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ROLE OF GALLIC ACID IN THE PREPARATION OF AN IRON–BASED INDIAN TRADITIONAL MEDICINE – *LAUHA BHASMA*

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ABSTRACT

Ayurveda is an ancient traditional system of medicine, which has been evolved and practised in India for several centuries. Metals used in herbo–metallic preparations (HMP) (*bhasmas*) are purified by repeated treatment with plant extracts and animal products. Horse gram extract (*kulatha kasaya*) is used in the initial stages of purification of iron in the preparation of *Lauha bhasma.* In this work, we have evaluated the role of gallic acid, which is one of the major constituents of *kulatha* in the purification of iron. Various physico–chemical techniques such as UV–Vis spectroscopy, Fourier transform infrared spectroscopy, thermal analysis and scanning electron microscopy were used to characterize the gallic acid–metal complex. Our results demonstrate that gallic acid readily formed a complex with iron(III) chloride through the phenolic groups; the carboxylic acid group of gallic acid was not involved in the coordination with the metal ion. Further, the gallic acid–metal complex was soluble in water and thus the treatment of iron with *kulatha kasaya*, results in the formation of water–soluble complex, which is removed during repeated washing. Thus we have established for the first time the scientific basis of using *kulatha kasaya* in the purification of iron and have demonstrated that during this process the trivalent iron, which is present in the raw material is removed thereby reducing the iron(III) content in the *Ayurvedic* preparation.

Keywords: Ayurveda, Herbo–metallic preparation, Bhasmas, Gallic acid–iron complex.

INTRODUCTION

Ayurveda and *Siddha* are traditional systems of medicine practised in India since ancient times. There are both herbal and herbo–metallic preparations that are used in these systems of treatment for different disorders [1]. *Bhasmas* are inorganic preparations that contain carbonates, sulphates, oxides of metals such as iron, copper, lead, gold, silver, zinc, etc. These metal–based preparations have been observed to have pharmacological efficacy when prescribed for different diseases [1]. One of the important herbo–metallic preparations used in the treatment anemia, oedema and immunomodulation is the iron based *Lauha bhasma* [1]. The preparation of *Lauha bhasma* requires expertise and no detailed information is available for the variations between different manufacturer techniques. These variations may be due to the improper preparation of *Lauha bhasma* and alter its biological activities turning it from medicine to toxin. All the iron preparations available commercially have been reported to possess some toxicity such as nausea, vomiting, diarrhea and gastro–intestinal disorders [2].

The presence of heavy metals in herbo–metallic preparations has been reported [2]. However, the oxidation states of the heavy metals used in the preparation have not been evaluated. Since, these HMPs are traditional medicines that have been used from time immemorial, the methods of preparation have been described in ancient scripts and hence, they should be non–toxic if they have been prepared properly. An understanding of the scientific concepts involved in the preparation procedures reported will enable one to appreciate the benefits of this unique form of treatment. Thus, HMPs are unique ayurvedic herbo–metallic preparations, which are used to cure some chronic illness or ailments.

Lauha bhasma is an iron–based bhasma, which is prepared from iron ore by a process known as *Bhasmikaran* [3]. The process of preparation involves several steps, which include *Shodan* (purification), *Maran* (powdering), *Chalan* (stirring), *Dhavan* (washing), *Galan* (filtering), *Putan* (heating) and *Mardan* (tirturating) [3]. The *Shodan* or the purification process removes the unwanted materials from the raw material and makes it suitable for the next step [4]. During this process, the raw material is heated to red–hot condition and dipped into various agents such as oil, buttermilk, cow's urine, rice gruel and horse–gram decoction [4]. One of the major constituents of *kulatha* (horse gram) is gallic acid [5]. Thus these purifying agents are believed to remove impurities and keep the metallic part of the raw material in some stable form.

Gallic acid (3,4,5–trihydroxybenzoic acid) is one of the naturally occurring polyphenols ubiquitously present in most of the herbal and ayurvedic preparations [6]. It possesses various pharmacological activities such as anti–inflammatory, anti–oxidant, anti–cancer, radio– protective, anti–hyper cholesterolemic, hepato–protective and anti–mutagenic activities [7,8,9,10,11]. Gallic acid is also used for the treatment of internal hemorrhage, albuminuria and diabetes [12]. The aim of the present study was to understand the role of gallic acid in the preparation of the *Lauha bhasma.* Iron–gallic acid complexes have been reported in literature and the metal to ligand ratio varies depending on the concentration of the precursors, temperature and most importantly, pH of the reaction medium [13]. We hypothesize that gallic acid present in *kulatha* may form a complex with iron and aid in the purification of the raw material.

