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Physico-chemical characterization of *kajjali*, black sulphide of mercury, with respect to the role of sulfur in its formation and structure



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ABSTRACT

Background: *Kajjali* is used as a base for Ayurvedic herbo-mineral medicines. It is a combination of mercury with sulfur in varying proportions. The ratio of sulfur (S) added to mercury (Hg) directly relates to the therapeutic efficacy of the compound.

Objective: To analyze the physico-chemical characteristics of *samaguna gandhaka kajjali* (Hg: S = 1:1) and *shadaguna gandhaka kajjali* (Hg: S = 1:6).

Materials and methods: X-ray diffraction, scanning electron microscopy, X-ray photoelectron spectroscopy (XPS), Fourier transmission infrared spectroscopy, thermo-gravimetry analysis, and atomic absorption spectroscopy were applied to characterize each type of *kajjali*.

Results: It was found that the particle size of the formed *kajjali* compound increases with a decrease in the mercury to sulfur ratio. The presence of excess sulfur does not change the surface oxidation states as revealed by the XPS analysis. No trace of mercury has been found in both *samaguna gandhaka kajjali* (SGK-1) and *shadaguna gandhaka kajjali* (SGK-6), indicating a complete Hg reaction with S.

Conclusion: *Kajjali* simulates nanomaterial of the modern era and possesses therapeutic efficacy as mentioned in classical Ayurveda texts. Complete trituration of mercury and sulfur combination ends up with this *kajjali* formation incorporating the potency of nanotherapeutics.

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1. Introduction

Ayurveda, the “science of life,” practiced for 5000 years has a holistic approach. The *Materia medica* inclusions suggest Ayurveda sources from herbal, metal/mineral, and animal origin [1]. Metals and minerals as such in an elemental form cannot serve the purpose of medicine due to embedded toxicity [2]. Mercury is one such element that primes a particular category of Ayurvedic pharmaceuticals called *Rasaushadhees* (herbo-mineral formulations) [3].

The therapeutic potency of *Rasaushadhees* dominates over herbal formulations [4]. *Kajjali*, a combination of mercury with sulfur constitutes a base for more than 90% of herbo-mineral formulations [5]. The persistent processing as trituration with mercury and sulfur need to be carried out till the classical quote specified criteria is achieved [6,7]. Avoiding the use of elemental mercury usage and combining it with sulfur after necessary processing is the uniqueness of Ayurvedic pharmaceuticals pointing out the advancement of science centuries before. Mercury and sulfur have to undergo a series of potentiation or purification processes before *kajjali* preparation [8]. This *kajjali* preparation starts with mercury trituration of sulfur and the final stage is with the saturation of mercuric pearls with sulfur and no further compounding is seen. Thus, the

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compound potentiation is directly proportional to the trituration time and saturation period [9]. The existing toxicity of mercury after pre-processing is neutralized by sulfur insertion [10]. Depending on the composition of mercury and sulfur, these compounds are termed *samguna* and *sadguna*. The proportion of mercury to sulfur in the same ratio or same weight fraction creates the *samguna kajjali*. The proportion of turning to six times sulfur to mercury gives *shadguna kajjali*. *Kajjali* is a technical term endeavored from this particular type of herbo-mineral preparations which means black as collyrium to the eyes. The *kajjali* in the final stage is black irrespective of the proportion of sulfur added to mercury. The medicine potency always reflects the constituent ratio and the different *kajjali* preparations depending on the mercury to sulfur proportion. Hence, it is advocated to prepare *kajjali* by mixing different ratios of sulfur in mercury.

The Ayurvedic *Rasa Shastra* texts have mentioned various parameters in terms of quality, safety, and efficacy of these metallic preparations. Further, different standard operating procedures (SOP) have been mentioned for therapeutic administration of these metallic preparations, in terms of mode of administration, dose, duration, etc. including their manufacturing process, right from selection of raw materials till the final stage [11]. Many times, some reports have come across regarding the preparations containing these heavy metals (particularly mercury) [12]. There is a dearth of studies pertaining to the effect of sulfur, in terms of structural, chemical, and pharmacological properties and the present study on *kajjali* attempts to elaborately understand the effects of sulfur with respect to the said properties. The authors have examined and studied two different proportions of *kajjali* (*Samaguna and Sadguna*), having different therapeutic properties in Ayurvedic texts and analyzed the same in the light of contemporary analytical tools which have not been reported elsewhere.

2. Materials and methods

2.1. Preparation of *kajjali*

Kajjali was prepared in two variants following a validated SOP according to the scheduled textbook, *Rasatarangini* [13]. In one preparation, an equal quantity of mercury and sulfur (*samaguna gandhaka kajjali*: SGK-1) was taken while in another, sulfur was taken six times in quantity to mercury (*shadguna gandhaka kajjali*: SGK-6). Before the preparation of *kajjali*, purification (*shodhana*) of sulfur [14] (Fig. 1 a), purification (*shodhana*) of cinnabar [15], and extraction of mercury from cinnabar [16] were done by adopting the standard Ayurvedic methodology (Fig. 1b). Raw cinnabar and sulfur were purchased from Khari Bawli market, Delhi. Cow's milk, cow's ghee, fruits of *Citrus medica* L. were purchased from a local market in Varanasi.

2.1.1. Processing of cinnabar (*Hingula shodhana*)

Five hundred grams of raw cinnabar was powdered in a mortar and pestle and levigated (*bhavana*) in the juice of *C. medica* L. The same process was repeated seven times.

2.1.2. Processing of sulfur (*gadghaka shodhana*)

The sulfur processing involved the melting and pouring (*galana/dhalana*) method. Five hundred grams of powdered sulfur was melted in cow's ghee and was poured into a vessel containing 2 L of cow's milk through ghee smeared cloth tied over the vessel. The process was repeated seven times, followed by washing in hot water and drying.

