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Preparation and characterization of a copper based Indian traditional drug: Tamra bhasma

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Abstract

The copper based Indian traditional drug 'tamra bhasma' is administered for various ailments since long. Its synthesis involves treating metallic copper with plant juices and then repeated calcination in presence of air so that the metallic state is transformed into the corresponding oxide form traditionally known as 'bhasma'. In this work, we present a systematic characterization of this traditional drug using various techniques like X-ray diffraction (XRD), scanning electron microscopy (SEM)–energy dispersive X-ray analysis (EDX), X-ray photoelectron spectroscopy (XPS), infrared spectroscopy (IR), thermogravimetry (TG) and surface area measurement. The results obtained were found to match very well with those of a standard copper oxide confirming the composition of the drug sample. In addition, some specific findings were also made which could help in interpreting the therapeutic properties of the traditional drug 'tamra bhasma'.

Keywords: Copper oxide; Tamra bhasma; Traditional medicine; XRD; XPS of copper oxide; Crystallite size

1. Introduction

The traditional medicinal system practiced in India for several centuries is well known as Ayurveda. According to this medicinal system, metal based drugs known as 'bhasma' involve the conversion of a metal into its mixed oxides. During these transformations, the zero valent metal state gets converted into a form with higher oxidation state and the most important aspect of this synthesis (known traditionally as 'bhasmikarana') is that the toxic nature (i.e. systemic toxicity causing nausea, vomiting, stomach pain, etc.) of the resulting metal oxide is completely destroyed while inducing the medicinal properties into it. The important step involved in the procedure for making 'bhasma' is repeated treatment of a particular metal with plant juices and high temperature calcination in an earthen pot. Various tests both physical and chemical for confirming the formation of metal oxides (bhasmas) have been described in the ancient Ayurvedic literature [1]. Some of such preliminary tests are: (i) Floating test: if a small quantity of the metal oxide (bhasma) is sprinkled on water surface it should float on the surface; (ii) Fineness test: on rubbing a small quantity of the sample between the fingers it should enter into the lines on the fingers; (iii) Loss of metallic luster: when visually examined preferably in presence of sun light no metallic luster should be observed in case of a metal oxide; (iv) Loss of metallic state: this involves heating of a very thin silver sheet (600 μm thickness) along with a small quantity of the metal oxide (bhasma) to red hot for about 5 min. After cooling the sheet to room temperature, no traces of this sample should permanently stick to the silver sheet indicating no alloy formation takes place, thus confirming the metal has totally transformed into its oxide form, 'bhasma'. However, all these are highly empirical and

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hardly provide any information on the composition and structural properties of these mixed metal oxides. Therefore, it is highly desirable that these drugs should be characterized with the help of modern instruments, such as X-ray photoelectron spectroscopy (XPS); X-ray diffraction (XRD); scanning electron microscopy (SEM); energy dispersive X-ray analysis (EDX); infrared spectroscopy (IR); thermogravimetry (TG); Brunauer, Emmett and Teller (BET) surface area measurement based on which the specifications of such drugs can be well standardized on a scientific basis.

One of the widely used example of such kind of metal oxide based Ayurvedic drug 'tamra bhasma' is derived from metallic copper that is recommended for different ailments of liver and spleen, abdominal pains, colitis, heart problems, anaemia, tumors, loss of appetite, dropsy, eye troubles and tuberculosis. Recently, pharmacological investigations have been reported on the use of 'tamra bhasma' for treating gastric ulcers and secretion, the management of lipid peroxidation in the liver of albino rats and free radical scavenging properties [2–4]. Patil et al. have examined the effect of 'tamra bhasma' on lipases and lypolitic activities in $CC1_4$ induced hepatic injury in rats [5]. The main objective of the present work was to carry out the structural characterization of traditional *Ayurvedic drug* (tamra bhasma) and compare these results with those of standard copper oxide. In case of 'tamra bhasma', crystallite size of CuO was found to be higher than that of a standard copper oxide, causing the reduction in its surface area. The preparation method of 'tamra bhasma' involved repeated calcination cycles, thus, facilitating agglomeration and hence bigger crystallites. These results together with XPS, XRD, EDX, SEM results showed that the 'tamra bhasma' of *Ayurvedic* origin consisted of mainly cupric oxide (Cu^{2+}) however, this form was significantly different from the standard copper oxide that may be partly responsible for its therapeutic applications.

