Research Article

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PHYSICOCHEMICAL CHARACTERISATION OF SUVARNA BHASMA AND SUVARNA PRASHA USING ADVANCED TECHNIQUES

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ABSTRACT

Background: Suvarna Prashana is an ancient Indian practice that boosts immunity, intelligence, memory, complexion, and virility and prevents infections in children. However, the characterisation of the drug is very limited. Material and Method: In this study, the physicochemical characterisation of Suvarna Bhasma is done with Scanning Electron Microscopy (SEM), Transmission Electron Microscopy (TEM), Energy Dispersive Xray Analysis (EDAX), X-ray Diffraction (XRD), X-ray Photoelectron Spectroscopy (XPS), and Suvarna Prash containing 15 mg of Suvarna Bhasma mixed with 0.25 ml of ghee and 0.75 ml of honey is characterised with Fourier Transform Infrared Spectroscopy (FTIR) with Suvarna Bhasma. Results: SEM revealed that gold particles of Suvarna Bhasma ranged from 27 nm - 8.1 µm and formed spherical aggregates of varied sizes with rough surfaces. EDAX suggested that Suvarna Bhasma contained Gold (86.3%), Oxygen (3.14%), and Carbon (10.52%). TEM reported that the particle size ranged from 2.2 to 18.9 nm, which existed in polycrystalline form as suggested by XRD. XPS confirmed the elemental state of gold Au(0), Silver-Ag $^{+}$oxidation state, Carbon C-C, C=O, and C-N, where carbon is bonding with carbon, oxygen and nitrogen. FTIR revealed that Suvarna Bhasma alone had a single functional group of alkynes. When Suvarna Bhasma was triturated with honey and ghee, many functional groups were added to Suvarna Bhasma, i.e. fats, carbohydrates and amino acids. Conclusion: In this novel study, FTIR of Suvarna Prasha is reported for the first time, and it can be used as a fingerprint for drug identification. Also, it proves that Suvarna Bhasma, used in the current study, is a pure form of gold without heavy metals like mercury.

Keywords: Suvarna Bhasma, Incinerated Gold Nanoparticles (InGNP), Ghee, Honey.

INTRODUCTION

Suvarna Bhasma plays a vital role in the clinical practice of Ayurveda as a wonder drug that can be therapeutically prescribed for multiple diseases. As per the Rasatarangini 15th chapter [1], it can be given in Jvara (fever), Pandu (anemia), Shawasa (respiratory disorders), Rajayakshma (tuberculosis), Garbhashaya and Rakta shodhana (purifies the uterus and blood), Unmada (schizophrenia), Apasmara (seizure), to improve medha (intelligence), garbhadharana (retention of fetus during pregnancy), stanya samvardhana (breast milk production), visha (poisoning), vrakkaroga, Asti (kidney disorders), shotha (edema) in children, garbhashaya shotha (uterine inflammation), Hridroga (heart diseases), in combination with various other drugs. As per Acharya Kashyapa, when Suvarna is given with ghee (butter oil) and honey for one month, the child becomes parama medhavi (very intelligent) and will not get any disease, and when given for six months, he becomes shruta dhara (lifetime memory after hearing just once) it improves longevity, virility, complexion, kills grahas (microorganisms of present context).[2,3]

Other Acharyas of Ayurveda have also given similar Suvarna yogas to be given in children with other herbal drugs. Suvarna Prasha has gained popularity due to its disease prevention, health promotion, memory, and ability to enhance intelligence in pediatric cases [2,4,5]. As its popularity has increased, it has become the need of the hour to produce evidence of drug composition in today's scientific era using modern technology. In this study, the Suvarna Bhasma was characterised through SEM (Scanning Electron Microscopy), TEM (Transition Electron Microscopy), XRD (X-ray Diffraction), EDAX (Energy Dispersed X-ray Analysis), XPS (X (tuberculosis), ray photo Electron Spectroscopy), FTIR (Fourier Transform Infrared Spectroscopy). The Suvarna Prasha containing 15 mg of Suvarna bhasma mixed with ghee (0.25 ml) and honey (0.75 ml) was characterized through FTIR.

A detailed description of the method of preparation and the tests for checking the completion of the Bhasmas are well explained in ancient Ayurvedic literature. It mainly explains visual organoleptic parameters like varitara (property of floating on water), nishchandratva (having no shining particles in bhasma), niswadu (having no taste), apunarbhava (from which the original metal cant be regained), dantagrakachkachabhava (produce kach kach sound while chewing), etc. All these parameters have their importance and limitations. Analytical study of the drug helps to understand the pharmacokinetics and pharmacodynamics of the same.