2.1.3. Extraction of mercury from cinnabar (*hingulotha parada*)

Five hundred grams of coarse powder of processed cinnabar was spread over a newspaper and folded to make a ball. The ball was wrapped with 500 gm of vertical-shaped cotton cloth strips and placed over an earthen casserole (*shrava*). It was placed over a steel plate and was ignited by coal. The big earthen casserole (*nada yantra*), was kept an inch above from the steel plate to allow proper ventilation. A wet cloth was kept on the upper outer surface of the big, earthen casserole for cooling. The cooling aids in the condensation of mercury and hence, throughout the process, cooling was kept intact. On termination of the heating process, the arrangement was left to cool down on its own. The condensed particles of mercury were eventually scrapped out, washed with lukewarm water, and finally collected.

2.1.4. Preparation of black sulphide of mercury (*kajjali nirman*)

The SGK-1 was prepared by triturating the cinnabar extracted mercury and processed sulfur till it fulfills the criteria of the *kajjali* preparation. Hundred grams of each mercury and sulfur were used for the *samaguna kajjali* preparation. The second preparation, the SGK-6, was prepared with 30 g mercury and six-time sulfur to this, i.e., 180 g. The final compound SGK-1 was prepared after 68 h of vigorous trituration while it took 52 h in the case of SGK-6. The process was carried out at room temperature. The completion of the process was determined using ancient testing methods like *rekhapurnatava*, *varitaratva*, *nischandra*, and attainment of the black color [17,18] (Fig. 1c and d).

2.2. The examination of attainment signs using classical quoted terminal points

The classically quoted terminal points include *varitaratva* (which will float on water), *rekhapurnatva* (which enters Dalton's line of the fingers when rubbed between), *sukshmatva* (extreme fineness), and *nishchandravam* (loss of metallic luster) (Table 1).

2.3. Characterization

The composition and the structure of both SGK-1 and SGK-6 were analyzed using X-ray diffraction (XRD), scanning electron microscopy (SEM), energy-dispersive X-ray (EDX) spectroscopy, X-ray photoelectron spectroscopy (XPS), Fourier Transform Infrared Spectroscopy (FTIR), Thermo-gravimetry analysis (TGA) / Differential Thermal Analysis (DTA), and Atomic Absorption Spectroscopy (AAS).

For powder XRD, a bench-top powder XRD diffractometer (Miniflex, Rigaku, Japan) operating at 10 kV and 15 mA was used. The pattern was recorded for the angle (2θ) ranging from 5° to 70° at a $0.083^\circ/\text{second}$ scanning rate. Morphological analysis of the formed particles was done using a SEM (EVO 18 Carl- Zeiss, Germany). XPS were obtained on an X-ray photoelectron spectroscope (AMICUS, Kratos Analytical, UK) using monochromated Mg- $K\alpha$ (1253.6eV) as an X-ray source. FTIR spectra of the liquid precursor were recorded on a thermo Nicolet model IS5 instrument (Thermo Fisher Scientific, USA), employing liquid precursor/gel/solid coatings on KBr pellets. No less than 32 scans were conducted for each spectrum. Spectrum range of $400\text{--}4000\text{ cm}^{-1}$ in a 2D format on a thin film and spatial resolution with 4 cm^{-1} was used. TGA was done using a thermogravimetric analyzer (TGA-50 Shimadzu Scientific Instruments, Japan) in a nitrogen atmosphere. The sample was placed in a Pt crucible, and the temperature was varied from 35° to 600°C with a heating rate of $10^\circ\text{C}/\text{min}$. AAS was performed using an atomic absorption spectrometer (AA-6300, Shimadzu Scientific Instruments, Japan).



Fig. 1. a: Purification of sulfur. b: Purification of cinnabar & extraction of mercury from cinnebar. c: Preparation of SGK-1. d: Preparation of SGK-6. e: Ancient testing methods for *kajjali*.

3. Results

Both samples of prepared *kajjali* were analyzed by traditional methods described in classical Ayurvedic texts and by modern analytical techniques. Both SGK-1 and SGK-6 fulfill the classical Ayurvedic criteria for product formation as shown in (Fig. 1e).

Characterization of *kajjali* was carried out using XRD, SEM, EDX, FTIR, TGA/DTA, and AAS techniques.

The powdered XRD patterns of both samples are displayed in Fig. 2. It is a two-phase mixture of β -HgS (cinnabar) and remaining α S (orthorhombic phase) at room temperature. The quantity of α S was found to be higher in SGK-6 as compared to SGK-1. No free



Fig. 1. (continued).

mercury was present. The average particle size of the SGK-6 was found to be 61 nm whereas SGK-1 was around 39 nm with a standard deviation of ± 2 nm. The crystallite sizes calculated from the Scherrer equation were 21 and 53 nm for SGK-1 and SGK-6 respectively. The morphology of the particles of both samples is shown in Table 2 and Fig. 3 and particle size distribution is shown in Fig. 4. XPS spectra analysis is shown in Table 3 and Fig. 5 showing the presence of only Hg and S in the final compound. No change is seen in the surface oxidation state even in the presence of an excess of sulfur in sample SGK-6. The FTIR spectrum of both samples was studied in the

region of 4000 cm to 400 cm and findings are presented in Fig. 6a and b. Details of the functional group present are given in Table 4. EDX analysis showed the percentage contribution of Hg: S in SGK-1 and SGK-6 as 59.40: 40.59 and 18.80: 81.19 respectively. The TG/DTA graphs of the kajjali are shown in Fig. 7a and b. Data related to AAS analysis is given in Table 5 which reveals the presence of heavy metals other than mercury within the acceptable range.

