Diffraction Analysis**

The PXRD pattern of the precursor and the final drug ‘Annabhedi’ is shown in Figure 1. Diffraction peaks at 2\(\theta\) are identical with those reported for the standard ferrous sulphate in the raw material. The strongest peaks identified from the diffraction pattern of the final drug attributes to the formation of iron oxide Fe\_2O\_3. The high intensity of XRD lines (0-2500) in the XRD pattern of the raw material indicate its crystalline nature where as the low peak intensity (0-350) of the final drug suggests the loss of crystalline nature of the material. The loss of luster is considered as a better quality for the final drug by a test nischandrika described in Ayurveda\(^10\).

<table>
<thead>
<tr>
<th>Table 1: Details of PXRD analysis of precursor and final drug</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample</td>
</tr>
<tr>
<td>---------</td>
</tr>
<tr>
<td>FeSO(_4)</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Final drug</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
</tbody>
</table>

FWHM: Full Width at Half Max

The particle size of the final drug ‘Annabhedi’ was calculated from XRD pattern following the Debye – Scherrer and it was compared with that of the starting material standard FeSO\(_4\) is given in the Table 1. It was found that the crystallite size of the drug ‘Annabhedi’ under study was lower than that of the raw material ferrous sulphate. The reduction of particle size from 12 nm to 7 nm facilitates absorption and assimilation of the drug in the system and also allows the Ayurvedic phenomena rekhaaparna and varitara\(^10\). This result confirms the role of preparation techniques mainly soaking in lime and the incineration on the crystalline nature and the particle size of the drug prepared. Some extra diffraction peaks are observed in the XRD pattern of the final drug confirming the influence of preparation steps and calcinations followed for the synthesis of the drug ‘Annabhedi’. The extra peaks may be due to the conversion of iron into heam iron with the help of ascorbic acid present in lime.

**Zeta Potential Analysis and Particle Analysis**

Zeta potential is the electrical potential developed at the solid–liquid interface in response to the relative movement of the particle and the solvent. The zeta potential of the drug was studied by dispersing the drug in double distilled water. Zeta potential of particles is a good indicator of their electrical potentials: the higher the zeta potential, the higher the surface potential of charged particles\(^11\). The charge of the material results from the charge on the particle surfaces, but it is strongly dependent upon the pore fluid chemistry. The reduction in surface energy is due to the decrease in the particle size already demonstrated by the XRD results\(^12,13\). The Zeta-potential of final drug shifted from -14.88 mV to -9.71 mV indicating greater surface potential after the pre- post synthetic conditions. The decrease in the average particle size of the material from 12 nm to 7 nm indicates the role of preparation conditions in the pharmacological activity of the drug.

The floatability (varitara) of the bhasma was performed by sprinkling the bhasma over surface of water\(^14\). The Annabhedi sindooram floated over water, this happens when the weight of the particle is overcome by the surface potential of the bhasma. This result confirms the particle size and surface potential plays an important role in the preparation of bhasma.

**Fourier Transform Infra Red Analysis**

The FTIR spectrum of raw material and final drug of Annabhedi were analyzed in the region from 4000- 500 cm\(^{-1}\) as shown in the Figure 2. The distinct peaks

![Figure 1: PXRD Pattern of raw material and Annabhedi Sindooram](image-url)
observed at 614.13 attributes to the presence of metal oxide bond of iron. The peak at 1102.91 is due to the presence of water molecules. The broad peak observed at 1625.23 for the raw material is characteristic of O-O bond due to the absorbed oxygenates. The FTIR spectrum of raw material and bhasma (Figure 2) shows a broad band between 3400 and 3500 cm⁻¹, characteristic of O-H bond.

DISCUSSIONS

Scanning Electron Microscopy
The photographs of SEM taken for the raw drug (a) and the drug ‘Annabhedi’ (b) are shown in Figure 3, which reveals the modification of the raw material into final drug. The raw drug (a) showed well-defined plate like structures while the drug ‘Annabhedi’ showed compact microcrystalline aggregates with loss of grain boundaries. The average particle size of sample (a) was found to be about 15-18µm while that of final drug (b) was in the range of 10-7µm due to decrease in particle crystallites. The few bigger particles seen are due to the agglomeration of smaller particles by the small dusty particles. The smaller particle size of the drug is in accordance with its strict preparation conditions. This result once again confirms the influence of method of preparation on morphology, particularly the calcinations temperature and duration.