MATERIALS AND METHODS

Ethical clearance: The characterisation of Suvarna Bhasma (InAuPs) was initiated after approval from the Institute Ethical

Committee. E.C.R./526/Inst/UP/2014/RR-20/dated 19/05/2020 via letter no. I.E.C. No.Dean/2021/E.C./2164 dated 24/09/2020.

Experimental Drug

Suvarna Bhasma (incinerated gold) (Batch No: SB00279) was purchased from the local market, Sehwal cow ghee (Indian cow breed) was prepared, and honey was purchased from the local market DIL, India, Lot No: BM4062. Suvarna Bhasma was characterised by SEM, TEM, XRD, EDAX, XPS, and FTIR. Suvarna Prasha, which contains ghee and honey, and Suvarna Bhasma were characterised by FTIR. FTIR of Suvarna Prasha was done for the first time compared to previous studies, and it can be used as a fingerprint for Suvarna Prasha.

SEM (Scanning Electron Microscopy)

SEM analysis of Suvarna Bhasma was carried out by Carl Zeiss, EVO-18 research model, Germany from the Department of Geology, BHU, Varanasi, Uttar Pradesh, India, to know the particle size, shape, and distribution of the drug. Suvarna Bhasma powder was fixed with double-sided carbon tape coated with palladium and gold alloy and subjected to SEM analysis.

TEM (Transition Electron Microscopy)

Tecnai G2 20 TWIN, FEI Company of USA (S.E.A.) PTE, Ltd from Central Instrumentation Facility, IIT, BHU, was used for the TEM imaging of Suvarna Bhasma to determine the microparticle size and nature of the drug. The sample was mixed in ethanol and sonicated for 3 hours to get the dispersed view of the particles over the copper grid. ImageJ and Origin 8.5 software were used to plot the histogram of the particles.

XRD (X-ray Diffraction)

Benchtop Powder X-ray diffraction, Rigaku, Miniflex 600, Tokyo from School of Material Science and Technology, IIT, BHU, was used for the XRD analysis of Suvarna Bhasma analysis to determine the elemental composition, average particle size, and crystalline structure.

100 mg of Suvarna Bhasma was placed into a sample holder and smeared uniformly onto a glass slide, ensuring a flat upper surface packed into a sample container and sprinkled on double sticky tape. Care was taken to make a flat upper surface and to get a random distribution of lattice orientations to create an oriented smear. Origin 8.5 software was used for raw data analysis of the XRD sample from which the peaks were procured, and from the peaks 2θ and FWHM values were obtained, which were used for average size calculation through XRD Crystallite (grain) Size Calculator (Scherrer Equation) software.

EDAX (Energy Dispersed X-ray Analysis)

The sample was analysed along with the SEM, and the sample preparation was the same as the SEM setup in the built EDX facility. EADX, AMETEK, USA, installed at the Department of Geology, BHU, Varanasi, was used for quantitative elemental drug analysis. Suvarna Bhasma powder was fixed with double-sided carbon tape and coated with palladium and gold.

XPS analysis of Suvarna Bhasma

X-ray Photoelectron Spectroscopy (XPS) from Thermo Fisher Scientific from Central Instrumentation Facility, IIT, BHU was used for the XPS analysis of Suvarna Bhasma for quantitative analysis of the elemental composition, chemical state, empirical formula, and electronic state of the elements in the sample. Comprehensive scans were acquired in the binding energy (BE) range 1200 – 0eV using a 500eV pass energy, while the core lines were acquired at 150 eV pass energy to increase the energy resolution.

For this, a thin layer of Suvarna Bhasma was made on a glass slide of 1x1 cm in length and breadth and subjected to spectroscopy.

Fourier Transform Infrared Spectroscopy (FTIR)

Fourier Transform Infrared Spectroscopy (FTIR), Model: Nicolet iS5, Company: THERMO Electron Scientific Instruments LLC from Central Instrumentation Facility, IIT, BHU was used for the FTIR analysis of Suvarna Bhasma and Suvarna Bhasma mixed with ghee and honey.

