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Pharmaceutical standardization and physicochemical characterization of traditional ayurvedic mineral drug red ochre roasted in cow's ghee (*Shuddha Gairik*)

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Rasashastra is a pharmaceutics branch of Ayurveda describing scientific methods to convert metals and minerals into bio-compatible formulations used individually or plant admixed to enhance their bioavailability and efficacy. In the present study, red ochre (*Gairik*) powder was processed in cow's ghee by textual method of roasting. The steps of preparation and changes in the properties therein were documented and validated in triplicate batches to develop a monograph. Ayurvedic and basic tests were performed to determine the properties of *Shuddha Gairik*. The physical characterization included Scanning Electron Microscopy (SEM), X-ray Diffraction (XRD), Fourier Transform Infra-red (FT-IR), Dynamic Light Scattering (DLS) and Thermo-gravimetric Analysis (TGA). Elemental composition was estimated by titration and gravimetric analysis while heavy metal limits were assessed using Inductively Coupled Plasma Optical Emission Spectrophotometry (ICPOES). This study depicted that crude red ochre, containing Kaolinite with high iron percentage, on roasting in cow's ghee led to the formation of fatty acids adsorbed red ochre particles. The developed monograph will be a guideline to the Ayurvedic industry for precise formulation of *Shuddha Gairik*. This will help researchers for better understanding the importance of Ayurvedic methods of pharmaceutical preparations and carry out their mechanistic studies in various diseases.

Keywords: Ferric oxide, Gairik, Kaolinite, Monograph, Red ochre, Roasting

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Red ochre, being a natural earth pigment, has been historically used for several purposes like painting walls, artworks and also for extraction of iron since Iron Age. Archaeological excavations at Caves of Altamira, Spain have revealed wall paintings dated 15,000 years back, which shows use of red ochre as a pigment since prehistoric era[1]. In India, it is traditionally used for wall painting and as a base of sacred floor design viz., *Rangoli*[2]. Ayurveda has mentioned use of red ochre, known as *Gairik* in *Charak Samhita* (200 BC) and *Sushruta Samhita* (200 AD), for treating several ailments through formulations like *Mahagandhahasti Agad* as anti-toxin; *Khadiradi Gutika* in stomatitis and oral cavity diseases; *Pushyanugchurna* in menorrhagia; *Dushivishariagadam* and *Tarkshyaagadam* as anti-toxins, *Laghusutashekhar rasa* and *Kamdudha rasa*, both in hyperacidity[3-8]. It has been mined traditionally and used for several purposes like, yellow or red pigment mixed in water or oil, topical application on oozing wounds, oral administration to decrease the sense of hunger, etc[9]. Red Ochre is an earthy variety of hematite mineral having mixture of iron oxide (Fe_2O_3) and clay hence called as *Svarnagairik* (Sanskrit), Reddle (English), *Geru* or *Gerumitti* (Hindi) and *Sonakava* (Marathi). Anhydrous iron oxide is red ochre (*Gairik*) while hydrated iron oxide is yellow ochre. Physically, it is present as massive clayey lumps with earthy luster, brittle and opaque showing reddish brown streak as well as with uneven fracture. It becomes magnetic on heating and does not easily fuse in a blow-pipe flame. It is soluble in hydrochloric acid and contains more than 16% Fe when analyzed by titration method[10].

Red ochre is described as one of the *Uparasa* (materials used in mercurial processing mentioned in *Rasashastra*) and group of soils (*Panchmruttika*) used in several Ayurvedic pharmaceutical processes[11]. It is also considered as *Upadhatu* (base mineral of a metal) of *Loha* (iron) having a synonym *Rakta Dhatu*[12]. It

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has sweet (*madhur*) and astringent (*kashaya*) taste, unctuous (*snigdha*) and cleansing (*vishada*) property, cooling (*shita*) potency as well as sweet effect after digestion (*madhur vipaka*). It helps to reduce burning sensation (*daha*) and has coagulating (*raktastambhan*), anti-toxin (*vishaghna*), anti-pruritic (*kanduhar*) and wound healing (*vranaropana*) actions[13]. It is indicated in diseases of eyes (*netraroga*), bleeding disorders (*raktapitta*), hiccup (*hikka*), vomiting (*vamana*), disorders due to toxins (*vishavikara*), menorrhagia or metrorrhagia or both (*raktapradara*), itching (*kandu*), fever (*jvara*) and diseases of abdomen (*udararoga*)[10]. Specifically, it is administered orally with honey and applied externally with turmeric for urticarial rash. In hemorrhagic disorders, it is indicated orally with sandalwood (*Santalum alba*), vetiver (*Vetivera zizanioides*) and coriander (*Coriandrum sativum*) decoction or with candy sugar and cardamom (*Elettaria cardamomum*) seeds[13]. Clinically, it has been assessed for oral contraceptive activity along with *Talispatra* (*Taxus baccata*)[14,15]. It has also been recommended for scalp rubbing in SARS CoV-2 positive patients[16]. In a recent study, red ochre has been used as a topical photo-protective agent against Ultra-Violet radiation based on evidences that red ochre probably was used for this purpose in Middle and Late Stone Age in African countries[17].

Rasashastra, being a pharmaceutical branch of Ayurveda, has stated two methods of processing red ochre before clinical use as oral administration or as an ingredient in formulations. Trituration of red ochre powder with cow's milk (*Bhavana*) and roasting with cow's ghee (*Bharjana*) are the two processes mentioned for converting it into bio-compatible form (*Shodhana*) with the therapeutic dose of 250 - 500 mg (i.e., 2 – 4 *Ratti*) per day[13].

Though *Shuddha Gairik* is one of the cost-effective medicines in Ayurveda, data on pharmaceutical standardization and characterization using modern parameters is meager. The experiment of trituration of red ochre with cow's milk has been published but without manufacturing details and characterization[18]. In another attempt, red ochre purified by both the methods, was analyzed, but with incomplete characterization[19]. The ratio of cow's ghee to red ochre in roasting method was studied but thorough characterization of roasted red ochre was not attempted[20]. Marketed tooth powder containing *Gairik* as an ingredient was analyzed by Numburi Phased Spot Test[21]. Apart from raw material specifications given in Ayurvedic Pharmacopoeia of India, neither cow's ghee roasted red ochre has been included in Ayurvedic Formulary of India nor an official monograph been published.

It is therefore essential to standardize the process of roasting red ochre with cow's ghee on a bulk manufacturing scale as per classical reference[13]. We analyzed three batches of red ochre processed in cow's ghee in the present study maintaining Quality Control (QC) and Quality Assurance (QA). All the steps were documented and the characterization was done to develop a monograph of the finished product to achieve batch to batch consistency on industrial level. The characterization denoted exact properties and highlighted the therapeutic relevance of processing iron oxide and kaolinite present in red ochre with cow's ghee.

Methodology

Procurement of materials

Gairik/ red ochre

Two samples were purchased from S. V. Ayurvedic Bhandar, Agriculture Produce Market Committee (APMC) Market, Vashi, Navi Mumbai, India and tested for basic Ayurvedic parameters as detailed in Ayurvedic Pharmacopoeia of India, Volume 7. Sample containing desirable content of iron was selected for the present study[10]. The reference specimen (BSDT/V-M&M/10) was deposited in the institutional repository for future reference.

Goghrut/ cow's ghee

Standard cow's ghee of Agriculture Marketing Information System Network (AGMARK) grade (Certificate of Authorization Number A1003440, Government of India) was purchased from local vendor, B.G. Chitale Group, Pune, India as per the Pharmacopeial standards[22]. The nutritional information declared by the vendor is given in Supplementary data Table S1.