MATERIALS & METHODS

Materials

Gallic acid (Sigma–Aldrich, USA), sodium hydroxide (Chemspure, India), anhydrous iron (III) chloride (Fischer Scientific, India), phloroglucinol (SD Fine Chemicals, India), catechol (Research Lab Fine Chem, India) were used in this study as such without further purification.

Preparation of Iron–Gallic Acid Complex (FEGAOH)

Gallic acid (1 g; 0.0059 mol) was dissolved in water. Anhydrous iron(III) chloride ($FeCl_3$, 0.32 g; 0.002 mol) was dissolved in water and added to the gallic acid solution while stirring continuously. Sodium hydroxide (NaOH, 0.7 g; 0.0175 mol) was dissolved in water and added to the above solution and stirred for 1 h. Alcohol was added to precipitate the complex and the precipitate was filtered under vacuum, while adding acetone to remove water and alcohol. The dry powder was further characterized. Similarly iron complexes with phloroglucinol (FEPGOH) and catechol (FECTOH) were prepared and characterized to determine whether the $–CO_2H$ group of gallic acid was involved in coordinating with iron.

Characterization of the Complexes

Iron was estimated colorimetrically by thiocyanate method using UV–vis spectrophotometry (Perkin Elmer, Lambda 25, USA). The Fourier transform infrared spectra were recorded between 4000–

400 cm^{-1} by KBr pellet technique using a Perkin Elmer, Spectrum 100 instrument averaging 10 scans. Electronic spectrum was recorded in Perkin Elmer, Lambda 25 and the thermal stability of the complexes was determined by measuring the thermograms under nitrogen atmosphere (TA Instruments, SDT Q 600, USA).

Raw material for *Lauha bhasma* was heated to red–hot condition and immersed in sufficient quantity of *kulatha kasaya* for two hours. The presence of Fe^{3+} was qualitatively detected using potassium thiocyanate (KCNS). Briefly, to 1 mL of horse gram decoction obtained after treating the raw material, 1 mL of potassium thiocyanate (KCNS) solution was added to observe the appearance of red color indicating the presence of free Fe^{3+}. In the absence of a red color, this assay was repeated after addition of concentrated nitric acid to decompose the gallic acid–iron complex. The surface morphology of the complexes was evaluated using a cold field emission scanning electron microscope (FE–SEM, JEOL, JSM6701F, Japan).

RESULTS & DISCUSSION

The FEGAOH complex formed was green in color and completely soluble in water. Figure 1 shows the proposed structure of the iron–gallic acid complex. The percentage of iron estimated colorimetrically using thiocyanate was found to be 10.00% and was in agreement with the calculated value (9.98%), which assumes a metal to ligand ratio of 1:3 (ML_3 complex). The precursor concentration, temperature and pH of the reaction medium were known to influence the stoichiometry of the iron–gallic acid complex.

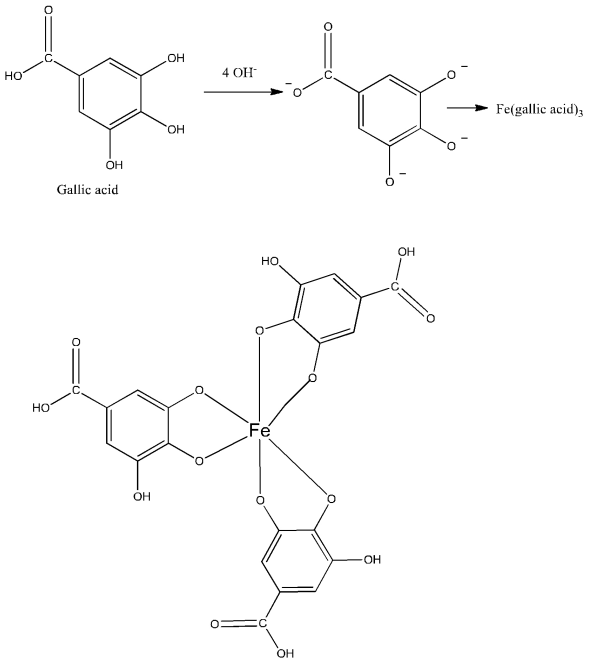

Fig. 1: Proposed structure of gallic acid–iron complex (FEGAOH).

The FTIR spectra of gallic acid, phloroglucinol, the iron complex of gallic acid (FEGAOH) and phloroglucinol (FEPGOH) are shown in Figure 2. The υ_{OH} of the phenolic groups and that of the $-CO_2H$ group merge and appears in the region 3400–3270 cm^{-1} in gallic acid (Table 1). These peaks do not appear in the iron complexes since the phenolic groups may have been converted to phenoxide ions by sodium hydroxide.