OBSERVATION AND RESULTS

Scanning Electron Microscopy (SEM) of Suvarna Bhasma

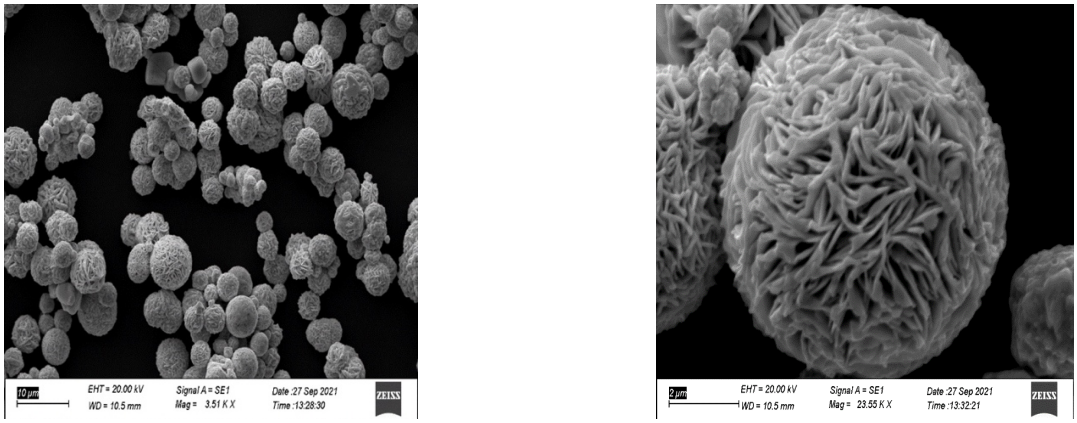

Figure 1: SEM images of Suvarna Bhasma showing (A) Heterogenous distribution of aggregated spherical gold nanoparticles (28 nm - 8.1 µm) (B) Rough spherical surface of Suvarna Bhasma particles (Incinerated Gold Nanoparticles)

As evident from the images, it was seen that Suvarna Bhasma contained agglomerated particles of gold forming spherical ball-like structures with particles ranging from 28 nm - 8.1 µm. The particles were heterogeneously distributed in the sample with rough surfaces.

TEM (Transition Electron Microscopy) of Suvarna bhasma [6]

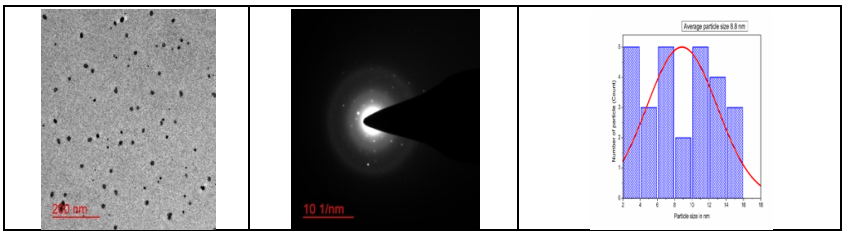

Figure 2: TEM images showing (A) The round shape of the gold nanoparticle ranging from 2 to 16 nm in heterogeneous distribution, (B) SEAD mode showing the polycrystalline nature of the incinerated gold nanoparticles, (C) Histogram Showing the heterogenous distribution of particle with an average particle size of 8.8 nm.

Table 1: Percentage of Different Particles ranging from 2 nm-16 nm present in the single field of TEM image

Size range (nm)	Number of Particles	Percentage
0-2	0	0
2-4	5	18.5
4-6	3	11.1
6-8	5	18.5
8-10	2	7.4
10-12	5	18.5
12-14	4	14.8
14-16	3	11.1

The results showed that the incinerated gold nanoparticles' size ranged from 2 nm - to 16 nm, and they were polycrystalline, as seen in the image as white particles illuminating in rings in selected area diffraction (SAED) mode of TEM. Histogram showing the heterogenous distribution of particles with an average particle size of 8.8 nm. Particles ranging from 2-4 nm, 6-8 nm, 10-12 nm existed in 18.5 %, particles from 12-14 nm existed in 14.8%, particles ranging from 4-6 nm, 14-16 nm existed in 11.1%, and particles ranging from 8-10% existed in 7.4%.