Materials and Methods

Instruments used

Industrial Khoa (thickened milk) machine (Solar Arks, Kolhapur, Maharashtra, India) having Stainless Steel (SS) vessel with dimensions as 0.52 m base diameter, 0.65 m top diameter, 0.24 m slanting height, volume 60 L and attached to an electric motor of 200 Watt rotating at a speed of 50 rotations per minute was used for the roasting process. This vessel had a liquefied petroleum gas burner beneath for heating and a fixed continuous stirring arrangement, as shown in Fig. 1. Accessory equipment like mixer grinder

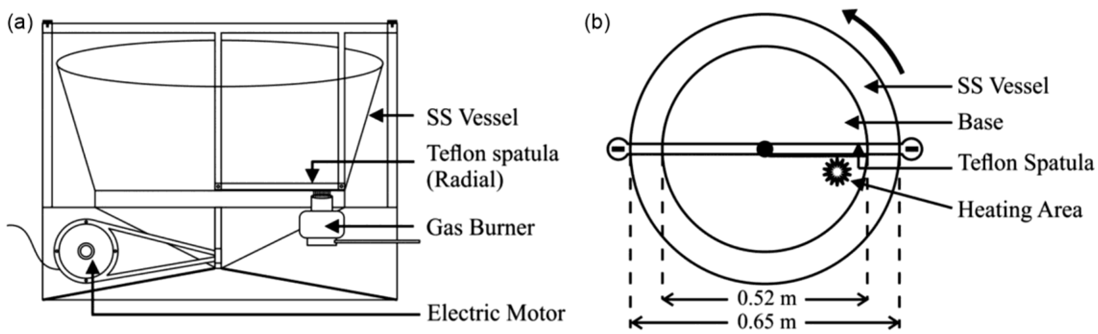

Fig. 1 — Schematic representation of the Khoa machine used for roasting powdered red ochre in cow's ghee: (a) Side view of the Khoa machine; (b) Top view of the Khoa machine showing heating area and vessel dimensions.

(Preethi make, 600-Watt motor, 2 L capacity vessel), SS vibro-sifter (0.76 m diameter), SS sieves of #200 mesh (American Society for Testing and Materials-ASTM), manual pounding instrument (made of iron) and Digital Infrared Thermometer (IR Non-contact LCD Laser meter gun style, Room Temperature – 1000°C, Gain Express Holding Ltd, Hong-Kong) for temperature recording were used.

Chemicals and standards

Nitric acid (Primer Plus Trace Analysis Grade > 68%, Fisher Chemical, Fisher Scientific UK Ltd., Loughborough, UK) and hydrogen peroxide 30% (J.T. Baker, Avantor Performance Materials, PA, USA) were used for microwave digestion of red ochre samples. Multi-element standard (for Cd and Pb) and As standard from Merck (Certipur, Darmstadt, Hesse, Germany) while Hg standard from Sigma Aldrich (TraceCERT, St. Louis, MO, USA) was purchased for ICPOES analysis.

Experimental details

Processing of red ochre (Gairik shodhana)

Three batches of 8 kg each of specifically selected raw material sample, which followed all the basic tests and parameters of red ochre as per the Ayurvedic Pharmacopoeia of India, were utilized for the process. The lumps of red ochre were pounded in an iron mortar by pestle. It was further grounded in a mixer grinder and sifted through mesh #200 in SS vibro-sifter. This powder is hereafter termed as "Crude Red Ochre" (CRO).

***Roasting* (*Bharjana*)**

The Khoa machine vessel was heated upto 100°C and cow's ghee was added to liquefy it. The Khoa machine was switched on to rotate. Powdered red ochre was then slowly added. The ratio of cow's ghee to powdered red ochre was kept as 1:10 (w/w) for each batch. The roasting process was continued till the powdered red ochre became free flowing and ghee started fuming mildly. The temperature was maintained between 140 – 170°C and recorded every 10 min. Time required for completion of process, and mass gains were recorded. Finally, the material was self-cooled in a closed SS vessel. It was again passed through sieve #200 and herein after termed as "Processed Red Ochre" (PRO). Three replicated batches were prepared separately in the same machine. All the experiments from purchase of raw material (CRO) to preparing final product (PRO) and their characterization were performed during the years 2016-2018.

Basic physicochemical analysis

The finished product of each batch was separately analyzed as per Ayurvedic methods like *Shabda*-Sound, *Sparsha*-Touch, *Rupa*-Appearance, *Rasa*-Taste and *Gandha*-Odor[23]. Basic physicochemical tests like loss on drying (LOD), loss on ignition (LOI), acid insoluble ash and bulk and tapped density were carried out as per the standard method. Compressibility of *Shuddha Gairik* was also calculated based on bulk and tapped density[24-26]. Total iron and silicon content were estimated by standard titration and gravimetric method, respectively[10].

Advanced analytical techniques

Scanning electron microscopy (SEM) analysis

Both, CRO and three batches of PRO were examined under Leica Cambridge 440 SEM (Leica Cambridge Ltd, Cambridge, UK) to elucidate the structure and particle size. The sample was prepared by spreading the powder on a double-sided carbon tape and sputter coated with gold for nullifying the charging effect. All the samples were observed with various magnifications such as 100x, 500x, 1000x, 2000x and 5000x and photomicrographs were captured.

X-Ray diffraction (XRD)

CRO and 3 batches of PRO were analyzed on PANalytical X'PERT Pro X-Ray Diffractometer (Lelyweg, Almelo, Germany) using Cu Kα radiation (λ = 1.5418 Å). XRD patterns were recorded in the 2Θ range of 10 - 80° at a scanning rate of ~2°/min. Powder XRD patterns of CRO and PRO were simulated from the known crystal structure parameters of ferric oxide (Fe_2O_3) and kaolinite reported in the literature, using the software 'Powder Cell for Windows' (Version 2.4, Federal Institute for Materials Research and Testing, Berlin, Germany, available at http://www.ccp14.ac.uk). Phase content was analyzed by the least squares' refinement of the experimental patterns. The error in the phase content analysis, if present for more than one phase, was ± 10%.

Fourier transform infra-red spectroscopy (FT-IR)

Both, CRO and all the three batches of PRO powders were analyzed on FT-IR (Spectrum GX, Perkin Elmer, Waltham, MA, USA) under Attenuated Total Reflection (ATR) mode with scanning range in mid IR from wavenumber 4000 – 600 cm^{-1}. A total of 10 scans were recorded in percentage Transmission mode with resolution 4.

Thermo-gravimetric analysis (TGA)

The TGA was done using 5 - 10 mg of CRO and three batches of PRO powders on TGA Q5000 (hanging pan machine) from TA Instruments Inc., New Castle, DE, USA. Temperature range of 40°C – 900°C with a heating rate of 10°C/min under nitrogen atmosphere was used for all the measurements. Universal Analysis software was employed for data analysis.

Dynamic light scattering (DLS) analysis

The particle size measurements were performed by dynamic light scattering technique using 90 Plus instrument from Brookhaven Instruments Corp., Holtsville, NY, USA equipped with 35 mW solid state laser and a highly sensitive Avalanche Photo Diode (APD) detector. Three replicates of PRO powder were suspended in filtered de-ionized water, ultra-sonicated for 2 min and diluted to avoid the effect of multiple scattering. The measurements were done at an angle of 90° for a period of 3 min at immediate, 8 min and 16 min interval for all the three batches. The particle size was obtained from the intensity auto-correlation function data. Volume weighted particle size distribution was obtained from the light scattering data and D10, D50 and D90 values were calculated. The span of the distribution was calculated using the formula, SPAN = (D90 - D10)/ D50.

Heavy metal assay by inductively coupled plasma optical emission spectrophotometer (ICPOES)

Both, CRO and PRO were analyzed and quantified for heavy metals (Hg, As, Cd, Pb) using ICPOES (Thermo Fisher Scientific, Waltham, MA, USA) by following methodology wherein the samples were closed digested in Microwave Digestion System (Ethos Easy Advanced, Milestone SRL, Milan, Italy). In this method, 100 mg sample was added with 8 mL HNO_3 and 2 mL H_2O_2 in the microwave vessel and allowed to react for 10 min. It was further digested for 15 min by gradually heating to achieve 200°C temperature, then maintained for 20 min and finally cooled to room temperature in 10 min. Each standard solution was diluted to 1, 5, 10, 25, 50, 100, 250 ppb concentrations for heavy metals. Digested samples of CRO and PRO were made up to 50 mL in de-ionized water. The final samples were aspirated in Argon gas flame in iCAP7200 Duo ICPOES system with auxiliary gas flow of 0.5 L/min. The wavelengths measured in axial view exhibiting R^2 value more than 0.999 were selected for calculation of the respective elements. The calibration graph for standards was plotted using Qtegra Software Version 2.6 (Thermo Fisher Scientific, Waltham, MA, USA) and final concentrations were converted into ppm.