2. Experimental

Copper wires (100 g) were preferred as the source of pure metallic copper (purity > 99.9%), and was made into fine powder by cutting and crushing. Four plants were selected for pretreatment of the metallic copper before calcination (known as 'bhasmikarana'), namely *Aloe vera*, *Phyllanthus emblica*, *Eclipta alba* and *Bacopa monnieri*. Their identity was confirmed with the help of an expert botanist at our institute. The standard copper oxide (99.9% pure) used for comparative studies was purchased from Aldrich.

The traditional preparation of the drug involved mainly trituration of the metallic copper with plant juices followed by repeated calcination of the mixture in presence of air following the procedure prescribed in the literature [6]. According to this procedure, metallic copper powder (100 g) was mixed with the above plant juices using an agate mortar and pestle. Fresh juices of four plants were mixed in equal quantities to make a total volume of 200 ml. This was added to the metal powder and the mixture was triturated manually with a pestle till a homogeneous paste was formed. The paste was then transferred to an earthen crucible covered with a lid and sealed with sealing clay. Then, it was subjected to calcination following the procedure described by Pandit et al. [7].

The XRD patterns of the solid samples were recorded on Rigaku cd-max II vc model X-ray diffractometer using Cu Kα radiation filtered by a nickel foil over the range of diffraction angle 3.0–50.0°. The wave length of the radiation used was 1.5405 Å.

The chemical composition of the sample was determined by EDX attached to SEM (JEOL JSM 200). IR spectra in the region (4000–450 cm^{-1}) were recorded on Perkin-Elmer FTIR spectrophotometer in KBr pellets. Far IR spectra in the low frequency region (600–50 cm^{-1}) were recorded on Nexus-870 model Nicolet). Thermograms were recorded in air atmosphere on a NETZSCH simultaneous thermoanalyzer STA–409 model with Pt and Rh thermocouples.

XPS were recorded with a nine channeltron CLAM4 analyzer under a vacuum better than 1×10^{-8} Torr, using Mg Kα radiation and a constant energy of 50 eV. The binding energy values (accurate to +0.2 eV) were charge-corrected to the C1s signal (284.6 eV). BET surface area analysis was done using Chembet 3000 surface area analyzer of Quantachrome USA.

3. Results and discussion

3.1. XRD

The XRD pattern of 'tamra bhasma' is shown in Fig. 1. Diffraction peaks at $2\theta = 35.7°$, 38.9° and 58.5° are identical with those reported for the standard cupric oxide [8]. No extra diffraction peaks are observed confirming that the drug 'tamra bhasma' is composed of cupric oxide.

The high intensity of XRD lines in the XRD pattern suggests that the drug is present as a crystalline material. The crystallite size of copper oxide in the drug 'tamra bhasma' was calculated from XRD pattern following the Scherrer Eq. (1) and it was compared with that of the standard CuO [9,10].

$$t = \frac{\lambda \times 0.9}{(\beta \times \cos\theta)} \quad (1)$$

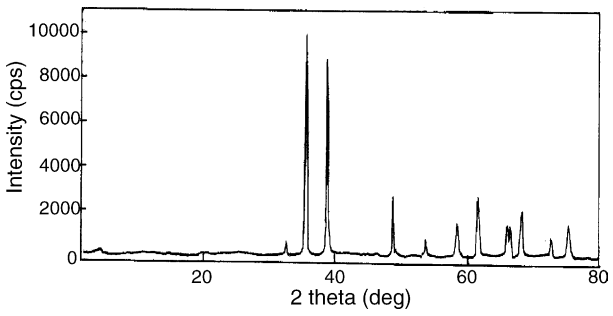

Fig. 1. XRD pattern of the drug sample.