Fig. 8 shows the Hg–S phase diagram. In the Hg–S system, the intermediate phase HgS is formed at ~50 at %S. It exists in three allotropic forms (α HgS, β HgS, and γ HgS) [19]. As mentioned earlier, the compositions of SGK-1 and SGK-6 were taken to be 50 and 83.33 wt% S. Both these samples under equilibrium conditions show a two-phase mixture β -HgS (cinnabar) and remaining α S (sulfur) in orthorhombic phase (Fig. 9) at room temperature. The quantity of α S is predicted to be higher in the case of SGK-6 as expected.

Table 1
Classical Ayurvedic characterization.

Sr. No	Test	Observation	Result	
			SGK-1	SGK-6
1.	Varitara	Floats on water	+ ve	+ ve
2.	Rekhapurnatava	Enters the furrows of the finger	+ ve	+ ve
3.	Susukshma	Reduced particle size	+ ve	+ ve
4.	Nishchandravam	Lusterless	+ ve	+ ve

3.1. Phase identification

The phase identification and crystalline behavior of two samples of kajjali were studied through powder XRD. Fig. 2 shows the XRD

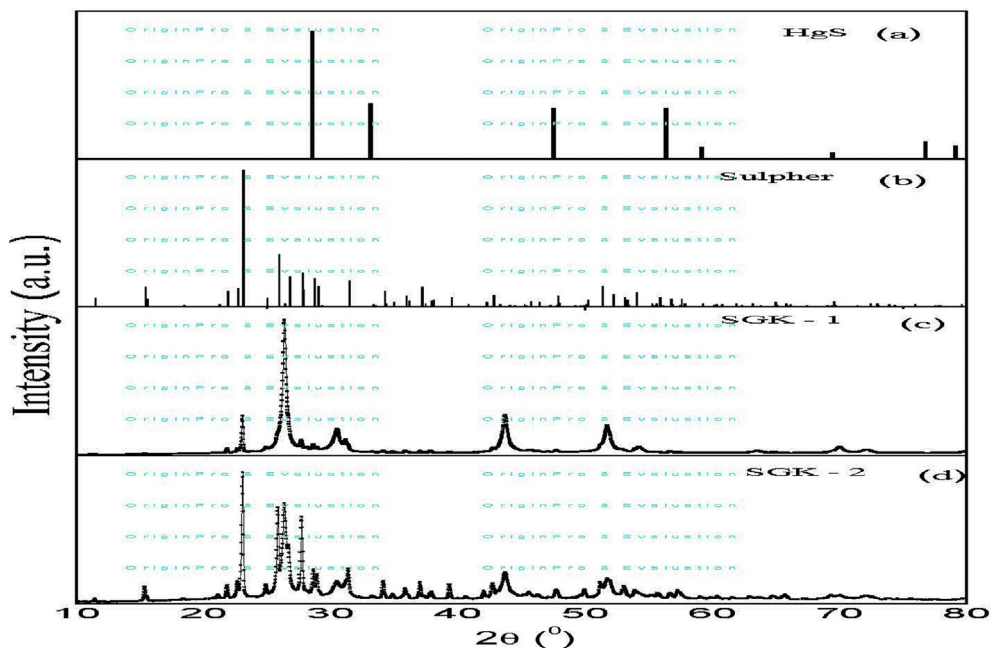


Fig. 2. Powder X-ray diffraction patterns of SGK-1 and SGK-6.

pattern of both the SGK-1 and SGK-6. Fig. 2 also includes the peak position of sulfur and β-HgS which followed up the identification of the peaks in the SGK-1 and SGK-6. It can be seen that Hg reacted well with the available sulfur and formed the compound HgS through the adopted preparation method. There is no trace of mercury that has been found in both SGK-1 and 2, indicating a complete reaction of Hg with S, thus removing the possible toxicity of nascent mercury. Since the available sulfur is much more than the atomically required quantity, the remaining sulfur is also seen in both the XRD patterns. As expected, sulfur’s peaks appear more prominently in SGK-2 because of its mere abundance being six times that of mercury.

The particle size of the samples was calculated using Scherer’s Equation $t = \frac{9\lambda}{\beta \cos \theta}$, where t is the crystal size, λ is the x-ray wavelength, β is the full width at half maximum, and θ is the peak position [20]. For the calculation of crystallite size, the highest intensity peak belonging to β-HgS phase was used. The crystallite size of the SGK-1 and SGK-6 *kajjali* was found to be 21nm and 53 nm, respectively.

3.2. SEM with EDX analysis

The particle sizes and morphology have been analyzed using the SEM, as shown in Fig. 3. The shown micrographs are at the same magnification. The particle size of the SGK-1 was less than that of

the SGK-6. A comprehensive size analysis was done using these micrographs with the help of ImageJ® software [21]. Particle size distribution graph analysis shows that the average particle of SGK-1 is around 39 nm, with a standard deviation of ± 2 nm (Fig. 4). The size calculated using SEM is slightly larger. This is normal behavior as the size obtained using an XRD pattern gives us the crystallite size, whereas the SEM gives us the secondary particle size. The average particle size of the SGK-6 is 61 nm with a standard deviation of ± 2 nm. This is in tune with the particle size obtained using the XRD pattern, where it was 21 nm and 53 nm for SGK-1 and SGK-6 respectively.. These results are summarized in Table 2.

A complete analysis of the elemental composition of *kajjali* was done by SEM-EDX (see Table 2). EDX analysis, the percentage contribution of Hg and S in SGK-1 and SGK-6 was 59.40: 40.59 and 18.80: 81.19 concludes that the presence of two elements, i.e., Hg and S. From the EDX analysis, the atomic ratio of Hg to S is 1:1 and 1:6. by wt.%.

3.3. XPS analysis

It may be possible that the better efficacy of SGK-6 comes from the charge state of the Hg and S in these compounds. To get a clue about these elements’ charge states, we performed XPS of both SGK-1 and SGK-6. Fig. 5 shows the x-ray photo spectra for both Hg and S. The samples show the presence of both mercury and sulfur.