Results

Raw Gairik analysis

Raw red ochre was dark red-colored lumps of variable size and shape. After powdering, it became fine, reddish-brown colored, insoluble in water and soluble in hydrochloric acid. Qualitative analysis for iron was positive and it showed magnetic properties after heating. Iron content as Fe was estimated to be 9.3 and 22.7% w/w for Sample 1 and 2, respectively, by titration method[10]. Sample with desirable Fe content (not less than 16% w/w) was used for further processing. Photographs of both, powdered raw red ochre samples are exhibited in Supplementary data Fig. S1.

Cow's ghee analysis

It was a yellow-colored thick viscous liquid having characteristic fatty odor. It showed specific gravity of 0.923 g/cc at 25°C and moisture content of 0.49% w/w. The saponification value, iodine value and Reichert Meissl value of cow's ghee were observed to

Table 1 — Stepwise mass changes during roasting of red ochre (*Gairik shodhana*)

Parameter	Batch 1	Batch 2	Batch 3
Qty. of red ochre lumps taken for pulverization (kg)	8.2	8.2	8.2
Qty. of red ochre powder obtained after pulverization (kg)	8.14	8.18	8.17
Weight loss during pulverization (%)	0.73	0.24	0.45
Qty. of red ochre powder taken for roasting (kg)	8.0	8.0	8.0
Qty. of cow's ghee used for roasting (kg)	0.8	0.8	0.8
Weight of ghee roasted red ochre (kg)	8.30	8.22	8.27
Weight gain in end product (%)	3.61	2.75	3.38

Table 2a — Analysis of 3 batches of PRO from Ayurvedic perspective

Ayurvedic Parameter	Observations
Shabda (Sound)	No sound produced when rubbed in a mortar with pestle (*Nishabda*)
Sparsha (Touch)	Soft in touch when rubbed between fingers and free flowing (*Shlakshna*)
Rupa (Appearance)	Red color powder (*Rakta varna churna*)
Rasa (Taste)	Sweet (*Madhur*) and astringent (*Kashaya*), like clay (*Mruttikavat*)
Gandha (Odor)	Characteristic of ghee (*Ghrutagandhi*)

Table 2b — Physicochemical analysis of 3 batches of PRO

Parameter	LOD %w/w	LOI %w/w	AIA %w/w	Density Bulk g/cc	Density Tapped g/cc	Compression ability %	Iron as Fe % w/w	Silicon as Si % w/w	Oil content % w/w
Batch 1	0.69	18.77	35.86	0.646	0.888	27.27	20.23	16.75	4.62
Batch 2	0.36	17.31	36.47	0.732	1.085	32.56	20.18	17.03	4.31
Batch 3	0.37	16.61	46.67	0.720	1.001	28.12	16.35	21.79	4.72
Mean	0.47	17.56	39.67	0.699	0.992	29.31	18.92	18.52	4.55
SD	0.19	1.10	6.07	0.05	0.10	2.84	2.23	2.83	0.21
SE	0.11	0.64	3.51	0.03	0.06	1.64	1.29	1.64	0.12

LOD: Loss on drying, LOI: Loss on ignition, AIA: Acid insoluble ash

be 247.9, 27.6 and 32, respectively with unsaponifiable matter as 4.3% w/w. Heavy metal contents were within permissible range of 20 ppm as performed by the limit test[22].

Red ochre powder (*Gairik churna*) analysis

It was bright red-colored, free flowing very fine powder with bulk and tapped density of 0.94 and 1.3 g/cc, respectively, and calculated compressibility of 27.5%. On sieve analysis, it passed through mesh #200 (73 μm) with 2.68% retention and dusting upto 97.3% as per the requirements for processed metal and mineral based Ayurvedic drugs[27].

Observations during roasting of red ochre

The total time required for powdering of 8 kg CRO was approximately 3 h (1 h for pounding and 2 h for grinding to pass through mesh #200). After adding powdered red ochre in heated cow's ghee, small lumps started forming due to sticky nature of ghee but disappeared gradually due to continual stirring. The bright red color of CRO turned to dark reddish brown and fluffiness of PRO increased towards the end of the process. Mild fumes emerged through the material especially at the temperature nearing to 170°C towards the end of the roasting process. The total duration required for roasting 8 kg batch of CRO was 1.5 h. The details of weight measures and yield are given in Table 1 while temperature record data in Supplementary data Table S2. The average mass loss during pulverization was 0.47% w/w while mass gain after roasting process was 3.24% w/w.

Ayurvedic and modern methods of physicochemical analysis of processed red ochre (*Shuddha Gairik*)

The appearance of crude, powdered and ghee processed red ochre is exhibited in Fig. 2. The color change was analyzed using standard Pantone Solid Coated Color Chart. The color assigned for CRO was Pantone 1795 C while for PRO was 1807 C. Ayurvedic parameters for testing *Shuddha Gairik* (PRO) as detailed in Table 2a indicated that PRO is a dark reddish-brown colored, free flowing very fine, soft powder, having characteristic oily odor of ghee. The basic parameters used for the assessment of batch-to-batch consistency; composition and estimation of oil content in PRO are shown in Table 2b. All the three batches of PRO did not show significant

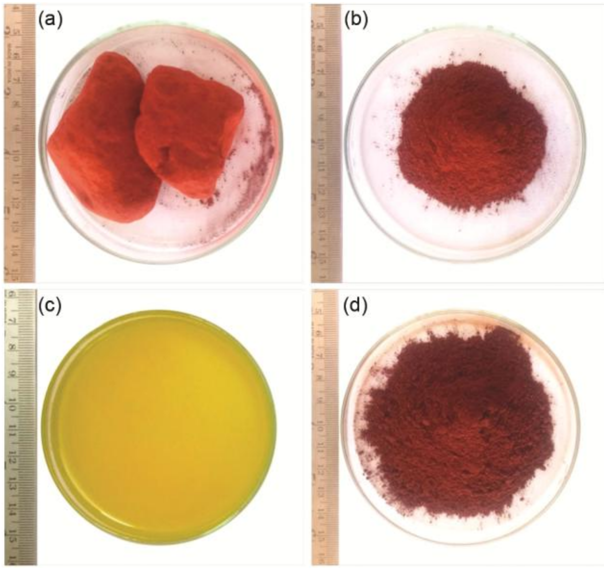

Fig. 2 — Raw material (CRO) and finished product (PRO): (a) RM1 lumps; (b) RM1 powder; (c) cow's ghee; (d) processed red ochre.

difference in loss on drying (0.5% w/w), loss on ignition (17.6% w/w) and oil content (4.6±0.2% w/w) but considerable variation in acid insoluble ash (40±6.1% w/w) was noticed. It was found that bulk and tapped density decreased (upto 0.3 g/cc) while compressibility increased (upto 2%) after processing with ghee as compared to those in CRO. The iron and silicon content showed a variation of 2 - 3% w/w within the three batches.

Analysis of CRO and PRO using modern techniques

Scanning electron microscope analysis

Both, mechanically powdered CRO and PRO did not show any significant difference with respect to shape and size when observed under SEM. Both showed particles as agglomerates having irregular shapes and edges. Smaller particles seemed to be adhered to the larger ones at several areas. The particle size showed agglomerates of variable range from less than 5 μm upto 25 μm as shown in Fig. 3, a representative figure of 1000x magnification for CRO and the three batches of PRO.

X-Ray diffraction study

The simulated pattern of CRO and 3 batches of PRO did not show any change in the crystalline phase after XRD analysis as given in Fig. 4. All the samples showed prominent peaks simulating with that of ferric oxide (Fe_2O_3) and kaolinite. In PRO 3 (GRK-S3-RM04) peaks near 20 and 40 2Θ degrees similar to kaolinite are more prominent than the other two PRO samples.

