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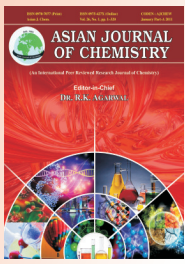

Aqueous Plant Extracts Increase Nanocrystalline Fe_3O_4/γ-Fe_2O_3 to α-Fe_2O_3 Transition Temperature: Insights into Traditional Calcination Process for Herbometallic Preparation

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A unique method of calcination for preparation of drugs from metallic and herbal ingredients has been in vogue in India since several centuries ago; however the same is not completely understood. Our experiments show that during the preparation of an iron-based herbometallic drug, transition from Fe_3O_4/γ-Fe_2O_3 to α-Fe_2O_3 is delayed to higher temperatures due to addition of light metals from the aqueous extracts of Indian gooseberry, *Belleric myrobalans* and *Chebulic myrobalans*. The repeated calcination cycles increase the composition of essential elements like potassium, sodium and calcium, while increasing the crystallinity of Fe_3O_4/γ-Fe_2O_3.

Keywords: Aqueous extract, Transition temperature, γ-Fe_2O_3, α-Fe_2O_3, Nanocrystalline, Calcinations.

INTRODUCTION

Thermal processing is widely used to alter the properties of alloys, ceramics, composites, polymers, *etc.*[1]. Use of several heat treatments like quenching, annealing, tempering, *etc*. in manufacturing testifies the importance of thermal processing[1]. More than 5000 years ago, the practitioners of an Indian system of medicine, *Ayurveda* had developed processes to prepare drugs from metal and herbal sources through extensive thermal processing. In a typical step meant for removal of surface contaminants, the metal is repeatedly quenched in a series of liquids that include sesame oil and aqueous solutions of plant and animal products[2,3]. The final step in the transformation of metallic ingredient to a herbo-mineral preparation, called *bhasma* is the calcination step, often referred as '*putapaka*' in ancient literature[2,3].

Lauha bhasma is a herbo-metallic drug, prepared from iron. The preparation of *Lauha bhasma* requires 60 cycles of calcination[4]. The steps involved in calcination are (i) wet grinding of a pre-treated mineral predominantly containing γ-Fe_2O_3, with the aqueous extract of Indian gooseberry, *Belleric myrobalans* and *Chebulic myrobalans* in a mechanized, stone mortar; (ii) preparation of flat discs of around 5 cm diameter from the ground mixture followed by drying under sunlight; (iii) loading of flat discs in an earthen, elliptical container and (iv) heating the elliptical container in a specially-designed chamber using cow-dung cakes. This step is widely believed to make *bhasma* a fine, non-toxic but efficacious drug. *Chebulic myrobalans* is well known for its antibacterial, antifungal activity and the fruit possess laxative property too[5,6]. Studies on *Belleric myrobalans* have revealed its anti asthmatic, antispasmodic, anti malarial, antioxidant activity apart from its laxative property[7-10]. Indian gooseberry also exhibits antifungal, antibacterial, antiviral, antioxidant activity and is used to cure diarrhea, mouth ulcers, nausea, dental and respiratory ailments[11-16]. Hence, *Triphala*, a combination of these three fruits in equal proportion possess therapeutic property. It is known for its anti-bacterial, anti-infective, anti oxidant and anti-cancer properties[17-20].

In our earlier works, scientific rationale for the various purification steps and *bhanupaka* step (exposure to sunlight) used in the preparation of *Lauha bhasma* were reported[21]. However, there are several scientific questions that remain unanswered in understanding the significance of calcination step. The present work is an attempt to answer the following question: What is the necessity for addition of aqueous plant extracts during each calcination cycle?

To address the above question, calcination experiment was carried out in traditional chamber with cow-dung cakes as heating medium, as prescribed in ancient texts. The role of calcination step in influencing elemental composition, crystallinity, phase, crystallite size and morphology were evaluated

using appropriate characterization techniques involving powder X-ray diffractometry, X-ray fluorescence spectroscopy and scanning electron microscopy. Thus, the present study will provide deeper insights into the understanding of calcination steps involved in the preparation of *Lauha bhasma*.