ICP–AES Analysis
The composition of the final drug was determined by ICP–AES analysis, the results are given in the Table 2. The material exhibited high percentage of iron in the form Fe₂O₃ and remaining metals like Ca, S, Na and silica in the range of 4.0 - 0.1. The percentage of sulphate and sulphur in the range of 3.2 - 2.0 % attributes the conversion of raw material into oxide. The formation of different compounds along with the final product is due to the oxidation and reduction reactions of the metals with sulphur and oxygen. The presence of silica may be due to the usage of earthen pots. These compounds formed during the preparation of final drug do not show any unwanted effect in experimental study which once again confirm the medicinal effect of iron in the final drug.

BET Surface Area Measurement
From the BET measurement, the specific surface area of the particles of the drug ‘Annabhedi’ was found to be 1.1799 m²/g which is about three times higher than the surface area of standard ferrous sulphate. This was consistent with the smaller particle size of the drug ‘Annabhedi’ as compared with that of pure ferrous sulphate determined by XRD studies, FTIR spectra and particle size determination. This result once again confirms the Ayurvedic quality tests and the role of preparation method on the formation of the final drug.

Table 2: ICP–AES percentage composition of Annabhedi

<table>
<thead>
<tr>
<th>Metal</th>
<th>% w/w</th>
</tr>
</thead>
<tbody>
<tr>
<td>Iron</td>
<td>89.298</td>
</tr>
<tr>
<td>Silica</td>
<td>3.924</td>
</tr>
<tr>
<td>Sulphate</td>
<td>3.13</td>
</tr>
<tr>
<td>Sulphur</td>
<td>2.35</td>
</tr>
<tr>
<td>Ca</td>
<td>0.417</td>
</tr>
<tr>
<td>Chloride</td>
<td>0.233</td>
</tr>
<tr>
<td>Na</td>
<td>0.105</td>
</tr>
<tr>
<td>Free sulphur</td>
<td>Nil</td>
</tr>
</tbody>
</table>

Scientific Basis of Traditional Method of Preparation
Iron in the form of ferrous Fe²⁺ is soaked in lime and dried. The ascorbic acid (vitamin c) present in the lime converts the non haem iron in the ferrous sulphate into haem iron. This is clearly observed in the XRD patterns of the samples. The conversion of non haem iron is required due to the better absorption nature of haem iron compared to non haem iron. The usage of ferrous salt is attributed to the better absorption nature of ferrous iron in the duodenum and proximal jejunum compared to all other forms of iron. Absorption of non-haem iron is enhanced by factors such as ascorbic acid (vitamin c), citric acid, sugars and hydrochloric acid. The drying and incineration is carried out to modify the oxidation number of iron from +2 to +3 through the formation of mixed oxide of iron (Fe₂O₃) from the raw material ferrous sulphate. The change in particle size and morphology plays an important role in the transport of iron in the duodenum and proximal jejunum compared to all other forms of iron. Absorption of non-haem iron is enhanced by factors such as ascorbic acid (vitamin c), citric acid, sugars and hydrochloric acid. The drying and incineration is carried out to modify the oxidation number of iron from +2 to +3 through the formation of mixed oxide of iron (Fe₂O₃) from the raw material ferrous sulphate. The change in particle size and morphology plays an important role in the transport of iron in the duodenum and proximal jejunum compared to all other forms of iron.
iron across the membrane. The results obtained from the characterization clearly explained the modifications required for the material to be pharmacologically important.

CONCLUSION

Iron based traditional drug popularly known as Annabhedi was prepared by traditional method from commercially available ferrous sulphate as precursor and was characterized by different techniques. The prepared drug exhibited excellent medicinal properties like hematinic and anti-ulcer properties. The PXRD patterns clearly revealed the conversion of the non haem iron into haem iron. The drying and incineration process converts the Fe²⁺ into mixed oxides of iron which consists of both +2 and +3 oxidation states. The FTIR spectra clearly confirm the presence of organic moieties along with the formation of iron complexes. BET surface area analysis, SEM and PXRD analysis once again confirms the reduction in particle size of the drug compared to the raw material used for the preparation of iron based traditional drug annabhedi sindooram.

ACKNOWLEDGEMENT

The authors greatly acknowledge Oushadhi Pharmaceutical Corporation (IM) Kerala Ltd and Hanza A, SUD, CHEMIE, Cochin that helped us to characterize our samples. We also express our heartfelt gratitude to Prof. Madan Thangavelu, Cambridge University who had inspired us to initiate this work.

REFERENCES


Cite this article as:

Source of support: Nil, Conflict of interest: None Declared