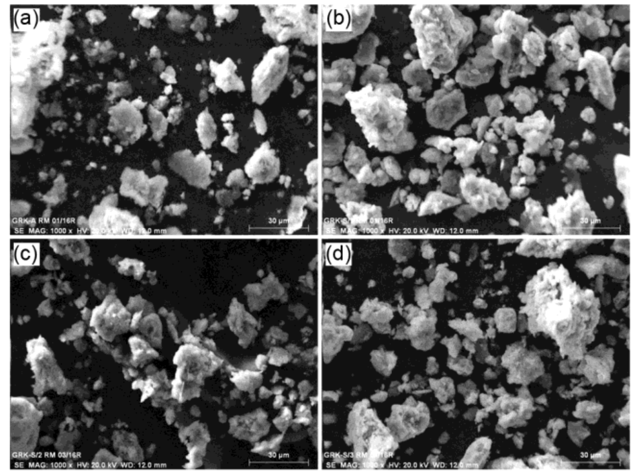

Fig. 3 — Scanning Electron Microscopy of red ochre: (a) CRO; (b, c and d) 3 batches of PRO, respectively, at 1000x magnification (Scale= 30 μm). In the figure, GRK-A represents CRO while GRK-S/1, GRK-S/2 and GRK-S/3 denote PRO 1, PRO 2 and PRO 3, respectively.

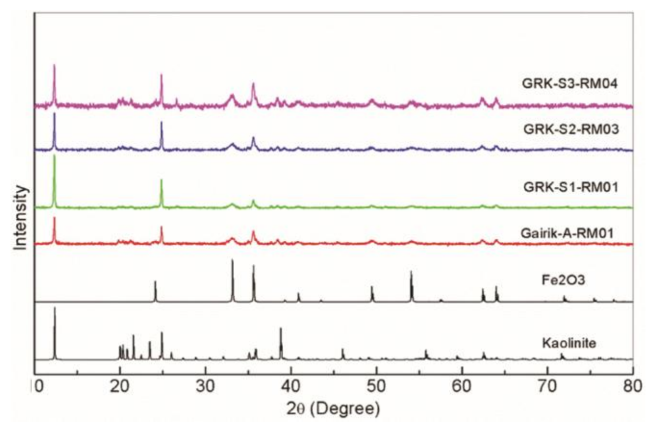

Fig. 4 — X- Ray Diffraction analysis of CRO and PRO simulated with ferric oxide (Fe_2O_3) and Kaolinite crystalline pattern. CRO is denoted by Gairik-A-RM01 while Batch 1, 2 and 3 of PRO are denoted by GRK-S1-RM01, GRK-S2-RM03 and GRK-S3-RM04, respectively.

Fourier transform infra-red spectroscopy study

The bands detected on FT-IR for CRO and PRO were plotted as transmittance (% T) *Vs* wavelength (cm^{-1}) as given in Fig. 5, while actual wavenumbers are given in Supplementary data Table S3. The CRO showed 11 prominent bands out of which, 10 were also seen in PRO viz., nearer to 3686, 3619, 1114, 1024, 998, 909, 795, 751, 642 and 629 cm^{-1}; except the wavenumber 1002.68 cm^{-1}. Similarly, a weak band at 692 cm^{-1} was observed in both, CRO and PRO. However, PRO additionally showed 5 bands, viz., nearer to 2920, 2851, 1741, 1465 and 935 cm^{-1}, which were not observed in CRO.

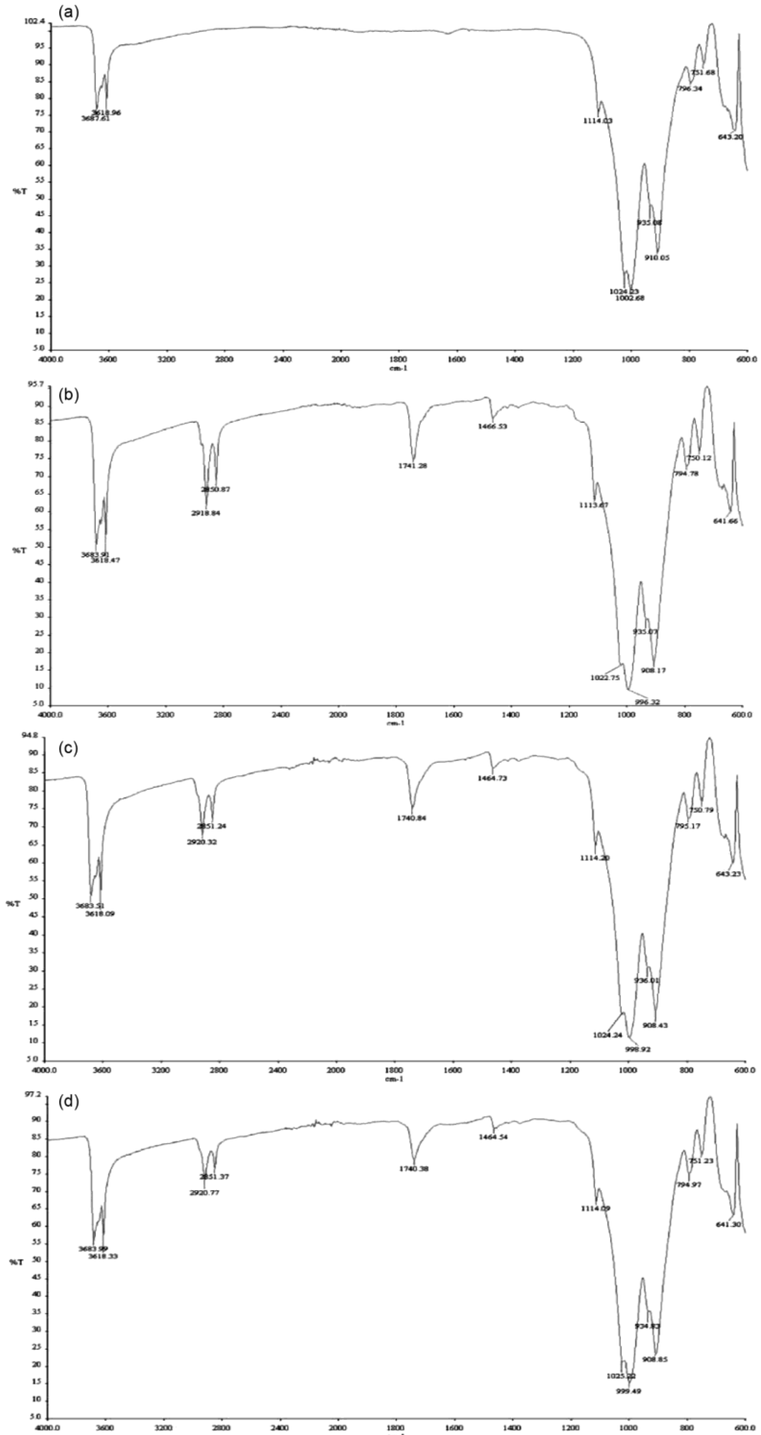

Fig. 5 — FT-IR analysis of CRO and PRO: (a) CRO showing 10 peaks; (b, c and d) PRO Batch 1, 2 and 3, respectively showing 5 additional peaks.

Thermo-gravimetric analysis

The graphs of mass loss in percentage and temperature range from 50 – 1000°C were plotted as exhibited in Fig. 6 for CRO and PRO. CRO showed sudden drop in mass while PRO showed interrupted mass losses, till it reaches the stable phase at the end of the experiment. The values obtained are given in Table 3 showing mass loss in sample at various temperatures. The loss in mass during TGA was seen in two phases for CRO and three phases for all the