XRD of Suvarna Bhasma

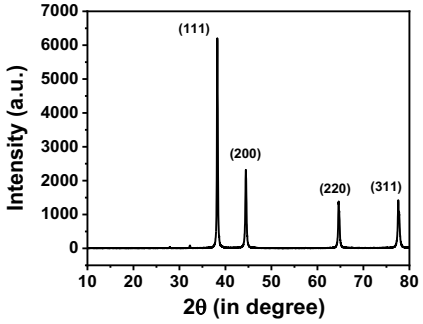

Figure 3: XRD of Suvarna Bhasma (InAuPs) showing peaks at (111), (200), (220), (311) representing pure gold

XRD analysis showed the crystalline nature of nanoparticles. The respective diffraction peaks at 38.25°, 44.4°, 64.65° and 77.58°, relating to (111), (200), (220), and (311) facets of the face-centred cubic (F.C.C.) crystal lattice is related to pure gold (JCPDS card no 04-0784). The average size of the crystal of gold particles in Suvarna Bhasma (InAuPs) was calculated by the Scherrer equation: $d = K\lambda/\beta\cos\theta$, where K is the shape factor between 0.89 and 1.1 ($CuK\alpha = 0.1542Å$), β is the full-width half-maximum of the prominent line (111) in radians, and θ is the position (38.25°) of that line in the pattern. (Figure 2 D) The average size of the crystal particle was found to be 27.58 nm.

EDAX of Suvarna Bhasma

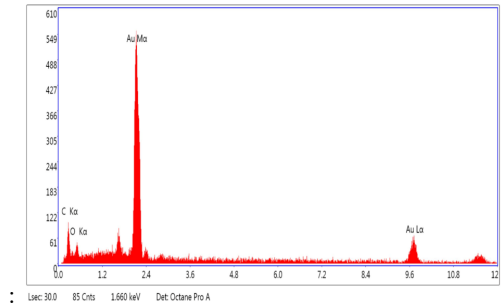

Figure 4: EDAX graph of Suvarna Bhasma (InAuPs) showing peaks of Gold, Oxygen, and Carbon elements.

EDAX report shows the presence of Gold (86.3%), Oxygen (3.14%), and Carbon (10.52%) in the sample of Suvarna Bhasma.

X-ray Photoelectron Spectroscopy (XPS)

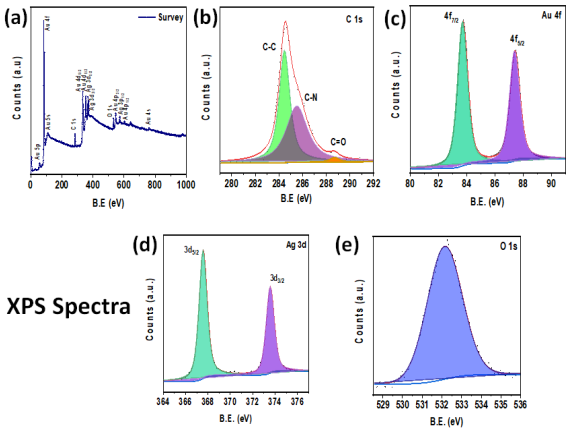

Figure 5: XPS spectra of Suvarna Bhasma a) SB survey b) Carbon 1s spectra c) Gold 4f spectra d) Silver 3dspectra e) Oxygen 1s spectra [7-9]

Table 2: XPS showing the different components of Suvarna Bhasma and their state with percentages in the sample

Name	Binding Energy (eV)	Species	Atomic percentage
Au4f	83.78	Au(0)	28.06
C1s	284.8 288.8	C-C, C=O,	51.68

	286	C-N	
Ag3d	368.5	Ag+	5.01
O1s	532.5	O2-	15.25

The quantitative analysis of the elemental composition, chemical state, empirical formula, and electronic state of the elements in the sample was studied using the XPS technique. Figure (a) shows the XPS survey of the Suvarna Bhasma sample. (b), (c), (d), and (e) show the Carbon, Gold, Silver, and Oxygen spectra. The C1s, O1s, and Au4f, Ag3d peaks were observed in the Suvarna Bhasma sample. The peak of C1s at 284.8 eV was used as the charge reference to determine core-level binding energies. Sample exposed to atmospheric air has carbon contamination. The core-level spectra of C1s provided more evidence that cell functional groups, for example, C-C (adventitious carbon) 284.8 eV, C-N (nitrates) 286 eV, C=O(ketones) 288.8eV,[10] this may be due to carbon deposition from the atmosphere also carbon involved in reducing and capping AuNPs. The Au 4f spectrum composed of doublet peaks corresponding to Au 4f7/2 and Au 4f5/2 at a binding energy of 83.78 and 87.47eV, respectively, with a difference of 3.69eV could be assigned to an elemental state of gold Au(0),[11] Oxygen spectrum with single peak corresponds to O1s and 532.5 eV binding energy corresponds to metal carbonate where oxygen is involved in bonding[12]. Peaks of silver Ag3d were observed at binding energies 368.5eV and 374.5eV, corresponding to the Ag^{+} oxidation state [13], but its percentage is only minimal. This may be because it is available as an alloy of gold in nature and might have been present along with gold, which was taken for bhasma preparation. XPS showed the ionic status of elements on the surface of the sample. The high percentage of carbon is caused by atmospheric carbon deposition while being handled.