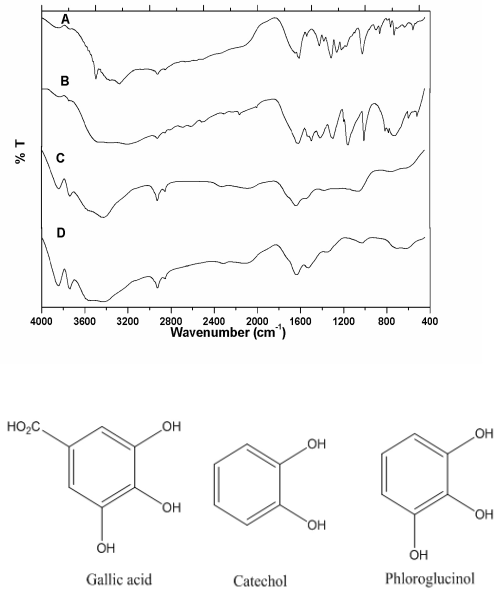

Fig. 2: FTIR spectra of [A] gallic acid; [B] phloroglucinol; [C] gallic acid–iron complex (FEGAOH); and [D] phloroglucinol–iron complex (FEPGOH) along with the chemical structures of gallic acid, phloroglucinol and catechol.

Table 1: Important bands in the FTIR spectra of ligands and complexes

Substance	υC–O	υO–H
Gallic acid	1025	3400–3270
Gallic acid–Iron complex	1055	3600–3400
Phloroglucinol	1009	3500–3200
Phloroglucinol–Iron complex	1034	3600–3400
Catechol	1095	3327
Catechol–Iron complex	1119	3500–3200

Carboxylic acid group is present in gallic acid, while it is absent in both phloroglucinol and catechol. The FTIR spectrum of gallic acid–Fe complex is similar to that of phloroglucinol–iron and catechol–iron complexes indicating that the $-CO_2H$ group of gallic acid is not involved in coordination (Figure 2). The blue shift of υ_{C-O} in all the iron complexes when compared to gallic acid may be attributed to the fact that phenoxide ion coordinates to the metal and the C–O bond gets some double bond character as explained in Figure 3. All these results demonstrate that only the two phenolic groups in gallic acid coordinate with Fe(III).

The electronic spectrum of FEGAOH is shown in Figure 4. The electronic spectrum of FEGAOH recorded in water showed a weak band at 19,048 cm^{-1}. This may be attributed to the $^4T_{1g}(G) - {}^4T_{2g}(G)$ transition suggesting an octahedral skeleton with D_3 symmetry to the complex[14].

The thermogravimetric (TG) and differential scanning calorimetry (DSC) analysis of FEGAOH is shown in Figure 5. The TG curve of FEGAOH in nitrogen atmosphere shows decomposition in the range between 100 and 120°C, which may be attributed to the loss of moisture (Figure 5). The complex was found to lose 30% of its weight up to 850°C after which it started to decompose. Further, this was further in agreement with the DSC data where two prominent endothermic peaks were observed at 120°C and 850°C (Figure 5) [15, 16].

Fig. 3: Schematic representations of iron complex formation with phenoxide group of gallic acid

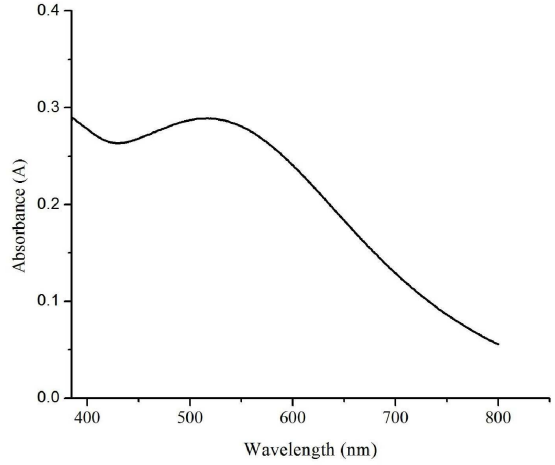

Fig. 4: Electronic spectrum of gallic acid–iron complex (FEGAOH)