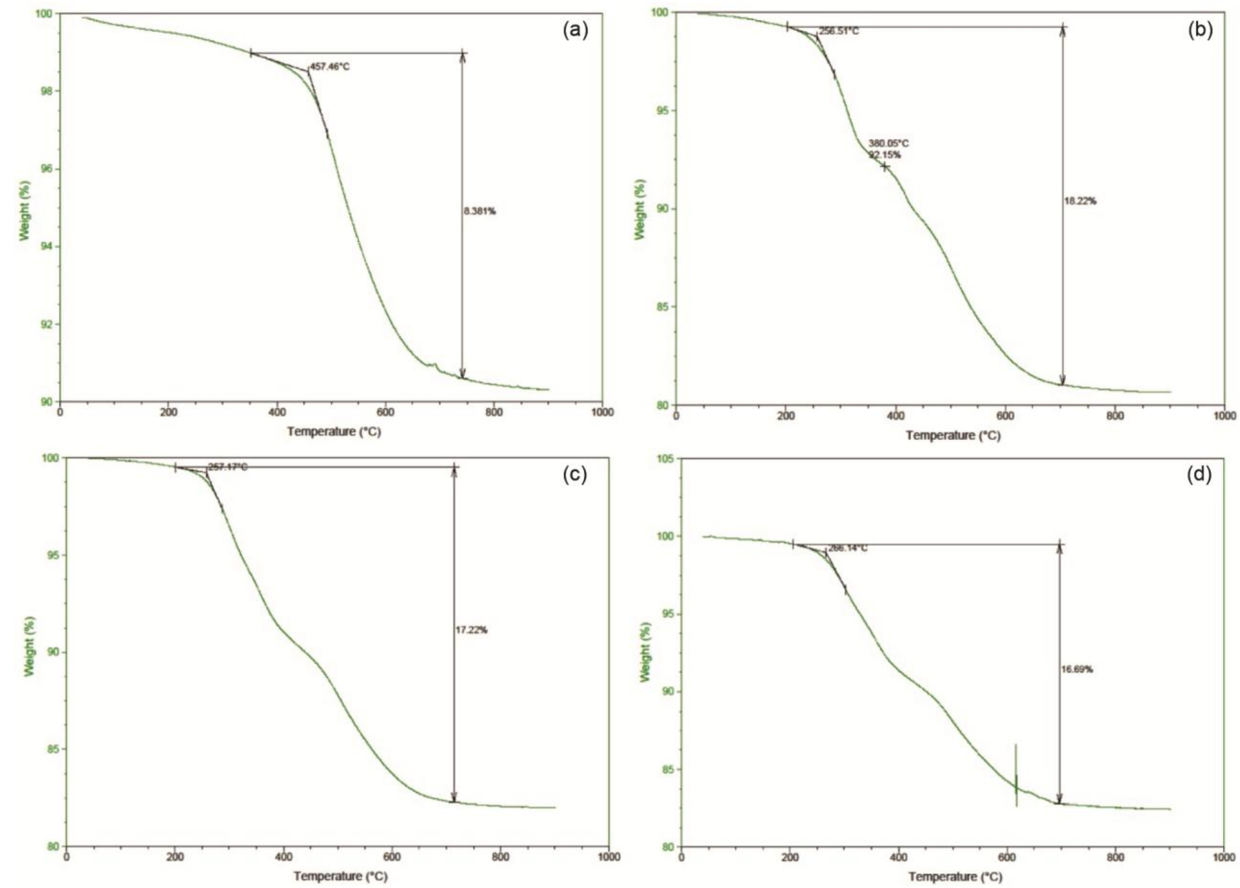

Fig. 6 — Thermo-gravimetric analysis of red ochre: (a) CRO; (b, c and d) 3 batches of PRO, respectively.

Table 3 — Thermo-gravimetric analysis of CRO and PRO

Sample	*T*1	L1	*T*2	L2	*T*3	L3	Total Loss
CRO	Nil	Nil	457.5	1.0	740	8.4	9.4
PRO 1	256.5	2.0	380	7.9	700	8.4	18.2
PRO 2	257.2	1.8	390	6.0	725	9.5	17.2
PRO 3	266.1	1.5	375	5.8	700	9.4	16.7

T= Temperature in °C. L= Loss in % w/w

three samples of PRO. CRO showed 1% mass loss at around 450°C while considerable mass loss of 8.5% above 750°C. PRO showed first mass loss of 1.8±0.3% w/w at temperature 250 - 260°C, while second mass loss of 6.5±1.2% w/w at temperature 400 - 450°C. The third significant mass loss of 9.1±0.6% w/w was seen at temperature 700 – 725°C. Both, CRO and PRO remained stable upto 900°C beyond 740 and 708.3±14.4°C, respectively. Thus, CRO showed upto 10% of total mass loss while PRO showed 17.4±0.8 % w/w of total mass loss in thermo-gravimetric analysis.

Dynamic light scattering analysis

The results obtained are enumerated in Table 4 for all the three batches. All the three batches showed effective diameter within the range of 900 – 1200 nm and mean diameter between 500 – 800 nm. The D10, D50 and D90 values for all the three batches ranged between 220 – 270 nm, 450 – 1000 nm and 900 – 1300 nm, respectively. Poly-dispersity and span value were found to be between the range of 0.3 - 0.5 and 1.4 – 1.7, respectively. The particle size analysis carried out at immediate, 8 min and 16 min interval showed mean effective diameter of 897±157.8 nm and mean diameter of 517±100.5 nm. The D10, D50 and D90 values were deduced as 209±28.7 nm, 508±131.5 nm and 917±144.5 nm, respectively. The poly-dispersity and span value were found to be 0.367±0.02 and 1.5±0.04, respectively.

Table 4 — Dynamic light scattering analysis of PRO after ultra-sonication

Factor	Immediately after sonication					8 min after sonication					16 min after sonication				
	1	2	3	Mean	SD	1	2	3	Mean	SD	1	2	3	Mean	SD
ED (nm)	952	1190	1091	1079	120	697	869	910	825	113	656	813	894	788	121
PD	0.36	0.43	0.35	0.38	0.04	0.35	0.42	0.34	0.37	0.05	0.33	0.38	0.33	0.35	0.03
MD (nm)	527	757	613	632	116	394	492	525	470	68.4	381	438	525	448	72.5
D10	226	233	266	242	21.5	172	176	233	194	34.5	171	164	237	191	40.5
D50	454	989	530	658	289	341	557	457	452	108	333	448	460	414	70.1
D90	913	1283	1056	1084	186	677	957	896	843	147	649	936	890	825	154
Span	1.51	1.65	1.49	1.55	0.09	1.48	1.64	1.45	1.52	0.10	1.44	1.54	1.42	1.47	0.07

ED: Effective Diameter, PD: Poly-dispersity, MD: Mean Diameter, D10-D50-D90: percentile values; 1, 2 and 3 denote batch numbers PRO 1, PRO 2 and PRO 3, respectively.

Table 5 — Heavy metal content (in ppm) estimated in CRO and PRO

Sample	As	Cd	Hg	Pb
CRO	1.6	8.9	1.9	21.6
PRO 1	Nil	7.5	3.2	19.5
PRO 2	Nil	7.8	2.4	20.6
PRO 3	Nil	7.8	0.7	19.3
Mean of PRO (n=3)	-	7.9	2.0	20.2
SD of PRO	-	0.18	1.30	0.67

Inductively coupled plasma optical emission spectrophotometry analysis

Both, crude and finished products when analyzed in duplicate samples had same levels of cadmium, mercury and lead while arsenic was not detected in PRO samples at all. The mean values are given in Table 5 while the details of sample weight, intensity of light emitted during ICPOES analysis, readings and calculated mean values are detailed in Supplementary data Table S4. Based on the ICPOES analysis of PRO, arsenic was not detected at all while the calculated Cd, Pb and Hg were 4, 10 and 1.02 μg/day, respectively, for 500 mg dose of PRO.

Monograph of Shuddha Gairik- cow's ghee processed red ochre (PRO)

A monograph was developed for commercial batch of 8 kg (n = 3 batches) based on the results obtained using manufacturing methods and analytical techniques detailed above. Table 6a and Table 6b show the parameters and master formula for manufacturing method and specifications of finished product, respectively.

Discussion

Ayurveda is a science of life that employs natural resources to transform them into therapeutically active, bioavailable palatable forms without any adverse effects (*Samskara*). *Bharjana (Agnisannikarsha),* i.e., roasting is one such process which is routinely used for making several recipes[5]. Cow's milk

Table 6a — Parameters and master formula for manufacturing of *Shuddha Gairik*

Parameter	Result
Crude red ochre to powder mass loss (% w/w)	Not more than 0.5
Quantity of red ochre powder (kg)	8.0
Quantity of cow's ghee (kg)	0.8
Time required for roasting (h)	1.5
Temperature during roasting (°C)	140 – 170
Mass gain (% w/w)	2 – 4

processed red ochre has a short shelf-life while that prepared by roasting in cow's ghee can be used for longer period. Moreover, red ochre processed in cow's ghee is extensively used in several Ayurvedic formulations, hence this method was selected in the present study.