EXPERIMENTAL

Preparation of feed for calcinations: An elaborate description of the method for preparation of *Lauha bhasma* is explained in our earlier work[2]. In brief, iron powder was subjected to a series of purification steps followed by reaction with *Triphala* (a mixture of three fruits) decoction under ambient conditions. The intermediate was subsequently roasted and obtained in dried form, which serves as the feed material (denoted as F) for calcination. *Triphala* decoction was prepared as an aqueous extract of equal mass of Indian gooseberry, *Belleric myrobalans* and *Chebulic myrobalans*.

Traditional calcination : The feed material (F) was subjected to wet grinding in a mechanized stone mortar in the presence of *Triphala* decoction, till the consistency of a paste was reached. Thin flat discs (denoted as FD) of about 5 cm diameter and 5 mm thick were made from this paste and dried under sunlight.

The thin flat discs were placed in a hemi-spherical earthen container and covered with another hemi-spherical container, with the interface between the upper and lower containers covered with a clay-smeared cloth. This arrangement is normally referred to as *Sarava Samputa* (denoted as SS) and is shown in Fig. 1a.

A brick-walled calcination chamber measuring 90 cm each in all three directions has been used for traditional calcination, in accordance with those proposed in ancient literature. This is referred to as traditional calcination chamber in the subsequent sections. About 150 cow dung cakes were stacked inside the pit over which the *Sarava Samputa* was placed. This was followed by stack of another 150 cow dung cakes, as shown in Fig. 1b, ensuing uniform heat supply for the contents of *Sarava Samputa*. This is referred to as traditional heating in the subsequent sections of this work. Calcination was initiated by igniting the cow dung cakes. *Sarava Samputa* was left undisturbed in the calcination chamber till all the cow dung cakes were burnt completely.

The calcined intermediate was removed from *Sarava Samputa*, after the same was cooled naturally. This process of grinding feed material (F) with *Triphala* decoction to make thin flat discs (FD) and calcination in traditional chamber was repeated 60 times, as per the traditional procedure. The temperature of *Sarava Samputa* during traditional calcination was measured using a K-type thermocouple fitted to a 3½ digital temperature indicator.

Characterization of intermediates: The feed material (F) and the intermediates obtained after each cycle of traditional calcination were characterized for surface morphology, elemental composition and crystallinity. The morphological characteristics of samples were observed using a cold emission scanning electron microscope (JSM 6701F, JEOL, Japan) at an acceleration voltage of 3 kV. The powder X-ray diffraction patterns of the samples were recorded using a X-ray diffractometer (D8Focus, Bruker, Germany). The elemental composition of samples was determined using an X-ray fluorescence spectrometer (S8Tiger, Bruker, Germany).

RESULTS AND DISCUSSION

Necessity for addition of aqueous plant extracts during each calcination cycle: Fig. 2 shows the temporal variation of temperature in the traditional calcination chamber. The temperature measured is the temperature on the top of *Sarava Samputa* container containing the thin flat discs. Hence, this is likely to be an overestimation of temperature of material subjected to thermal processing. Nevertheless, this is the temperature measurable closest to the material. A near-reproducible pattern in temporal variation of temperature may be observed from Fig. 2. The temperature increases steeply to about 800-900 °C in 60-70 min. This temperature range (800-900 °C) is sustained only for a short period of time, say about 20-30 min after which the temperature drops to 200 °C in about 3 h. The variation in the maximum temperature between different calcination cycles carried out in traditional calcination chamber with cow dung cakes as the heat source may probably be attributed to the differences in the mass of cow dung cakes used in different cycles.