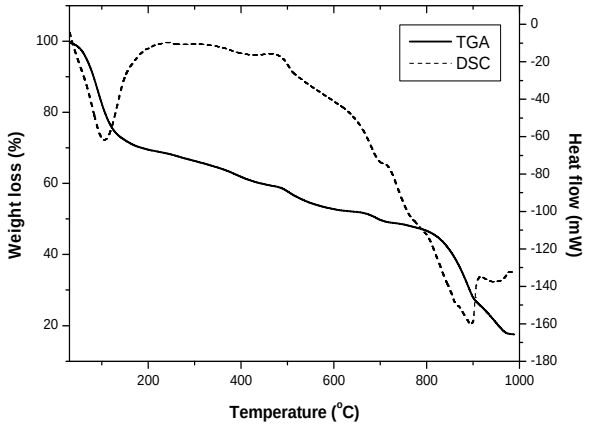

Fig. 5: Thermal analysis of gallic acid–iron complex (FEGAOH)

When 1 mL of KCNS solution was added to 1 mL of horse gram decoction obtained after treating the raw material, red color was not obtained, which indicates the absence of free Fe^{3+}. However, when the test was repeated after addition of few drops of concentrated nitric acid red color was observed. This indicates the presence of Fe^{3+}, which is formed due to the decomposition of the gallic acid–iron complex by concentrated nitric acid. These results clearly demonstrate that the role of *kulatha kasaya* is to remove Fe^{3+} from the raw material. This process is critical since Fe^{3+} contributes to the toxicity in biological systems by enhancing the formation of free radicals [4].

The scanning electron micrograph of raw iron and FEGAOH are shown in Figure 6. The high magnification image of raw iron exhibits lumps with a smooth texture, while the gallic acid–iron complex showed aggregates of fine structured grains (Figure 6A & B). Surface morphology and size have an important role in determining the biological interactions and fate of a molecule. The presence of fine nano–structured grains can aid in faster dissolution of a preparation, which has been well established in the case of many therapeutic preparations [17]. Further the images reveal the presence of micro cracks, which may also contribute to the faster dissolution (Figure 6B). Thus, the conversion of raw iron to iron–gallic acid complex during the preparation of *Lauha bhasma* results in the solubilization and removal of Fe(III) which otherwise would be insoluble.

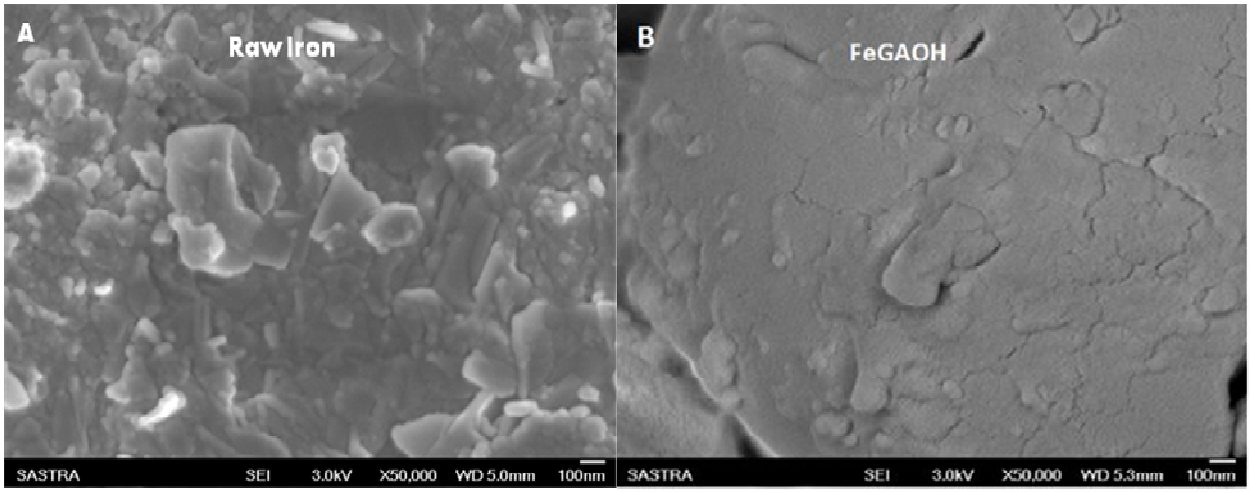

Fig. 6: Scanning electron micrographs for [A] raw iron and [B] FEGAOH.

CONCLUSIONS

Gallic acid is one of the major ingredients in horse gram decoction or *kulatha kasaya*, which is used as one of the purifying agents during the preparation of *Lauha bhasma*. In this study we evaluated the role of gallic acid in the purification process and we have demonstrated that gallic acid, which has one $–CO_2H$ and three phenolic groups, coordinates with iron through the two phenolic groups giving rise to an octahedral complex with D_3 symmetry. The thermal analysis showed that the complex was stable up to 850°C and the complex formed was soluble in water. Thus gallic acid present in *kulatha* is responsible in removing Fe (III) present in the raw material thereby reducing the toxicity of *Lauha bhasma.*

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