Table 2
Morphology of particles in SEM analysis.

Test drug	Morphology	Mineral/Compound Name (x-ray analysis)	Crystal structure	Average Crystallite Size
SGK-1 (Fig. 4a)	Rounded, Oval, Cubic, Circular	Metacinnabar Mercury sulfide Cinnabar Sulphur	Cubic Cubic Hexagonal Orthorhombic	21 nm
SGK-6 (Fig. 4b)	Rounded, Oval, Cubic, Circular	Meta cinnabar Cinnabar Mercury Manganese Sulphide Sulphur	Cubic Hexagonal Cubic Orthorhombic	53 nm

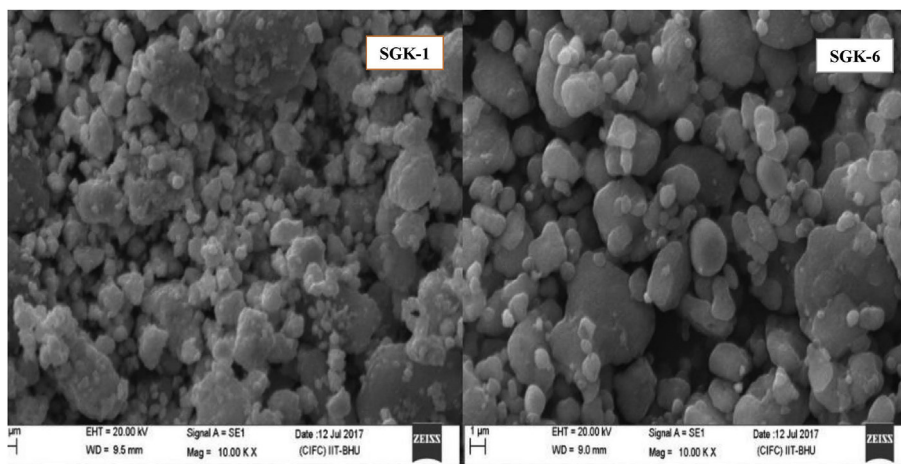


Fig. 3. SEM photomicrograph.

High-resolution spectra at the Hg core level showed the presence of the peaks at 100.49 eV, 100.46 eV for SGK-1 and 101.19 eV, 105.19 eV for SGK-6, corresponding to Hg (4f) (Table 3) [22].

Similarly, the sulfur peaks are shown at 163.39 eV, 163.93 eV in SGK-1 and at 164.36, 164.44 for SGK-6. Thiols are a group of hydrocarbon organic components with sulfur containing a sulfohydryl group and are important antioxidant components. It is a reduced form of sulfur as in a mercaptan [23]. These values are well-matched with the reported data of binding energies of HgS [24] and confirm the formation of HgS [25] and list all the values and the corresponding association of these compounds' XPS peaks.

3.4. FTIR spectra

To identify the functional group and organic ligands [26], both samples of *kajjali* were analyzed using FTIR spectroscopy. FTIR spectra were observed in the region of 3442.54 to 617.06 cm^{-1} (Fig. 6a and b). A total of 3 peaks in SGK-1 and seven peaks in SGK-6 were observed, including two peaks in the hydrogen stretching region (4000–2700 cm^{-1}) in SGK-1, SGK-6. One and four peaks were found in the fingerprint region (1500–700 cm^{-1}) in SGK-1 and SGK-6, respectively. Strong and sharp peaks obtained at 3442.54, 2920.86 cm^{-1} indicate stretching vibrations between O–H

bonds and represent the presence of alcohols, phenols, secondary amines, and primary amines functional group. A peak observed an aliphatic amine functional group at 1077.96, 1020.44 cm^{-1} , which is raised due to C–N stretching vibrations. Still, the appearance of the peak is not much clear and represents either primary or secondary amines. C=O stretching vibrations observed at 1743.25 are assigned to alpha-beta-unsaturated aldehydes. C–Cl or C–Br stretching vibrations observed at 748.78 are assigned to alkyl halides. The FTIR spectrum was measured from the reference spectrum library. Their details and functions have been summarized in Table 4.

3.5. Thermogravimetric analysis (TGA)

TGA is useful in characterizing a compound's thermodynamics, especially the sublimation enthalpy, which is a measure of its volatility and is typically measured by a method of TGA [27].

The TGA technique helps in analyzing the difference between the thermal degradation pattern and stability profile. The TGA curves display three stages of decomposition. There are two temperatures in the reaction, T_i (initial temp.) representing the stage where decomposition starts while T_f (final temp.) representing the final temperature at which the process has been completed. There

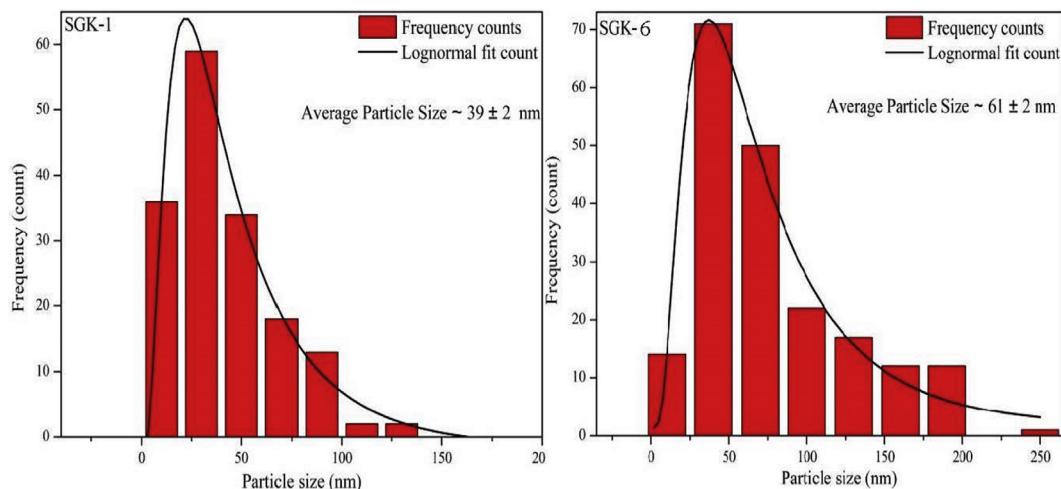


Fig. 4. Particle size distribution graph.