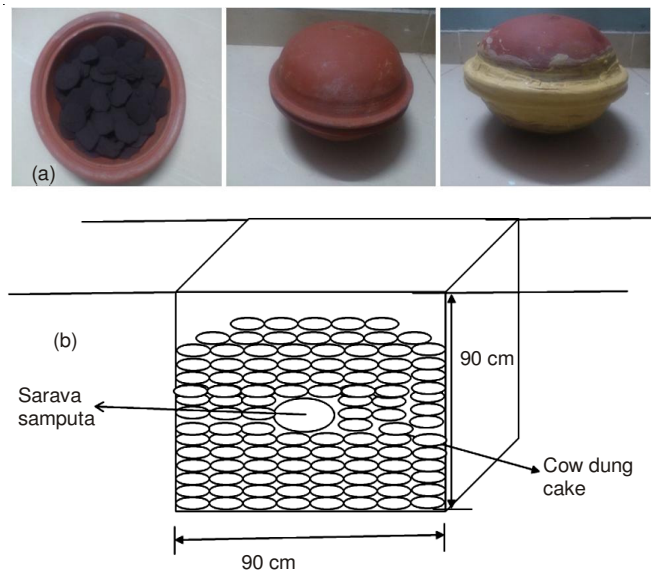

Fig. 1. Photographs showing (a) *Sarava Samputa*; (b) Schematic diagram of traditional calcination chamber showing the placement of *Sarava Samputa* surrounded by cow dung cakes

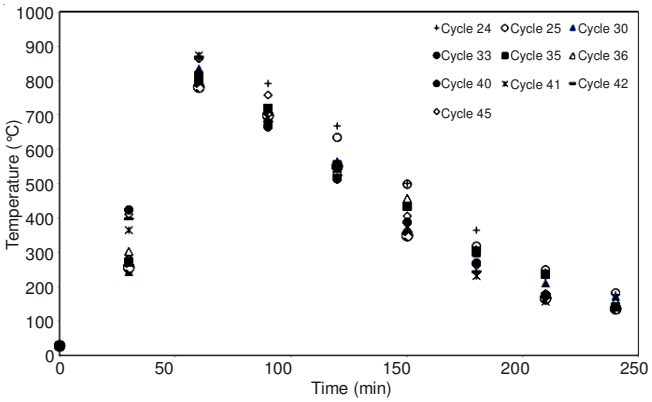

Fig. 2. Temporal variation of temperature during calcination in conventional calcination chamber

Fig. 3a and 3b show the powder X-ray diffraction patterns of feed material (F) and the intermediates obtained after different calcination cycles. The diffraction patterns of feed material (F) shows Fe_3O_4/γ-Fe_2O_3 to be the predominant phase with intensity ratio I(36:Fe_3O_4/γ-Fe_2O_3)/I(33.3: α-Fe_2O_3) being 2.38. It is to be noted that Fe_3O_4 and γ-Fe_2O_3 phases cannot be distinguished based on powder X-ray diffraction patterns as both these phases have same interplanar spacing[22]. Hence we prefer to use Fe_3O_4/γ-Fe_2O_3 for describing the phase with maximum diffraction intensity at 36°. The grain size estimated using Scherrer formula from the X-ray diffraction pattern was 32.69 nm. The diffraction patterns of intermediates from different calcination cycles match reasonably well with that of Fe_3O_4/γ-Fe_2O_3 with very little α-Fe_2O_3.

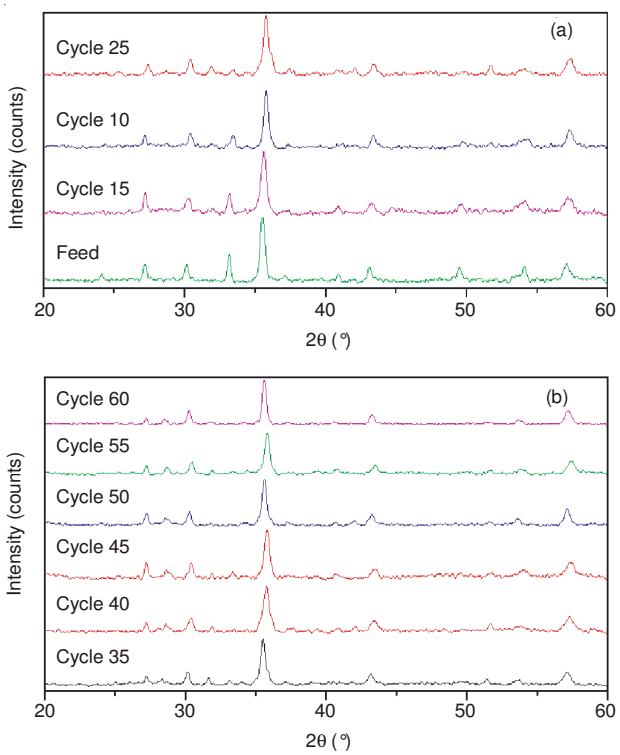

Fig. 3. Powder X-ray diffraction patterns of intermediates obtained after different calcinations cycles: (a) 1 to 25; (b) 35 to 60