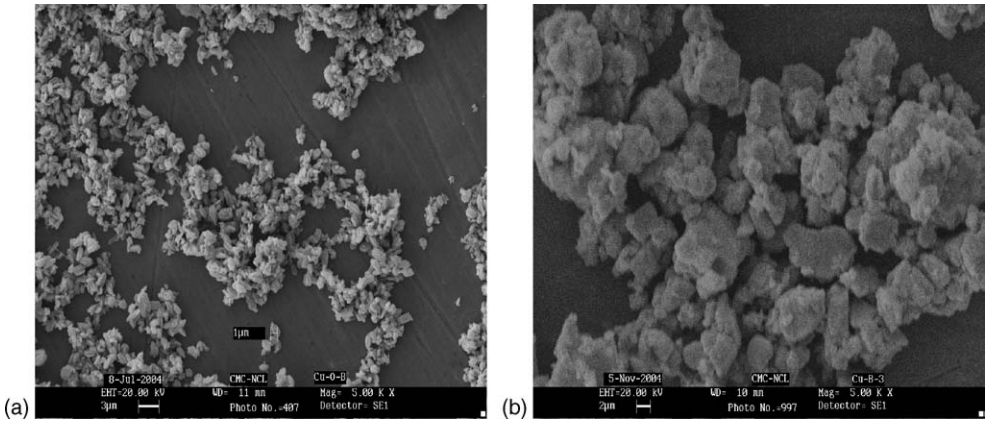

Fig. 2. SEM of standard copper oxide (a) and the drug 'tamra bhasma' (b).

where t is the crystallite size for ($h\,k\,l$) phase, λ the X-ray wavelength of radiation for Cu Kα (0.1542 nm), β the full-width at half maximum (FWHM) at ($h\,k\,l$) peak in radian and θ is the diffraction angle for ($h\,k\,l$) phase. It was found that the crystallite size of the drug 'tamra bhasma' under study was bigger (32.2 nm) than that of the synthetic cupric oxide (23.6 nm).

3.2. SEM and EDX analysis

The photographs of SEM taken for the standard cupric oxide (a) and the drug 'tamra bhasma' (b) are shown in Fig. 2 which reveal distinct features of both the samples. The standard cupric oxide (a) showed well-defined plate like structures while the drug 'tamra bhasma' showed spongy, relatively compact microcrystalline aggregates with loss of grain boundaries. The average particle size of sample (a) was found to be about 1 μm while that of sample (b) was in the range of 8–10 μm obviously due to agglomeration of plate-like crystals which were also covered by the small dusty crystallites. The bigger particle size of the drug 'tamra bhasma' (sample b) is in accordance with its preparation method which involved repeated calcinations. The influence of method of preparation on morphology, particularly the calcination temperature and duration, have been reported in the literature for oxides of other metals also [11].

The elemental composition of the drug sample analysed by EDX analysis is presented in Table 1. From this analysis, 60% copper present in 'tamra bhasma' was equivalent to 75% CuO (as per the stoichiometry) while, other major elements were carbon and stoichiometric excess oxygen. The large amount of carbon was due to repeated cycles of calcination in presence of plant juices and also this procedure would lead to excess amount of oxygen being chemisorbed on the copper surface which was also evident from the XPS analysis (see Section 3.3). The presence of carbon would be responsible for the passivity of the cupric oxide and may result in decreasing its toxicity and on the contrary, imparting the therapeutic value to the copper oxide, thus prepared. However, more detailed systematic investigations are necessary to ascertain the role of carbon present in this drug.

3.3. XPS

XPS measurements provided valuable information about the surface state of the drug sample which was also compared with that of the standard cupric oxide sample. Results presented in Figs. 3 and 4 show the Cu $2p_{3/2}$ and O1s core level spectra, respectively. The characteristic CuO peaks in the standard cupric oxide as well as in the prepared drug sample were observed at 933.6 eV which is close to that previously reported for CuO [12,13]. The other two peaks at 941 and 943 eV were the characteristic shake-up satellites which accompany the main Cu $2p_{3/2}$ line of CuO. The ratio of the area of the shake-up satellites to that of the $2p_{3/2}$ line was ca 0.43 and 0.49 for the prepared and the standard commercial CuO samples respectively, which is similar to that reported for CuO particles [14]. These observations confirmed that all the copper in the prepared drug sample was present in the Cu^{2+} state. We did not observe in our sample any reduction of Cu^{2+} to Cu^{0} due to ultra-high vacuum and X-ray irradiation, indicating the stable nature of CuO in the given composition. However, the surface Cu/O ratio observed in our prepared drug sample was <1.0, lower than the expected stoichiometric ratio. This discrepancy could reflect the presence of adsorbed

Table 1
Elemental composition by EDX of the drug sample

Element	Wt.%[a]	At.%[a]
C	9.78	23.64
O	24.72	44.87
Mg	0.59	0.70
Si	0.75	0.77
S	0.99	0.90
Ca	0.34	0.25
Fe	2.58	1.34
Cu	60.26	27.54
Total	100	100

[a] Based on ZAF quantification (standardless).