Table 3
Peak positions in XPS spectra of SGK-1 and SGK-6.

Test drug	Peak Positions in XPS spectra	No.of Peaks	XPS Spectra Core level group	Peaks	
				Binding Energy (eV)	Intensity (%)
SGK-1	Carbon peak positions	1	C1s	285.357	39185.3
	Mercury Peak positions	2	Hg 4f	100.49	24929.77
			Hg4f	100.46	26418.2
			S2p	163.39	19230.6
	Sulphur Peak positions	2	S2p	163.93	19267.9
S2p			163.93	19267.9	
SGK-6	Carbon peak positions	1	C1s	285.61	37311.7
	Mercury Peak positions	2	Hg 4f	101.19	11259.8
			Hg4f	105.19	9621.9
			S2p	164.36	12186.5
	Sulphur Peak positions	2	S2p	164.36	12186.5

are three distinct transitions/weight loss associated with the temperature range of room temperature to 500 °C. The first loss appears at ~120 °C, which is generally related to the loss of moisture. The second loss at 300 °C has been assigned to the sublimation of sulfur, and the third and final loss has been assigned to the sublimation of HgS, which occurs at 440 °C. The TGA/DTA curve is shown in Fig. 7a and b. For SGK-1, the loss of unreacted sulfur has turned out to be 45.93%, and the remaining 54.07% loss was for HgS.

Similarly, for SGK-6 the loss associated with unreacted sulfur has been found to 80.53% in the expected line due to 6 times more sulfur presence. The loss related to HgS sublimation has been found to 19.65%. The TGA/DTA analysis shows that if the focus of the

medicine is on the HgS, then these samples must be treated at 300 °C for a few minutes to remove S and keep the HgS intact.

3.6. Atomic absorption spectrometry (AAS)

Heavy metals like Pb, Cd, and trace metals like Zn were determined using flame AAS, and heavy metals such as As were determined by the hydride generation technique (cold vapor AAS). Their concentration in the *kajjali* was found to be within the limit [28] (Table 5).

Another heavy metal, Hg, was found to be in abundance in the formulation as the formulation's chief ingredient. But, the present

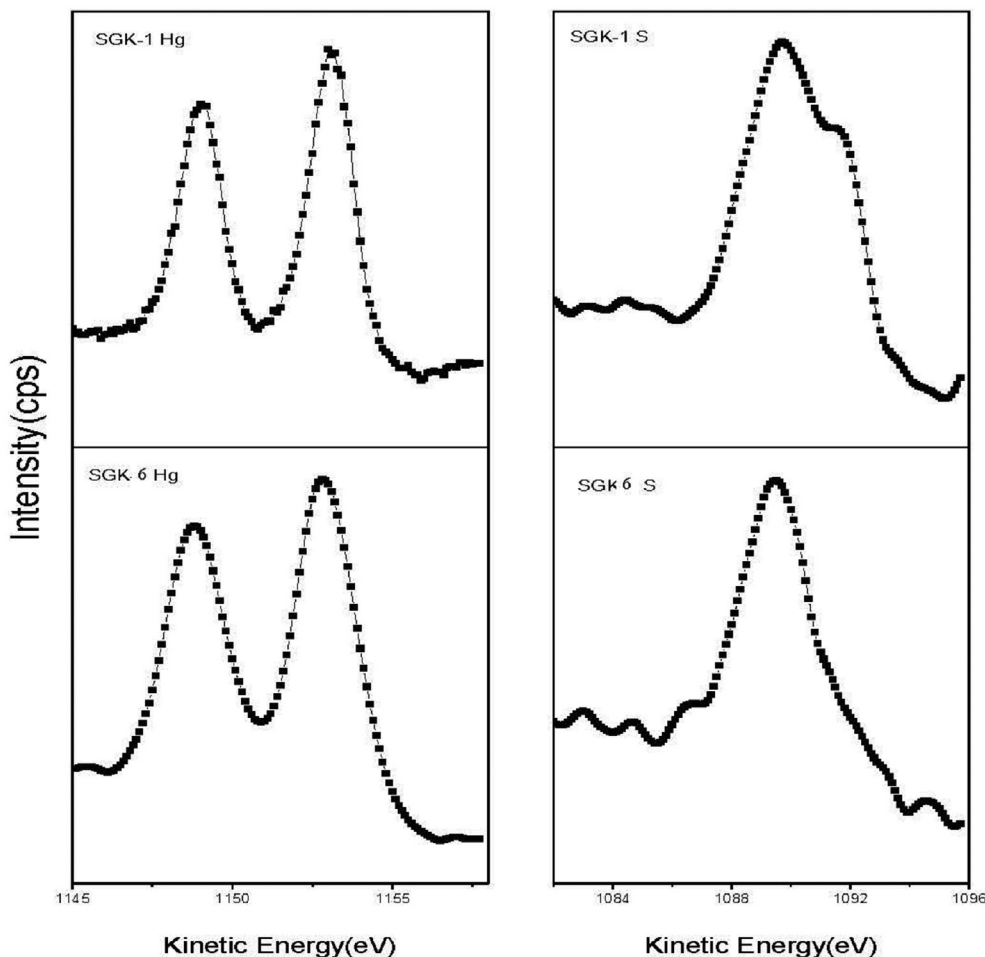


Fig. 5. XPS: (S-p1/2 has higher energy).