With increase in calcination cycles, the relative intensity of peak corresponding to Fe_3O_4/γ-Fe_2O_3 increase in comparison with others. For instance, the ratio of I(36:Fe_3O_4/γ-Fe_2O_3)/I(33.3: α-Fe_2O_3) for the feed, intermediate after 5th calcination cycle and that after 10th calcination cycle were 1.21, 1.25 and 1.47 supporting the relative increase of Fe_3O_4/γ-Fe_2O_3 contentwith respect to α-Fe_2O_3. During subsequent calcination cycles, α-Fe_2O_3 content seems to have vanished. The broadening of the peaks indicates that the product contains nanocrystallites. The crystallite size, was found to be between 25 and 35 nm for the intermediates obtained after different calcination cycles.

Upon heating Fe_3O_4, the transition from Fe_3O_4 to α-Fe_2O_3 takes place through the formation of intermediate phase γ-Fe_2O_3, depending on the temperature. It is well known that transition of γ-Fe_2O_3 to α-Fe_2O_3 takes place at 500-600 °C for bulk material, while this transition temperature ranges between 300 and 450 °C for nanocrystalline material[23,24]. The temperature during calcinations cycles stays above 600 °C for atleast 1 h. Hence, one would expect the phase transition from nanocrystalline Fe_3O_4/γ-Fe_2O_3 to α-Fe_2O_3 during the calcinations step. The fact that the product obtained after each calcinations cycle was Fe_3O_4/γ-Fe_2O_3 indicates that the transition to α-Fe_2O_3 was shifted to higher temperatures. The role of metal dopants in increasing the temperature of transition of nanocrystalline γ-Fe_2O_3 to α-Fe_2O_3 transition temperature has been reported[25,26]. Doping of Mn(III) in the range of 5 to 8.5 wt % increased the temperature of complete transition to above 600 °C[26]. Presence of sodium at 0.9 wt % was found to increase the temperature for complete transition to 750 °C[26]. These attempts to increase the γ-Fe_2O_3 to α-Fe_2O_3 transition temperature were aimed at preparing crystalline γ-Fe_2O_3 at high temperatures owing to its technological importance in magnetic storage, optical devices, *etc*.[25,26]. The elemental analysis of lyophilized *Triphala* decoction shows the presence of potassium, magnesium, calcium, *etc*. (Table-1). During the wet grinding of Fe_3O_4/γ-Fe_2O_3 containing material with *Triphala* decoction and subsequent drying under sun, these metals are probably incorporated in flat discs of Fe_3O_4/γ-Fe_2O_3. Also, wet grinding is believed to decrease the particle size and hence increase surface area, facilitating better interaction between solid (feed material) and liquid (*Triphala* decoction)[27-30] leading to incorporation of ingredients from *Triphala* decoction. This was confirmed from the elemental analyses of thin flat discs shown in Table-2, from which the incorporation of elements like potassium, magnesium and calcium during wet grinding is evident. We believe that the presence of these metallic ingredients as dopants increases the activation energy for the transition, in line with the observations of earlier works[25,26]. This has probably led to increase in the transition temperature ensuring that the material was retained as Fe_3O_4/γ-Fe_2O_3. The addition of *Triphala* decoction for every calcinations cycle may be attributed to prevent the transition of Fe_3O_4/γ-Fe_2O_3 to α-Fe_2O_3 during any of the calcinations steps.