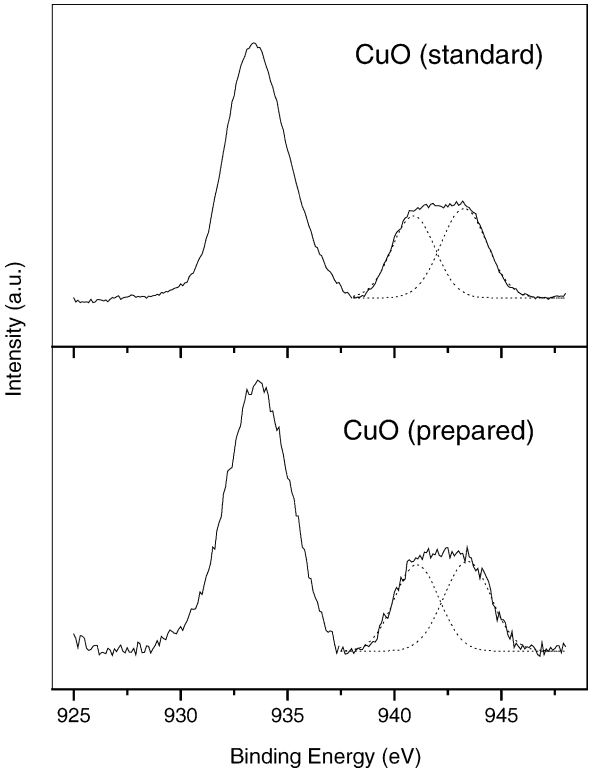

Fig. 3. Cu $2p_{3/2}$ photoelectron spectra of standard CuO and the prepared drug sample.

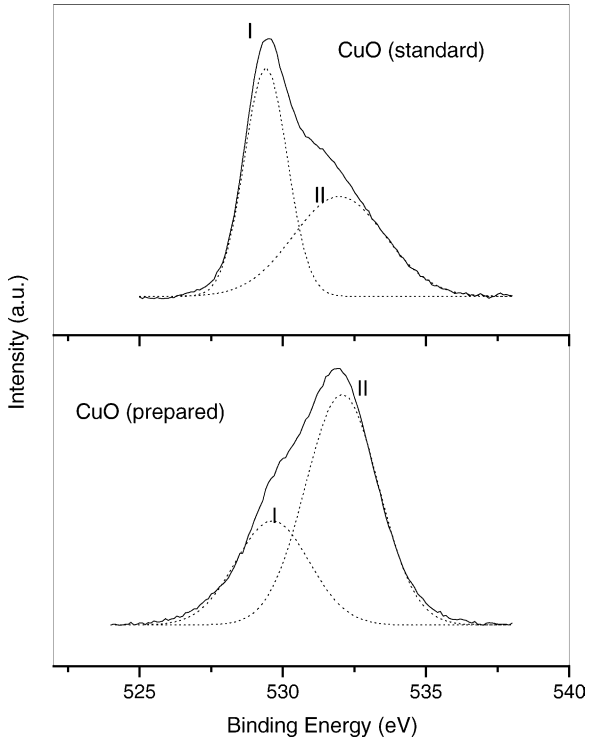

Fig. 4. O1s photoelectron spectra of the standard CuO and the prepared drug sample.