FTIR Spectra of Suvarna Bhasma and Suvarna Prasha

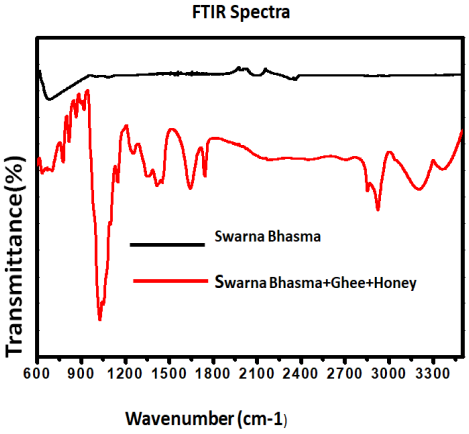

Figure 6: FTIR spectra of Suvarna Bhasma and Suvarna Prasha containing Suvarna Bhasma+ghee+honey showing transformation with multiple functional groups.

Table 3: FTIR Showing different functional groups in Suvarna Bhasma alone and Suvarna Prasha

S.No	Peaks	Functional Group	Chemical Composition
FTIR of Suvarna Bhasma			
	668cm-1	C≡CH	Alkynes
FTIR of Suvarna Prasha (Suvarna bhasma 15mg+ghee0.25ml+honey0.75ml)			
1	1022cm-1	C-O C-H	Carboxylic group Alkylehalide CH2X
2	1249cm-1	C-O C-H	Carboxylic group Alkylehalide CH2X
3	1351cm-1	C-H	Alkenes
4	1429cm-1	C-C stretch ring	Aromatics
5	1621cm-1	ACH=CHR	Alkenes
6	1747cm-1	C=O stretch	Ketones
7	2844cm-1, 2928cm-1, 3209cm-1, 3371cm-1	RCO-OH, C=CCOOH	Carboxylic group
8	33371cm-1 belonged of	RCO-OH, C=CCOOH	Carboxylic group, Ar OH bond of Phenols

The Suvarna Bhasma FTIR spectra showed a single peak at 668cm-1, representing the C≡CH bend of Alkynes.[14]

The Suvarna Prasha containing Ghee (0.25 ml), honey (0.75 ml) Suvarnabhasma 15 mg showed a peak at 1022cm-1 which belonged to C-O of carboxylic group, C-H group of alkyl halide CH2X, peak at 1249cm-1 [15] is belonging to C-O of carboxylic group, [16] C-H group of alkyl halides CH2X, peak at 1351cm-1 C-H group of alkanes, 1429cm-1 belonged to C-C stech ring of Aromatics,[17] peak at 1621cm-1 belonged to ACH=CHR of Alklens, peak at 1747cm-1 belonged to C=O stretch of ketones, peaks at 2844cm-1, 2928cm-1, 3209cm-1, 3371cm-1 belonged to RCO-OH, C=CCOOH of Carboxylic group, [18] peak at 3371cm-1 belonged to RCO-OH, C=CCOOH of carboxylic group, ArOH bond of phenols.

From the above, it can be inferred that Suvarna Bhasma alone had a single functional group of alkynes. When Suvarna Bhasma was triturated with honey and ghee, many functional groups were added to Suvarna Bhasma, i.e. fats, carbohydrates, and amino acids.

DISCUSSION

The SEM analysis was done to see the particle size, which revealed the particle size ranged from 28 nm to 8.1 µm. The particles aggregated with each other to form the spherical nano-to-micro balls, which are heterogeneously distributed. TEM analysis showed the polycrystalline nature of the Suvarna Bhasma; also, on further magnification, it was seen that the particle size ranged from 2 nm - 16 nm; the difference in size was due to sample preparation as Suvarna Bhasma was dissolved in ethanol and sonicated to get good particle spread on a copper grid. XRD analysis provided information about pure gold's face-centred cubic crystalline structure; the average crystalline size of the gold particles in Suvarna Bhasma was 27.58 nm, along with the elemental composition of Au. EDAX report suggested the presence of Gold (86%), Carbon (10%), and Oxygen (3%) in the sample. To identify the state of Gold, XPS was done, which