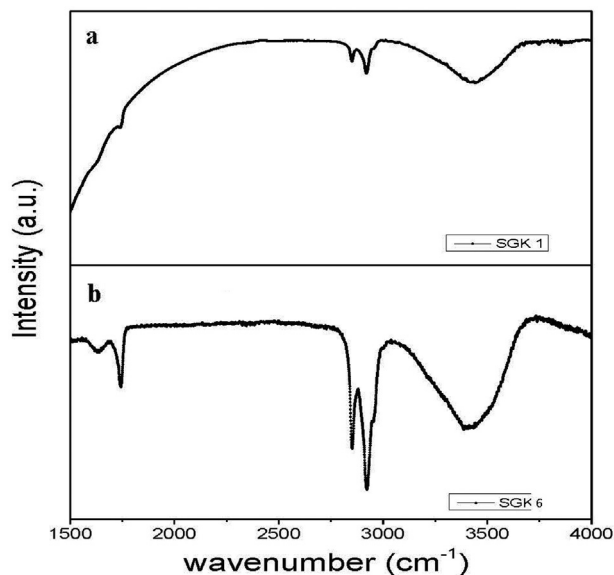


Fig. 6. a and b: FTIR Graph of SGK-1 and SGK-6.

study explains the formulation's safety despite the excess concentration of mercury in it.

4. Discussion

The prepared *kajjali* were subjected to the aforementioned classical end-points such as *varitaratva*, *rekhapurnatava*, *susukshma*, and *nishchandratvam*. These parameters quantify the physical nature of the formulation redirecting to the drug dissolution and drug absorption particulars. *Varitaratva* specifies the ability of the formulation to retain specific gravity less than to the water and also mentions intermolecular forces among the particles of the drug. The intermolecular forces are influenced by the density of the particles and this indirectly relates to the crystal form and phases which got revealed by the XRD pattern of the formulation [29]. *Rekhapurnatva* indicates fineness and the fine particles influence the free movement of the particle and facilitates absorption, assimilation to the body system [30]. The SEM technique reveals the desired specification of fineness or *rekhapurnatva*. The technique termed *nishchandratvam* is most valuable to know the binding of particles in *kajjali*. Spectroscopic methods like FTIR, TGA, XPS, and AAS play a significant role in studying binding affinity, binding ratio, and binding mechanisms [31].

Heavy metals such as arsenic, cadmium, lead, mercury, and nickel are scarcely used in Western medicine due to their toxicity [32]. On the contrary, the Indian system of medicine has used these metals and minerals in abundance for a long time. These herbo-mineral combinations are given paramount importance in

treatment practices, where metals and minerals are used in the form of *rasaushadhees* (herbo-mineral preparations). *Kajjali* is one such medicine that is an ingredient of these herbo-mineral preparations. It has been reported that nanoparticle geometry can significantly impact the interactions with biological targets, thereby bringing about notable differences in formulation efficacy [33].

It is evident from particle size analysis that particle size increased on trituration with an increased sulfur ratio in mercury. Due to less quantity of sulfur in the case of SGK-1, the available material for growth is less and hence, the growth rate is slow and particle size of SGK-1 is less in comparison with SGK-6 where sulfur is six-time more in comparison with SGK-6. The solubility of the drug is intrinsically related to the particle size, and the smaller the particle size better is the absorption [34,35]. However, it seems that below a particle size, the drug's solubility decreases [36]. It will not be out of place to mention that the solubility is optimal in a particular size range and is a matter of investigation. As mentioned in ancient Ayurvedic texts, there is a supremacy of SGK-6 over SGK-1 [37]. It seems that the SGK-1 size is below the optimum limit of the size for better cellular uptake. In contrast, the size range in SGK-6 lies in the optimal size range for better drug efficacy. Some studies showed that particle size significantly affects the nanotherapeutics biodistribution and toxicity but does not support the conclusion that smaller particles are better for clinical application [38]. In clinical trials, it has been confirmed that the use of medicines manufactured from SGK-6 has been found to benefit more than SGK-1 [9].

Nano-metals have attracted increasing interest for their potentiality in the treatment of many diseases [39]. It is worth mentioning here that the particle size of *kajjali* in both variants lies within the range of 100 nm. In the SEM study, particles being in agglomerated form shows larger size when calculated by using ImageJ software. However, by the DebyeScherrer equation using XRD, the crystallite size was found to be within nano ranges (SGK-1 - 21 nm and SGK-6 -53 nm). All the prevailing data including the therapeutic potency thus presumes *rasaushadhis* are to be understood as an ancient version of nanomedicine.

The analysis of XPS reveals no changes in the surface oxidation states of the SGK-6 due to the excess presence of sulfur. This rules out the role of any change in the charge state of these compounds in determining the efficacy of SGK-6 thus, strengthening our hypothesis of the particle size's optimal size for better efficiency. Sulfur XPS peak of SGK-1 and SGK-6 difference is due to a higher concentration of unreacted free sulfur in the case of SGK-6.

The processing before final trituration to *kajjali*, cinnabar, and sulfur received was with organic compounds like lemon juice, milk, *goghrita*, etc. making them homologous to the body tissues. Organic quantity cannot be exactly determined in this case due to the *kajjali* preparation procedure used. However, the water-soluble ash test has revealed that approximately 2.4 wt.% materials in the *kajjali* samples. The presence of these functional groups, adds to the

Table 4
Functional group present in SGK-1 and SGK-6.

