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Original Research Article (Experimental)

Mercury based drug in ancient India: The red sulfide of mercury in nanoscale

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ABSTRACT

Mercury is one of the elements which had attracted the attention of the chemists and physicians of ancient India and China. Among the various metal based drugs which utilize mercury, we became interested in the red sulfide of mercury which is known in ancient Indian literature as rasasindur (alias rasasindura, rasasindoor, rasasinduram, sindur, or sindoor) and is used extensively in various ailments and diseases. Following various physico-chemical characterizations it is concluded that rasasindur is chemically pure α-HgS with Hg:S ratio as 1:1. Analysis of rasasindur vide Transmission Electron Microscopy (TEM) showed that the particles are in nanoscale. Bio-chemical studies of rasasindur were also demonstrated. It interacts with Bovine Serum Albumin (BSA) with an association constant of $(9.76 \pm 0.56) \times 10^3\ M^{-1}$ and behaves as a protease inhibitor by inhibiting the proteolysis of BSA by trypsin. It also showed mild antioxidant properties.

1. Introduction

Ayurveda, meaning the science of life, is one of the ancient medical systems of the Indian subcontinent. The principles and practices of the subject are documented in a large number of age-old texts; Charaka Samhita and Sushruta Samhita being two main Ayurvedic classics [1,2]. Ayurvedic material medica is dominated by substances of vegetable, animal and mineral origin. Metals and minerals used include mercury, gold, silver, copper, iron, tin, zinc etc. An extensive range of chemical and physical processing of these metals and their compounds has been elaborated in texts which are generally known as Rasashastra [3–7].

Mercury is one of the metals which attracted wide attention of ayurvedic chemists and physicians [8]. Indeed the documentation of chemical and physical processes involving mercury is truly enormous in ancient texts of which classics by Vagabhatta and Nagarjuna are noteworthy. Among the various procedures which utilize mercury, we became interested in the one that involves mercury and sulfur. The process is divided in three distinct steps, namely (i) pre-treatment of mercury and sulfur with herbal and milk products, (ii) mixing of mercury and sulfur along with other herbal ingredients resulting in the formation of black sulfide of mercury, (iii) thermal treatment of black sulfide of mercury at 600–650 °C [9]. The sublimed red sulfide of mercury is termed as rasasindur (alias rasasindura, rasasindoor, rasasinduram, sindur, or sindoor) in Rasashastra and is used extensively in various ailments and diseases [10].

The context of toxicity in metal based drugs in general, and of mercurial preparation in particular is an important issue [11]. It needs to be emphasized that the Rasashastra texts elaborately emphasize the concept of specific attributes in starting materials, intermediates, and products which lead to toxicity and adverse

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effects in a patient [12]. However, such narrations are at times difficult to interpret in equivalent modern scientific terms. The elaborate preparative protocol in the synthesis of rasasindur prompted us to study the physico-chemical properties of rasasindur. As presented below, we are delighted to find that the synthesis protocol described in the ancient text is indeed a case of bottom-up synthesis of red sulfide of mercury in nanoscale [13]. In light of the results of the present study and those by others [14,15] the context of nanotransformation (and its plausible implication on bio-efficacy/bio-availability/in-vivo toxicity) in metal-based ayurvedic drugs warrants a relook.