TABLE -1
ELEMENTAL COMPOSITION OF LYOPHILIZED *Triphala* DECOCTION

Elements	K	O	Cl	Ca	Si	Al	Mg	P
Composition by mass (%)	53.06 ± 0.62	21.85 ± 0.06	8.97 ± 0.02	7.18 ± 0.56	2.25 ± 0.01	1.78 ± 0.01	1.20 ± 0.01	1.06 ± 0.04

TABLE-2
ELEMENTAL COMPOSITION OF THIN FLAT DISCS SUBJECTED TO CALCINATION

Elements	Fe	O	K	Si	Cl	Ca	Mg	Al	Na
Composition by mass (%)	60.05 ± 0.04	30.89 ± 0.03	3.14 ± 0.03	2.31 ± 0.02	0.92 ± 0.01	0.53 ± 0.01	0.42 ± 0.02	0.34 ± 0.03	0.31± 0.01

It is pertinent to mention that Fe_3O_4 and γ-Fe_2O_3 have higher biocompatibility than α-Fe_2O_3[31] and hence may be the more preferred forms of iron oxide for biological applications.

During repeated calcination cycles, the material undergoes substantial changes in the elemental composition (Figs. 4, and 5a-b). The relative percentage of iron decreases from 62.6 % in the 1st calcination cycle to 50.18 % in the 60th calcination cycle. The reduction in percentage of iron is not due to the removal of iron oxide, but due to the incorporation of elements like potassium, sodium, calcium, magnesium, chlorine. These are confirmed from elemental analysis shown in Figs. 4 and 5a-b.

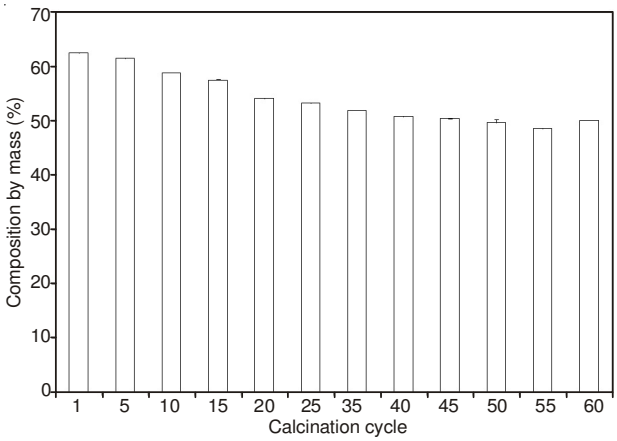

Fig. 4. Variation of percentage of iron in the products obtained after different calcination cycles

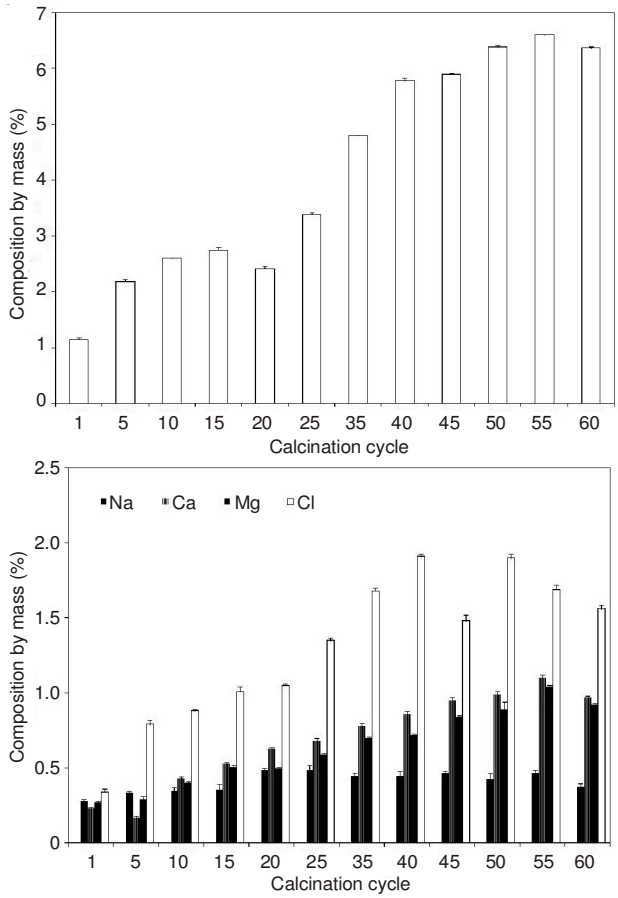

Fig. 5. Variation of percentage of intermediates from different calcination cycles (a) K; (b) Na, Ca, Mg and Cl

Fig. 6 shows the scanning electron micrographs of intermediate obtained from conventional calcination at three different magnifications.