revealed that Gold was present in the elemental neutral state Au(0). Acharyas have not mentioned how long the mixing has to be done and with what pressure either on stone or in khalva yantra (stone mortar and pestle). The quantity of honey and ghee is also not mentioned. As per the concept of viruddhahara, honey and ghee should not be mixed in equal quantities while explaining the concept of matra viruddha[19]. Hence, 15 mg of Suvarna Bhasma (human dose as per Rasatarangini 15/ 81) was mixed with 0.25 ml of ghee and 0.75 ml of honey and subjected to analysis. FTIR of Suvarna Bhasma showed the presence of the CH group, whereas upon mixing with ghee and honey, many functional groups were added to Suvarna Bhasma, i.e., fats, carbohydrates and amino acids.

Probable composition and Biological action: Neutral spherical heterogeneous gold nanoparticles of average size 27.58 nm, when triturated with ghee and honey, get surrounded by lipids and sugars, which act further as capping and reducing agents as they surround them. Previous studies have shown that upon treating gold nanoparticles with lipids, a lipid bilayer was seen under TEM, revealing the possibility of functionalising the surface of gold clusters with specific cell-targeting membrane proteins, which upon treating with live cells showed that while being internalised into cells, Fluorescence-tagged AuCLs retained their lipid layers.[20] In Suvarna Prasha, the size of Incinerated Gold Nano Particles is further reduced by the trituration process, and they get suspended in lipid and sugar colloidal suspension. This stabilises the particles from clustering and helps in cell internalisation as the cell membrane comprises a phospholipid bilayer. This is given in paste form and used as lehana (electuary), consumed by children from birth to 16 years to increase intelligence, memory, complexion, virility, longevity, and memory.