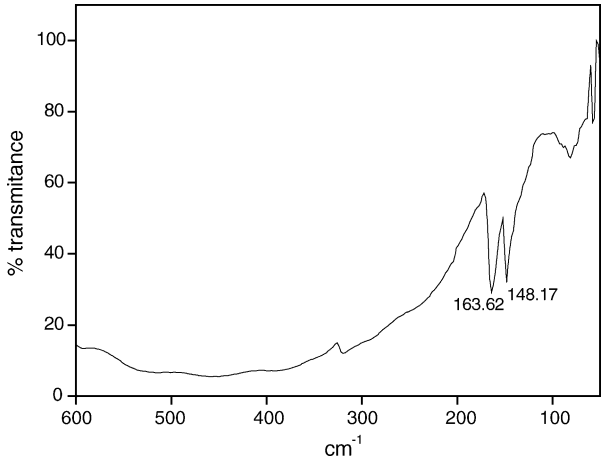

Fig. 5. Far IR spectrum of the drug sample.

oxygen on the surface of the sample. As shown in Fig. 4, the O1s core-level spectrum is broad and two O1s peaks (I and II) can be resolved by using a curve-fitting procedure. Peak I, at a lower energy of 529.8 eV, is in agreement with O^{2-} in CuO, while peak II, at a higher energy of 531.6 eV, is attributed to O adsorbed on the surface of CuO particles. It was also observed that the area under curve of O adsorbed species in the prepared drug sample was higher than that for the standard CuO sample, as a result of a different procedure of its preparation involving repeated cycles of calcination.

3.4. IR and TG results

Different FTIR spectra of the drug sample are presented in Figs. 5 and 6. The presence of cupric oxide (CuO) was confirmed by the characteristic doublet at 163 and 148 cm^{-1} in the in the low frequency region (far IR, Fig. 5) [15]. The strong and predominant peaks in the mid IR region (468–488 cm^{-1}) for the drug sample (Fig. 6b) also matched very well with that of standard copper oxide (Fig. 6a). Additional peaks in the region 1140–1100 cm^{-1} for the drug sample in Fig. 6b could

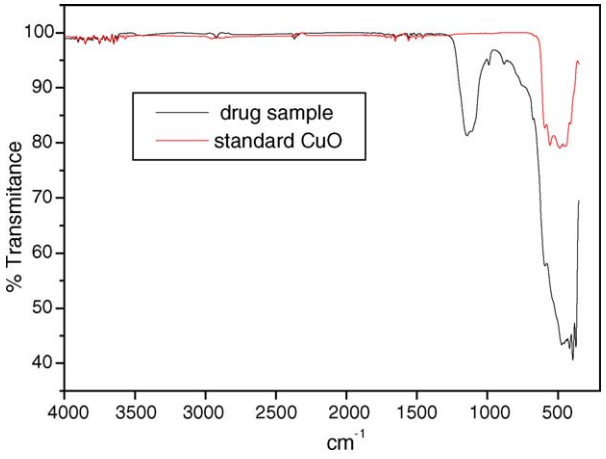

Fig. 6. FT-IR (a) standard cupric oxide (b) drug sample.

be due to the characteristic frequencies of O–O bond arising from the adsorbed oxygenates [16]. The thermogram of the drug sample was recorded in air atmosphere in the temperature range of 30–900 °C which showed 0.638% weight loss at 329 °C indicating the loss of adsorbed dioxygen species from the sample. This is again in accordance with the XPS results about the chemisorption of dioxygen during typical calcination procedure.

3.5. BET surface area measurement

From the BET measurement, the specific surface area of the particles of the drug sample 'tamra bhasma' was found to be 0.769 m^2/g which was about 3.75 times lower than that of the standard cupric oxide (2.895 m^2/g). This was consistent with the bigger particle size of the drug 'tamra bhasma' as compared with that of pure cupric oxide determined by XRD.

4. Conclusion

In order to establish the composition of an Indian traditional drug 'tamra bhasma' various characterization techniques such as XRD, XPS, SEM-EDX, BET surface area measurement were used and these results were compared with those of a standard copper oxide sample. From XRD phases and XPS studies it was confirmed that the drug contains copper with +2 oxidation state in the form of cupric oxide. An interesting feature of the adsorption of dioxygen species on the surface of the drug sample was revealed by XPS and supported by other characterization studies. Also, the particle size is bigger than the standard sample, due the repeated calcination procedure followed in its preparation leading to agglomeration of the smaller CuO particles. These results contribute to standardization of specifications of the traditional drug and provide useful hints on its therapeutic properties.

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