S. No	Peak	Actual Peak	Bond	Functional group	Appearance
1.	3500–3200	3442.54	O–H	Alcohols, Phenols, Secondary amines, Primary amines	Short and broad
2.	3300–2500	2920.86	O–H	Carboxylic acids, Alkyl	Medium to strong
3.	2850	2852.10	C–H	Alkyl	Medium to strong
4.	1710–1665	1743.25	C=O	Alpha, beta–Unsaturated Aldehydes, ketones	Strong
5.	1250–1020	1077.96 1020.44	C–N	Aliphatic amines	Often overlapped
6.	1000–650	617.06	N–H	Primary Amines, Secondary Amines	Short and broad
7.	850–550	748.78	C–Cl or C–Br	alkyl halides	Medium

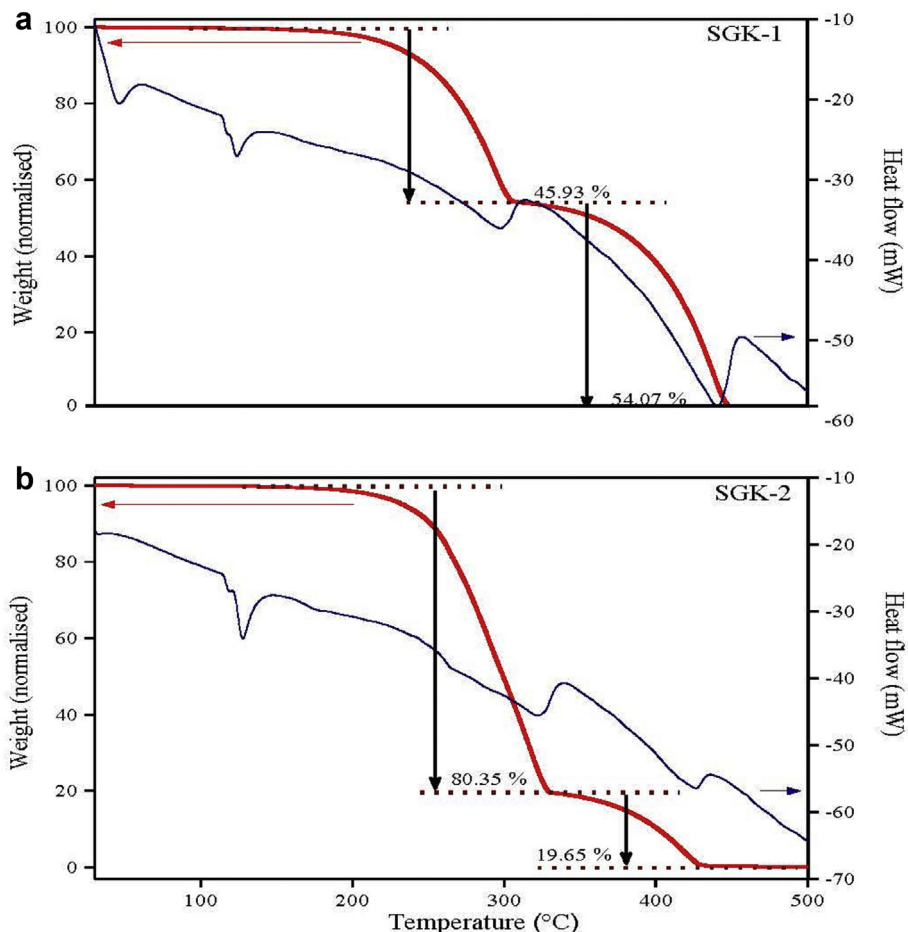


Fig. 7. a and b: Thermogravimetric Analysis (TGA).

therapeutic potential of metals and minerals used in Ayurveda. Moreover, their presence makes difference in these metals and minerals' characteristic features, as understood by modern analytical tools. The safety aspect and efficacy seem to be interrelated in any drug aspect. Both SGK-1 and SGK-6 were devoid of free

mercury. Heavy metals other than mercury were found to be within range. Excess of mercury is obvious, being a compound of mercury itself and it was in the form of HgS. Several studies have been done in which similar traditional pharmaceutical studies in the processing of *hingula* or *gandhaka* are done and found to be safe

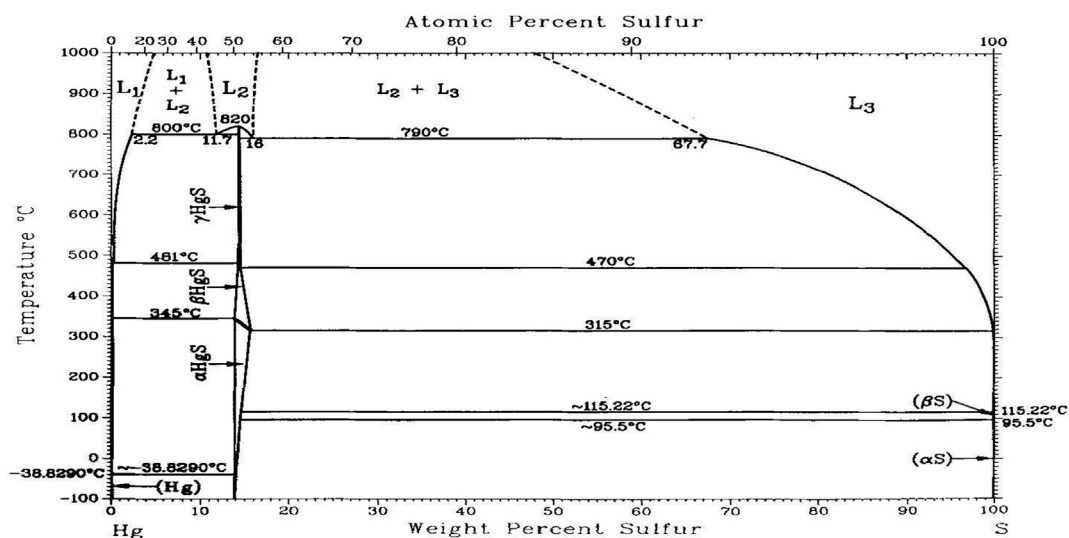


Fig. 8. Hg–S phase diagram [19].