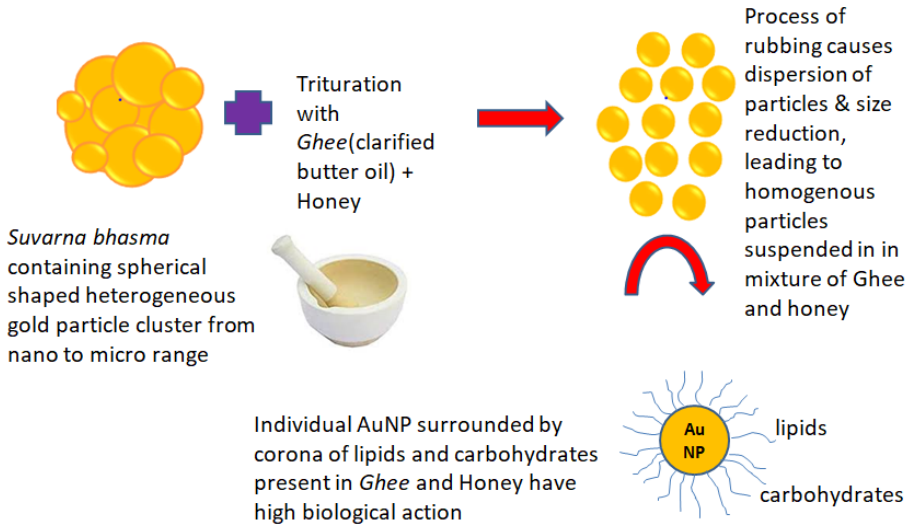

Figure 7: Probable changes in Suvarna Prasha

The investigated sample of Suvarna Bhasma was purchased from the market which claims to prepare the Suvarna Bhasma as per the 4th reference of Suvarna Marana process (calcination of gold) as explained in Rasatarangini (textbook of Indian Alchemy) which makes use of Gold (Au), Mercury (Hg) in equal quantity, amalgam prepared in the presence of kanji (fermented rice (*Oryza sativa*). This mixture is heated in an earthen crucible with an equal amount of sulfur powder covered from above by another earthen crucible, and the two are joined with mud and cloth. This is dried and heated with 30 cakes of cow dung in a pit called puta. The mixture is allowed for self-cooling. This is repeated 14 times by reducing 1/16th of mercury in each step. The final product becomes a powder of yellowish-brown colour called 'Suvarna Bhasma'. EDX and XPS were carried out to detect elements in Suvarna Bhasma and especially to check for the presence or absence of mercury (Parada) and sulfur, which have been used in the manufacturing process of this sample, and it showed that mercury and sulphur are absent in the final product which makes it safer for biological uses. They detected the presence of Gold, Oxygen, and carbon. The boiling point of sulphur is 444.6 ^{0}C, and that of mercury is 356.7 ^{0}C. During heating 14 times and due to a high temperature above 450 ^{0}C, the mercury and sulphur would have evaporated, so they are undetected. The amalgam of Au, Hg, and S, in the presence of fermented preparation like kanji, which contains many microorganisms responsible for fermentation, all contribute to particle size reduction and caping, which stabilises the particles. The space created between the elements during the evaporation of mercury and sulphur due to high temperature reduces the particle size. The acidic nature of kanji and microorganisms act as a catalyst to augment this process. It is a known phenomenon in modern science to synthesise gold nanoparticles using different acids and microorganisms.[21] As Suvarna Prasha is given, even in neonates, it should be devoid of other toxic metals like mercury. Unlike the previous studies, which detected the presence of many other minerals in the sample of Suvarna Bhasma, like Ag, Al, Ca, Cu, Fe, K, Mg, Mn, Na, P, Si, Sr, Ti, and Zn in traces,[21] these elements were not seen in this sample owing to a different method of synthesis and ingredients of the preparation. Also, the particle size range differed from sample to sample. Hence, it is the need of the hour to scientifically evaluate Suvarna Bhasma produced from different preparation methods and standardise the pressure of trituration to get the desired particles of therapeutic significance that have good biological effects. The optimum pressure, duration of trituration, and temperature of incineration for different therapeutic effects are a huge area of research for Ayurveda. Collaboration with IIT and pharmacology would help provide the most scientific input into this area. FTIR detects the chemical bonds in a compound or sample by generating an infrared absorption spectrum. The spectra give a sample profile, a specific molecular fingerprint that can be used to scan and screen samples for many different functional groups. FTIR is a sensitive analytical technique for

characterising and detecting covalent bonding functional groups without much sample preparation. The presence of organic functional groups is important. FTIR spectra revealed that Suvarna Bhasma alone had a single functional group of alkynes, but when Suvarna Bhasma was triturated with honey and ghee, many functional groups were added to Suvarna Bhasma, i.e. of fats, carbohydrates and amino acids making it biological easy for absorption. The powder XRD method is the most sensitive method for understanding the phase of the particle. XRD confirmed that Suvarna Bhasma is not in oxide, carbonate or sulphide form but is pure gold. The accelerated growth of gold nanoparticles is evident in XRD. The nanosize and agglomeration of particles are evident in TEM and SEM. The property of nanoparticles to make bigger agglomerates is already reported in the literature. [22] The agglomerates are clusters of gold nanoparticles in large micrometric sizes. The main advantage of Suvarna Bhasma is its stability and claimed safety, which its use has been understood since ancient times as a medicinal agent. Particle size is significant for the circulation of nanomaterials in the bloodstream, absorption across physiological drug barriers, site and cell-specific localisation, and induction of cellular responses. Apart from gold, the Suvarna Bhasma marked the presence of other elements like Ag, C, and O. Organic and inorganic drugs used during the processing of incineration, raw materials (kanji, a fermented rice preparation) used during the amalgamation of Au Hg, contributed to the presence of these elements in the final bhasma of gold. The presence of silver peaks may be due to the existence of silver along with gold in ore form, which was in traces. Suvarna Bhasma, when given in different combinations of herbal and mineral drugs, can cure different diseases like Jwara (fever), Sangrahani (bowel disorders), Pandu (anemia), Rajayakshma (tuberculosis), Garbhashaya shodhana (cleansing uterus), Phiranga (syphilis), Rakta shodhana (blood purification), Amlapitta (gastritis), Apasmara (seizure), speech disorders, memory disorders, stanya vardaka (increases breast milk), Vishahara (cures poisoning), Asti shotha, Kshata, Vakrata (inflammation, breaking, bending), Vrukka roga (kidney disorders), Andashota (scrotal swelling, orchitis), Hrudroga (heart disorders), etc. [23] This indicates the repurposing of the same drug in different drug combinations in multiple diseases whose mechanism is the topic of further research. Gold Bhasma has shown beneficial results in clinical studies for cancer management [24]. Various studies conducted on Suvarna Bhasma have focused on the use of gold nanoparticles in Suvarna Bhasma in the management of cancer bioavailability, a cellular entry *in vivo*, on cancer cell lines; toxicity study has proved it to be an effective, safer drug so far when prepared as per textual guidelines.[25-27] Unlike the lab-generated gold nanoparticles, which have shown toxicity in animal models due to the chemicals used in the preparation, the Suvarna Bhasma has been proven safe up to 30 mg/kg for 90 days without any histopathological changes.[28] The results are specific to the investigated sample prepared with specific ingredients and processes. Results cannot be generalised. The toxicity of contemporary AuNPs has led scientists to discover new methods for AuNP synthesis. Suvarna Bhasma, when prepared correctly per Rasa Shastra guidelines, is safe and time-tested. Due to the rapid development in technologies for the chemical synthesis of gold nanoparticles over recent years, a great variety of particles with different sizes, shapes, structures, and optical properties are now available to contemporary researchers. The long-term safety of Suvarna Bhasma is a major issue for the scientific world. Hence, it must be studied scientifically for its judicious use.