In this study, industrial Khoa machine was utilized due to its dual advantage of having a rotating vessel for constant stirring and simultaneously controlled heating arrangement. The process of roasting 8 kg red ochre mixed with 0.8 kg of cow's ghee has been optimized in 60 L capacity vessel though the exact ratio is not given in classical texts[28]. Neither experimental end point has been mentioned in *Rasashastra* texts nor detailed researches have been reported, hence the duration and temperature for the method have also been standardized. Duration for roasting 8 kg of red ochre was around 1.5 h, while observational parameters like generation of fumes, change in color and achieving free flowing nature were finalized to determine the end point. It has been reported experimentally, that heating ghee upto 140 - 150°C does not affect its natural molecular composition and this range can be used for household cooking/frying purposes. Even upto 170°C, it can be used with less deterioration of the ingredients. However, above 180°C, it deteriorates and becomes rancid at 250°C which is its smoking point[29]. Hence, the temperature attained during roasting of red ochre

Table 6b — Parameters and specifications of finished product- *Shuddha Gairik*

Parameter	Specification
Description	Color- dark reddish brown (Equivalent to Pantone 1807 C) Consistency- free flowing, very fine, soft powder Odor- characteristic oily
Loss on drying (% w/w)	0.25 - 0.75
Loss on ignition (% w/w)	16.25 - 18.75
Acid insoluble ash (% w/w)	35 – 50
Bulk density (g/cc)	0.62 - 0.75
Tapped density (g/cc)	0.85 - 1.10
Flow-ability, compressibility (%)	Poor, 25 - 35
Fe content by titration method (% w/w)	15 - 22
Si content by gravimetric method (% w/w)	16 - 22
Oil content (% w/w)	4 – 5
SEM analysis at 1000x	Irregular shaped agglomerates ranging from 5 - 25μm
XRD analysis	Fe_2O_3 and kaolinite
FT-IR analysis (cm^{-1})	3686, 3619, 2920, 2851, 1741, 1465, 1114, 1024, 998, 935, 909, 795, 751, 642
TGA (mass loss) (w/w)	upto 2 % at 250 - 260 °C, upto 8 % at 400 – 450 °C, upto 10% at 700 – 725 °C, upto 19% of total loss
Particle size analysis-DLS (nm)	Effective diameter 900 – 1200 Mean diameter 500 – 800 D10: 220 – 270 D50: 450 – 1000 D90: 900 – 1300
Heavy metal content by ICPOES (μg/day)	Within permissible limits Hg<20, As< 10, Cd< 6, Pb< 10

in the present study was adequate to retain the structural, nutritional and medicinal properties of ghee intact in the finished product.

Further, several analytical tools were employed to enlist the precise properties of the end product. The raw material complying with the specifications of API was selected while the parameters of cow's ghee roasted red ochre are unique for this standardized process (Table 6a and Table 6b). Though red ochre is a clay mineral and ought to show natural variation, the parameters were consistent and within 5% limit of acceptable variation for all the three batches, except for acid insoluble ash content. It was also noted that the initial CRO to cow's ghee proportion of 10:1 changed to approximately 10:0.5 in PRO, probably due to evaporation of ghee during roasting.

The SEM analysis did not show any structural changes in both, pre- and post-roasting stages while the particle size of the agglomerates in both CRO and PRO were in similar range. Likewise, the XRD pattern also did not show any chemical and crystalline phase changes. This is mainly due to the low temperature (*mruduagni*) used for roasting[13]. Interesting observations were noted in FT-IR and thermo-gravimetric analysis. Additional bands on FT-IR analysis in PRO as compared to the raw material represented cow's ghee fats that got mixed with or deposited on the red ochre particles during roasting process as shown in Supplementary data Table S5.

The reported major and minor bands in the FT-MIR (Fourier Transform-Mid Infrared) spectra of cow's and buffalo's ghee are at 3005, 2922, 2853, 1744, 1466, 1418, 1377, 1236, 1161, 1114, 1098, 966, 870 and 721 cm^{-1}. Out of these bands, the strong band centered at 2922 cm^{-1} arises from the asymmetric stretching bands of C–H in CH_2 and terminal CH_3 groups of fatty acid aliphatic chains, whereas, the strong band centered at 2853 cm^{-1} is attributed to the symmetric stretching bands in the same groups. Further, the very strong single band of the C=O stretching of carbonyl groups of the triglycerides is reported at about 1744 cm^{-1} while the medium band at 1466 cm^{-1} is attributed to H–C–H bending (scissoring) of CH_2 and CH_3. The weak band appearing at 965

cm^{-1} is due to C–H bending of *trans* double bonds[29,30]. These five bands viz., 2922, 2853, 1744, 1466 and 966 cm^{-1} of cow's ghee were also recognized in PRO in the present study as shown in Fig. 5 (b, c and d). Thus, the chemical and structural properties of triglycerides in the cow's ghee processed red ochre remained unchanged.

The wavenumbers 3670 and 3645 cm^{-1} indicates Al---O-H stretching and OH stretching present in crystalline kaolinite. Both the bands at 1030 and 1005 cm^{-1} are assigned to Si-O stretching in clay minerals. Band at 918 cm^{-1} represents the OH deformation linked at $2Al^{3+}$ while 788 and 799 cm^{-1} denote Si-O quartz and 642 cm^{-1} as Si-O-Si bending, respectively[31]. In another reported data, the spectra for kaolinite (Al_2O_3•$2SiO_2$•$2H_2O$) are designated by 3685, 3650, 3619, 1114, 1024, 1002, 912, 787, 749, 673, 641 and 525 cm^{-1}[Ref. 32]. Out of these, 3687, 3618, 1114, 1024, 910, 796, 751 and 643 cm^{-1} were found in both, CRO and PRO in the present study which is attributed to the presence of kaolinite. On the other hand, wavenumbers 726, 692 and 629 cm^{-1} are represented by ferric oxide out of which 692 and 629 cm^{-1} were shared by both CRO and PRO in the current study (Supplementary Data Table S5)[32].

The thermo-gravimetric analysis of kaolinite shows four stages viz., dehydration of adsorbed water (85 - 110°C), goethite de-hydroxylation (295 - 350°C), kaolinite de-hydroxylation (500 - 560°C) and kaolinite re-crystallization (930 - 1002°C). A differential mass loss ranging from 6.5 to 19.5% w/w has also been reported[33]. In the present experiment, about 10% total mass loss of the CRO from 500 to 900°C represents kaolinite pattern of thermal behavior. All the three batches of PRO revealed upto 2% w/w mass loss which is attributed to the oxidation of cow's ghee present in PRO at 250 to 260°C due to smoking[29]. The second loss of about 8% w/w in PRO at around 350 to 400°C can be considered due to de-hydroxylation reactions of goethite (α-FeO•OH) resulting into hematite (α-Fe_2O_3)[33]. Though TGA showed a difference of upto 10% w/w, the changes are seen only after 250°C. However, in the present methodology the temperature of PRO was not allowed to cross 170°C, hence there is less possibility of structural changes in kaolinite containing high percentage of iron oxide at this temperature. This highlights that roasting red ochre in cow's ghee does not change the structure of either but is essentially carried out to combine triglycerides with kaolinite containing ferric oxide.

The particle size analysis was performed to check the uniformity of the end product. The acceptable particle size for Ayurvedic metallic and mineral products is such that it should pass completely through mesh #200 (73 μm), but the upper limit or distribution of particles is not mentioned[23]. In the present study, all the processed batches showed uniform particle size distribution ranging from 200 nm (D10 value) to 1100 nm (D90 value), typically representing clay mineral category[34]. Further studies may be conducted to assess the suitability of this particle size in several therapeutic applications.

Presence of heavy metals as expected content in red ochre is due to its natural origin. The permissible limits of heavy metals in natural health products for per day consumption are Arsenic < 10, Cadmium- < 6, Lead- < 10 and Mercury- <20 μg/day[35]. Based on the ICPOES analysis of PRO, the derived contents of heavy metals converted into µg/day intake of the drug were within permissible limits for the maximum therapeutic dose[13]. Hence, *Shuddha Gairik* being a natural health product is safe for oral consumption. Moreover, low and similar heavy metal contents in the crude and processed red ochre support the Good Manufacturing Practice compliance.