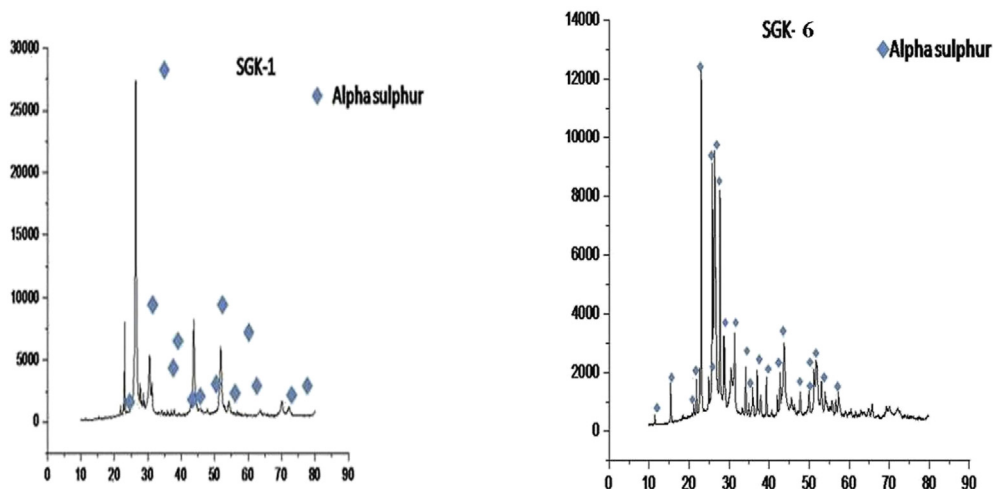


Fig. 9. Alpha sulfur of SGK-1 and SGK-6.

[40,41]. The compound was also tested in a clinical study in the form of *kana kajjali* which shows the role of *gandhaka* in the enhancement of therapeutic efficacy of the *kajjali* with no adverse effects reported or observed during the entire study period. Moreover, an *in vitro* toxicity study in the bovine shrimp model and osmotic fragility test was done on *kajjali*, which reported it to be safe [41]. However, more advanced studies in terms of safety studies in mammalian animal models like acute, subacute, and chronic toxicity; carcinogenicity; reproductive and developmental toxicity; mutagenicity; and biopharmaceutical studies like stability study, bioavailability study, and pharmacokinetics study are still required to come to any conclusion.

Rasashastra texts signify utmost importance for quantity/ratio of sulfur to mercury in *kajjali* preparation for desired therapeutic effect. It is mentioned that more the quantity of sulfur to mercury, more potent is the final product i.e., *shadaguna kajjali* > *samaguna kajjali*. *Samaguna kajjali* is supposed to be 100 times more potent than purified mercury (*shodhita parada*) [42] and cures only the common ailments [43] while *shadaguna kajjali* cures all types of diseases (*sarvarogahara*) [44] and brings extraordinary power [45]. Based on the present study, it has been found that based on traditional tests and physico-chemical characterization, both variants of *kajjali* are more or less the same. XRD, TGA, SEM analysis give a picture of the formation of stable compound HgS, despite the presence of an excess of sulfur. The only difference found in the presence of α -S which was found to be higher in SGK-6. α -S crystals have been studied for their significant potential for increasing the activity of any particle through surface modification and nanoscaling. α -S crystals have been found to generate hydroxyl (OH radicals) group [46]. Hydroxyl radicals are capable of reducing disulfide bonds in proteins. Biologically reactive hydroxyl radical causes hydroxylation of various biomolecules of unsaturated bonds found in their structures. This reductive principle itself makes the compound effective scavengers of hydroxyl radicals and thus exert an antioxidant effect [47]. This might be

the reason why *shadguna kajjali* is claimed to be more therapeutically potent than *samaguna kajjali*. Moreover, excess of sulfur may further enhance the anti-oxidant potential of the former compound, being plasma thiols (S-containing compounds) having an anti-oxidant effect [48]. The sulfur-containing amino acids including methionine, cysteine, cystine, homocysteine, homocystine, and taurine play critical roles in protein synthesis, structure, and function with their additional anti-oxidant activity [49,50].

5. Conclusion

From the present study, it may be concluded that *kajjali* in its independent form is scarcely used as a therapeutic agent. It is always used in combination as an ingredient with other inclusions in a formulation. No trace of mercury has been found in both SGK1 and 2, indicating a complete Hg reaction with S, thus removing the possible toxicity of nascent mercury. The average particle size of SGK-1 and SGK-6 was found to be 39 nm and 61 nm, respectively. It is worth mentioning here that the particle size of *kajjali* in both variants lies within the range of 100 nm. It may also be concluded that the size reduction is required to an optimum level, as evident in SGK-6 where particle size was more due to the presence of an excess of sulfur. The presence of excess sulfur does not change the surface oxidation states, as evident in the case of the SGK-6. This rules out the role of any change in the charge state of these compounds in determining compound efficacy. In SGK-6, more functional groups such as alcohols, phenols, secondary amines, and primary amines functional groups were found. The presence of these functional groups adds to the therapeutic potential of metals and minerals used in Ayurveda. The TGA/DTA analysis shows that if the medicine's focus is on the HgS, then these samples must be treated at 300 °C for a few minutes to remove S and keep the HgS intact.

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Conflict of interest

None.

Table 5 Heavy metal analysis.

Sample code	Pb (ppm)	Cd (ppm)	As (ppm)	Zn (ppm)
SGK-1	0.6884	0.0024	0.006	0.1995
SGK-6	0.4580	0.0026	0.007	0.3812

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jaim.2021.05.006>.

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