Biological application of Suvarna Prasha

A prospective randomised controlled trial on preterm infants with a gestational age of 34 weeks who were administered with medically graded bee honey added to milk at a dose of 5, 10, 15, and 0 g/day for 2 weeks in groups A, B, C, and D with 10 subjects in each group respectively, for 0, 7, and 14 days showed that. Compared with group D, all 3 intervention groups demonstrated a significant increase in weight ($P<0.0001$). Head circumference increased in groups B and C. Enterobacter stool colonisation decreased in groups A and B, whereas *Bifidobacterium bifidum* colony counts increased in groups A, B, and C, and *Lactobacilli* colony count increased in group B($P<0.0001$). Applying real-time polymerase chain reaction, *B bifidum* and *Lactobacilli* increased in group C . [29] The gut flora of the neonates is sterile during the intrauterine life due to contact with maternal vaginal flora in vaginal delivery, skin flora in cesarian section, nosocomial infection, breastfeeding and it eventually reaches the adult flora at the age of five. [30] Honey helps in building the gut flora by acting as a prebiotic. Ghee containing short-chain fatty acids like butyric acid helps in maintaining gut flora and also prevents pathogenic bacteria; research shows that adequate production of butyric acid supports the production of killer T cells in the gut and, thus, a strong immune system. [31]

CONCLUSION

Suvarna Bhasma was characterised by scanning electron microscopy (SEM) and transition electron microscopy, which revealed that the particle size ranged from 3 nm - 8.1 µm, and the particles were aggregated with the heterogeneous distribution. The aggregates of nanoparticles formed spherical balls of various sizes with rough surfaces and were polycrystalline. XRD analysis showed the crystalline nature of nanoparticles and face-centred cubic (FCC) crystal lattice are related to pure gold (JCPDS card no 04-0784). The average size of the crystal d of gold particles in Suvarna Bhasma was 27.58 nm. EDX report revealed that the Suvarna Bhasma sample contained Gold (86.3%), Oxygen (3.14%), and Carbon (10.52%) sample of Suvarna Bhasma. XPS analysis showed the presence of Carbon, Gold, Silver, and Oxygen spectra. The Gold peaks belonged to the neutral state of (0) along with C-C, C-N, and C=O peaks, which may be due to carbon deposition from the atmosphere also from carbon involved in reducing and capping AuNPs during the process of Bhasma correct polycrystalline as polycrystalline preparation. Oxygen single peak corresponds to metal carbonate, where oxygen is involved in bonding. FTIR spectra revealed that Suvarna Bhasma alone had a single functional group of alkynes, but when Suvarna Bhasma was triturated with honey and ghee, many functional groups were added to Suvarna Bhasma, i.e. of fats and carbohydrates and amino acids making it biologically active for absorption. The FTIR and XPS results are being reported for the first time, making it a unique study. Suvarna Prasha, containing gold nanoparticles, ghee and honey, acts as a perfect nutritional supplement, antioxidant, anti-inflammatory, antimicrobial, anti-cancerous, and nootropic drug for neonates, infants and children and proves to be a boon when used judiciously. It must be mentioned here that the results obtained are specific to the investigated sample prepared with specific ingredients and processes. Results cannot be generalised to any sample of Suvarna Bhasma, honey, and ghee but would be a prototype for future research in drug standardisation.

Limitations

The limitation of this method is controlling the size and shape of particles, which is easily controlled in conventional synthesis methods and has a specific action. However, in the case of bhasma having a heterogeneous distribution, each particle would have different activities. The smaller particles could penetrate even into the genetic level, while bigger particles would not even cause cellular entry and may be excreted as they are. The process of giving the drug with specific anupanas (drugs used for mixing

and administration) and the trituration process just before the administration would solve this problem by reducing the particle size further, preventing them from aggregating, homogenising, and making them biologically compatible with effective drug delivery systems.

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