Red ochre is a mixture of ferric oxide and silicate of alumina. The Ayurvedic concept of roasting such iron-rich clay mineral with fat can be explained based on need of fatty acids for enhancing iron absorption. Iron absorption studies in suckling and weaning rats by feeding them combination of iron with several types of fats reported that Fe uptake and hemoglobin regeneration were higher in those consuming saturated fat. A positive association was also observed between oleic, linoleic and total unsaturated fatty acids with that of alteration in fatty acid composition in the intestinal brush border membrane. Long-chain fatty acids further increased ferrous iron uptake by this membrane. It purports that formation of fatty-acid iron complexes in the intestinal lumen helps to maintain iron in solution and favors its absorption. Further, it is also suggested that fatty acids could complex with other cations that compete with iron for chelation or transportation, thus reducing the competition with iron for favorable absorption[36]. In another study, short-chain fatty acids like butyrate and propionate were found to enhance iron absorption in proximal colon, irrespective of the valency of iron[37]. Even medium-chain fatty acids enhance iron absorption and increase serum ferritin level[38]. In one of the reported studies, ferrous fumarate sustained-

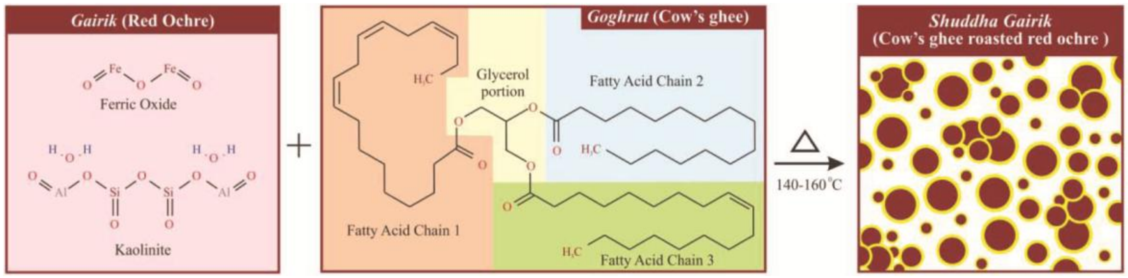

Fig. 7 — Schematic representation of concept of *Gairik Shodhan* in cow's ghee.

release pellets were prepared using cow's ghee as a hot-melt coating agent. The dissolution data indicated that the drug release follows zero order profile indicating success of this step while the pellets remained stable during stability study[39]. Thus, fatty acid iron complexes provide stability and better absorption of iron, affirming the concept of roasting red ochre with cow's ghee.

On the other hand, silicon (Si) is a tetravalent metalloid having low reactivity and high chemical affinity to oxygen. Hydrated Si can bind to organic molecules or form complexes with other inorganic compounds. It mostly occurs in nature as silica or silicate minerals or amorphous bio silica in algae, sponges and crop and horticultural plants. The reported mean uptake or recommended intake of Si varies between 10 and 62 mg/day and is non-toxic, even if consumed in higher doses. Si affects connective tissue, bones, skin, blood vessels, immune and nervous systems. Silica-based micro- and nanoparticles are being extensively explored as novel drug delivery systems[40]. Clay minerals are being extensively used in pharmaceutical and cosmetic industries because of high adsorption capacity, large specific surface area, reactivity toward acids, high heat retention capacity, astringency, non-toxic nature, etc. Kaolinite [$Al_2SiO_5(OH)_4$] is a phyllosilicate used orally as gastrointestinal protector and anti-diarrheal, and topically as anti-inflammatory, dermatological protector and local anesthetic. Kaolinite, by adhering to the gastric and intestinal mucous membrane, diminishes irritation and gastric secretions and take up gases, toxins, bacteria and even viruses. Hence, it acts as a gastrointestinal protector by increasing the viscosity and stability of the gastric mucus while by decreasing the degradation of glycoproteins in the mucus. Moreover, kaolinite remains undissolved and is essentially excreted through feces[34].

Ayurvedic texts and current literature corroborates the need of coating ferric oxide and kaolinite particles with triglycerides so as to facilitate their easy transportation and absorption. The present process of *Shuddha Gairik* has been schematically represented in Fig. 7. The physicochemical characterization in the current study has indicated that coating of ferric oxide and kaolinite with cow's ghee in *Shuddha Gairik* did not alter chemical and structural forms in either of them. Thus, roasting red ochre with cow's ghee can be considered as a profound drug delivery system.

Ayurvedic practitioners recommend iron containing medicines to be consumed with cow's ghee as a vehicle because it is unctuous, laxative and increases appetite, as well as improves digestion[41]. Additionally, combining iron containing medicines with ghee may ameliorate constipation which is a common side effect of iron supplements. Such *Shuddha Gairik* is used individually or as an ingredient in formulations like *Kamdudha Rasa*, which is highly effective in hyperacidity, fever, hemorrhagic disorders, burning sensation, appetite loss, indigestion, vertigo and insanity along with alleviation of radiotherapy- and chemotherapy-induced adverse effects in cancer patients[8,42,43].

Conclusions

The concept of roasting red ochre with ghee highlights an optimum drug delivery system. Industrial batch of 8 kg requires 1.5 h roasting at 140-170°C to get *Shuddha Gairik*. It contains Fe and Si upto 22% and oil upto 5%. It is a red-colored fine powder having agglomerates of irregularly shaped particles with size between 220 – 1300 nm. XRD identified Fe_2O_3 and Kaolinite phases, confirmed by FT-IR along with peaks of cow's ghee. This Standard Operating Procedure gives batch to batch consistency. The monograph will be helpful for quality control as well as to design efficacy studies.

Supplementry Data

Supplementry data associated with this article is available in the electronic form at http://nopr.niscpr.res.in/jinfo/ijtk/IJTK_21(02)(2022)303-316_SupplData.pdf

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Conflicts of Interest

The authors declare no conflict of interest.

Authors' Contributions

The research was conceptualized by SC, VD and SS; formal analysis was done by SC and VG whereas VD and SS were involved in funding acquisition and provided all the resources. Original draft of the manuscript was written by SC; reviewed and edited by VG, VD and SS while all the authors have read and approved the final manuscript.

References

1 Alonso L P, Caves of Altamira, In: *Ancient History Encyclopedia*, https://www.ancient.eu/Altamira/ (2015).

2 Anonymous, Rangoli Art Circular, In: *Directorate of Education, Govt. of NCT of Delhi*, http://www.edudel.nic.in (2007).

3 Meulenbeld G J, Caraka, his identity and date, A History of Indian Medical Literature, In: Groningen Oriental Studies, Volume IA, Part I, Chapter 10, edited by Groningen EF (Groningen, The Netherlands), Proc IInd Workshop of the European Ayurvedic Society, 1999, p. 404– 407.

4 Loukas M, Lanteri A, Ferrauiola J, Tubbs R S, Maharaja G, *et al*., Anatomy in ancient India: A focus on the Sushruta Samhita, *J Anat*, 217 (6) (2010) 646–650. DOI:10.1111/ j.1469-7580.2010.01294.x.

5 Charakacharya, Charak Samhita, Chikitsa Sthanam, 23rd Chapter, 79th Shloka; 26th Chapter, 210th Shloka; 30th Chapter, 91st Shloka; *Vimana Sthanam* 1st Chapter, 21st Shloka, 2006.

6 Sushrutacharya, Sushruta Samhita, Kalpa Sthanam, 3rd Chapter, 51st Shloka; 5th Chapter, 67 & 77th Shloka, 2009.

7 Anonymous, Laghusootshekhar Rasa, In: Rasa Tantra Saar va Siddhaprayogsangrah, Vol 1, (Krishnagopal Ayurved Bhavan, Ajmer, RJ), 1999, p. 546.

8 Pandit H, Kamdudha Vati, In: Rasayogasagar, Vol 1, (Chowkhamba Krishnadas Academy, Varanasi, UP), 2004, p. 260.

9 Desai V G, Bharatiya Rasashastra (The Ancient Chemistry of India), (Raghuvanshi Prakashan, Mumbai, MH), 1928, p. 232-233, 260-263.