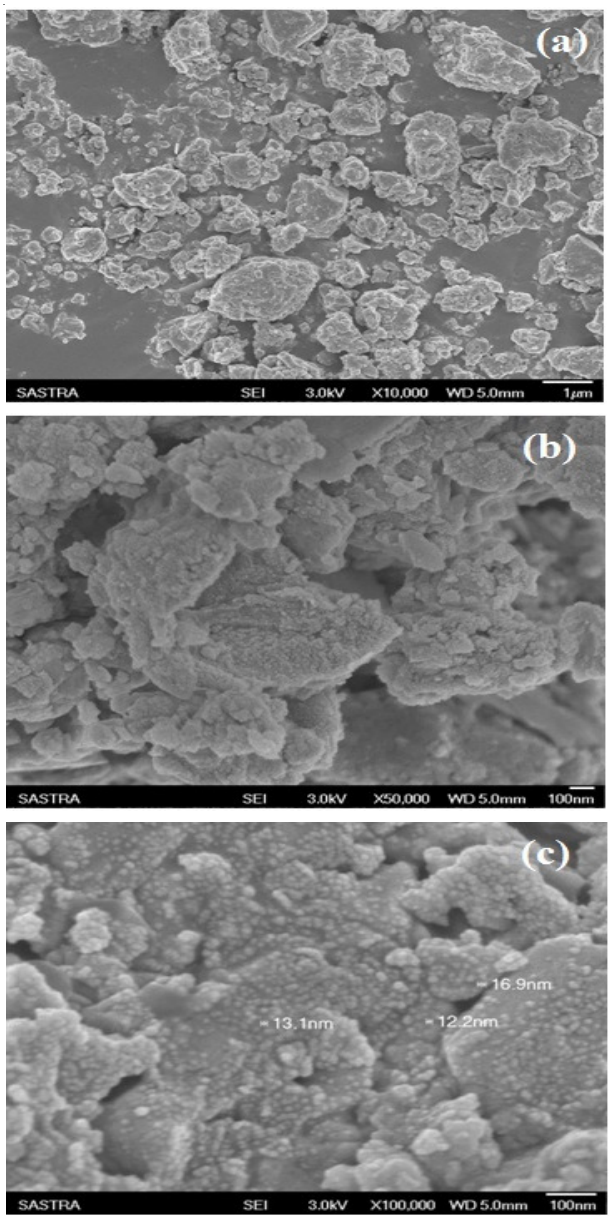

Fig. 6. Scanning electron micrograph of intermediates obtained from (a-c) conventional calcination

The scanning electron micrographs at magnification of 10,000 X show that the particles of the intermediate obtained from traditional calcination are well-separated despite varying in size. The nanoparticles decorating the surface of larger particles are about 11-16 nm in the product from traditional calcination.

Conclusion

The addition of aqueous extracts of three fruits namely, Indian gooseberry, *Belleric myrobalans* and *Chebulic myrobalans* increases the temperature for transition from Fe_3O_4/γ-Fe_2O_3 to α-Fe_2O_3 during a traditional calcination process. This is probably due to the presence of metals like potassium and calcium in the material subjected to calcination, made available from the

aqueous fruit extract. These light metals are believed to increase the activation energy for transition from Fe_3O_4/γ-Fe_2O_3 to α-Fe_2O_3. The better biocompatibility of Fe_3O_4 and γ-Fe_2O_3 compared to that of α-Fe_2O_3, provides yet another justification for addition of fruit extracts and unique calcination method. It might be possible to mimic traditional calcination using muffle furnace, provided the temperature profile obtained during traditional calcinations, is reproduced in the furnace.

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