10 Anonymous, The Ayurvedic Pharmacopoeia of India, Part 1, Vol 7, (The Controller of Publications, Department of Ayurveda, Yoga- Naturopathy, Unani, Siddha & Homeopathy, Ministry of Health and Family Welfare, Delhi), 2008, p. 6-7.

11 Rasa Vagbhat, Rasa Ratna Samuchchaya, 3rd Chapter, 1st Sholka, 10th Chapter, 81st Shloka, 1996.

12 Trikamji Y, Rasamrutam, 3rd Chapter, 164th Shloka, 1951.

13 Sharma S, Rasa Tarangini, 22nd Chapter, 110th-132nd Shloka, 1979.

14 Shete K, Clinical study of contraceptive activity of Talispatra and Gairik, *Int J Ayur Pharma Res*, 4 (9) (2016) 39-43.

15 Bhatt N & Deshpande M A, Critical review and scientific prospective on contraceptive therapeutics from Ayurveda and allied ancient knowledge, *Front Pharmacol*, 12 (2021) 629591. DOI: 10.3389/fphar.2021.629591.

16 Bhadlikar D, Role of Ayurvedic herbal medicines and ayurvedic therapies in prophylaxis and therapeutic management of SARS CoV-2 (Covid-19) Coronavirus, *Int J Rec Innov Med Clin Res*, 2 (4) (2020) 113-123.

17 Rifkin R F, Dayet L, Queffelec A, Summers B, Lategan M, *et al*., Evaluating the photo-protective effects of ochre on human skin by *in vivo* SPF assessment: Implications for human evolution, adaptation and dispersal, *PLoS One*, 10 (9) (2015) e0136090. DOI:10.1371/journal.pone.0136090.

18 Suman L, Gupta L, Vardhan S, Kapil P & Sharma S, Concept of Shodhana of Gairik in Godugdha, *Int J Ayurveda Pharm Chem*, 8 (3) (2018) 39-43.

19 Pallavi Y C & Nagaraja T N, Standardization of Laghu Suta Shekhara Rasa – An analytical study, *Int Ayurvedic Med J*, 4 (12) (2016) 3534-3539. http://www.iamj.in/posts/images/ upload/3534_3539.pdf

20 Sharma V, Vaghela D B, llarisha C R, Shukla V J & Prajapati P K, Physicochemical evaluation of LaghuSutasekhara Rasa tablets with special reference to Gairik Shodhana, *SLJIM*, 2 (1) (2012) 101 – 146.

21 Palbag S, Singh V K, Singh N K & Gautam D N S, Qualitative evaluation of marketed Ayurvedic red tooth powder by Numbudri phased spot test, *Int Res J Pharm*, 4 (3) (2013) 128-130. DOI: 10.7897/2230-8407.04324.

22 Anonymous, The Ayurvedic Pharmacopoeia of India, Part 1, Vol 6, (The Controller of Publications, Department of Ayurveda, Yoga- Naturopathy, Unani, Siddha & Homeopathy, Ministry of Health and Family Welfare, New Delhi), 2008, p. 204-205.

23 Lohar D R, Protocol for Testing Ayurvedic, Siddha and Unani Medicines, (Department of AYUSH, Ministry of Health and Family Welfare, Pharmacopoeial Laboratory for Indian Medicines, Ghaziabad, UP), 2008, p. 31.

24 Anonymous, Pharmacopoeial Standards for Ayurvedic Formulations, (Central Council for Research in Ayurveda and Siddha, Yugantar Prakashan (P) Ltd., New Delhi), 1987, p. 464-467.

25 Anonymous, Bulk and Tapped Density, In: *United States Pharmacopoeia*, Revision <616> http://www.usp.org/ harmonization-standards/pdg/general-chapters/bulk-density-and-tapped-density-of-powers (2015).

26 Lachman L, Lieberman H A & Kanig J L, Pharmaceutical Dosage Form Design, In: *The Theory and Practice of*

Industrial Pharmacy, (Varghese Publishing House, Mumbai, MH), 1987, p. 184.
27 Anonymous, General Guidelines for Drug Development of Ayurvedic Formulations, http://www.ccras.nic.in/ content/ general-guideline-series, (2018).
28 Vaidya S S, Dole V A, Gairik Shodhan, In: Rasashastra Pratyakshik, (Anmol Prakashan, Pune, MH), 2003, p. 29.
29 Ahmad N & Saleem M, Studying heating effects on desi ghee obtained from buffalo milk using fluorescence spectroscopy, *PLoS One*, 13 (5) (2018) e0197340. https://doi.org/10.1371/journal.pone.0197340.
30 Antony B, Sharma S, Bhavbhuti M, Mehta K, Ratnam K D, *et al*., Study on FT-MIR spectra of ghee (anhydrous milk fat), *Br Food J*, 119 (1) (2017) 181-189. http://dx.doi.org/ 10.1108/BFJ-06-2016-0276.
31 Saikia B J & Parthasarathy G, Fourier transform infrared spectroscopic characterization of kaolinite from Assam and Meghalaya, Northeastern India, *J Mod Phys*, 1 (2010) 206-210. DOI:10.4236/jmp.2010.14031.
32 Vahur S, Teearu A, Peets P, Joosu L & Leito I, ATR-FT-IR spectral collection of conservation materials in the extended region of 4000-80 cm^{-1}, *Anal Bioanal Chem*, 408 (13) (2016) 3373–3379. DOI: 10.1007/s00216-016-9411-5.
33 Diko M, Georges E G & Ogola J, Fourier transform infrared spectroscopy and thermal analyses of kaolinitic clays from South Africa and Cameroon, *Acta Geodyn Geomater*, 13 (2) (182) (2016) 149–158. DOI: 10.13168/AGG.2015.0052.
34 Carretero M I & Pozo M, Clay and non-clay minerals in the pharmaceutical and cosmetic industries Part II Active ingredients, *Appl Clay Sci*, 47 (2010) 171–181. DOI:10.1016/j.clay.2009.10.016.
35 Anonymous, Quality of natural health products guide, Natural Health Products Directorate, Canada, https://www.canada.ca/ content/dam/hc-sc/migration/hc-sc/dhpmps/alt_formats/pdf/consultation/natur/consult_quality-qualite-eng.pdf, (2015).
36 Pabon M L & Lonnerdal B, Effects of type of fat in the diet on iron bioavailability assessed in suckling and weanling rats, *J Trace Elem Med Biol*, 15 (2001) 18-23. http://www.urbanfischer.de/ journals/jtraceelm.
37 Bougle D, Vaghe-Vaezzadeh N, Roland N, Bouvard G, Arhan P, *et al*., Influence of short-chain fatty acids on iron absorption by proximal colon, *Scand J Gastroenterol*, 37 (2002) 1008–1011.
38 Furuse M, Yang S I, Hayashi M, Iwasaki T & Oxumura J, Effect of iron-medium chain fatty acid complexes on iron metabolism in pregnant rats, *Anim Sci Technol (Jpn)*, 63 (9) (1992) 912–916.
39 Sakarkar D M, Dorle A K, Mahajan N M & Sudke S G, Design of sustained release pellets of ferrous fumarate using cow's ghee as hot-melt coating agent, *Int J Pharm Investig*, 3 (3) (2013) 151-156. DOI:10.4103/2230-973X.119225.
40 Gotz W, Tobiasch E, Witzleben S & Schulze M, Effects of silicon compounds on biomineralization, osteogenesis, and hard tissue formation, *Pharmaceutics*, 11 (2019) 117. DOI: 10.3390/ pharmaceutics11030117.
41 Vagbhatacharya, Ashtanga Hridayam, Sutra Sthanam, 5th Chapter, 37th Shloka; 16th Chapter, 1st Shloka, 2007.
42 Deshmukh V, Effectiveness of Ayurvedic treatment in alleviating side-effects of radiotherapy in patients suffering from oropharyngeal cancer and its relationship with improvement in immune status of the host, In: *J Clin Cell Immunol*, 5 (5), Proc IIIrd International Conference and Exhibition on Clinical & Cellular Immunology, 2014.
43 Deshmukh V, Kulkarni A, Bhargava S, Patil T, Ramdasi V, *et al*., Effectiveness of combinations of Ayurvedic drugs in alleviating drug toxicity and improving quality of life of cancer patients treated with chemotherapy, *Support Care Cancer*, 22 (2014) 3007–3015.