2. Experimental

2.1. Materials and methods

X-ray powder diffraction (XRD) patterns were obtained in a Bruker D8 Advance powder diffractometer using Cu Kα radiation (λ = 1.5406 A°). The optical absorption spectra of the sample were measured in the range of (500–800 nm) using PerkinElmer UV WinLab 5.2.0.0646/1.61.00 Lambda 900. Raman spectra were recorded with LabRAM HR800 micro-Raman spectrometer (Manufacturer HORIBA JobinYvon) using 632.8 nm laser excitation wave length. All measurements were made in a backscattering geometry, using a 50× microscope objective lens with a numerical aperture of 0.7. Typical laser power at the sample surface was 2.0 mW with spot size of 2 μm. The acquisition time for all the spectra was 2 s. Emission measurements were performed (excited at 270 nm) at room temperature with a Fluorolog-3 (HORIBA JOBINYVON) Spectrofluorimeter. Transmission electron microscopy (TEM) studies were carried out with a Jeol, Ultra high resolution Transmission Electron Microscope (PP resolution: 0.19 nm), at 200 KeV. Rasasindur powder was first dispersed in isopropanol to prepare a 0.1 mg/mL suspension, which was sonicated for 1 h. One drop of the suspension was taken on a copper coated grid, dried at room temperature and submitted for TEM. X-ray photoemission spectra (XPS) were recorded on a KRATOS AXIS 165 with a dual anode (Mg and Al) apparatus using the Mg Kα anode. Lens Mode: Electrostatic; Resolution: pass energy 80, Anode: A1 (150 W), step size is 100.0 meV. The pressure in the spectrometer was about 10^{-9} Torr. For energy calibration, we have used the carbon 1s photoelectron line. The carbon 1s binding energy was taken to be 285.0 eV. Spectra were deconvoluted using the Sun Solaris based Vision 2 curve resolver. The location and the full width at half-maximum (FWHM) for a species were first determined using the spectrum of a pure sample. The location and FWHM of the products, which were not obtained as pure species, were adjusted until the best fit was obtained. Symmetric Gaussian shapes were used in all cases. SEM analysis was carried out in a Zeiss Merlin Compact Oxford instrument. EDS analysis was performed at 20 kV. The working distance was set in the range 4–5 mm. The image was analyzed with secondary 1 detector. Energy of X-ray is characteristic of the difference in energy between the two shells or the atomic structure of the element.

2.2. Preparation of rasasindur

Rasasindur was prepared at Arya Vaidya Sala, Kottakkal, Kerala, India following validated standard operating procedure according to rasatarangini, which is one of the classics of Rasashastra [9]. The same procedure has been also cited in other reports [10,14]. Briefly, the preparation involves the following major steps each of which takes days to complete. Initially 350 g mercury and 350 g lime were ground on a mortar after which the mercury was filtered through a muslin cloth. The mercury thus obtained was again ground on a mortar with garlic and rock salt. Finally it was washed with water and kept ready for the next step. The processing of sulfur involved melting it, pouring the liquid sulfur in milk and in the juice of *Eclipta alba* in stages. After washing with water the sulfur was dried. 310 g of detoxified mercury and 310 g of detoxified sulfur, obtained as above, were ground together to a fine paste in the presence of the juice of *Ficus benghalensis*. The resulting black sulfide of mercury (called Kajjali in traditional texts) was then sun-dried. 250 g of Kajjali was taken in a porcelain reactor and the lid was closed. The reactor was smeared with five layers of clay and dried. Finally the reactor was heated in an open-hearth furnace at 650 °C for 33 h 15 min. The sublimed red crystals were milled on a mortar for such duration till the powder of Rasasindur passed through the standard quality control (QC) parameters as in Ayurvedic texts.

3. Results & discussion

3.1. Structure and morphological study

The crystalline behavior of rasasindur was established through Powder X-Ray Diffraction. The PXRD pattern was shown in Fig. 1. On comparison with standard JCPDS (Card No. 00-006-0256) data base values of all peaks, it is indexed to be a pure hexagonal lattice (cinnabar phase) and space group P3221. The lattice parameters are a = 0.415 ± 0.005 nm, c = 0.949 ± 0.005 nm. There are no impurities detected from the PXRD pattern, suggesting the high purity of rasasindur.

A complete analysis of elemental composition of rasasindur was possible by SEM-EDX (Fig. 2 (e)). EDX analysis concludes that the presence of only two elements e.g. Hg and S in the red HgS where Hg (80%) is present as a major element. Presence of other elements C and O are due to use of carbon tape for support and oxygen due to adsorption of atmospheric oxygen on the surface of the sample. Atom balance shows that both Hg and S are present ~50%. Thus from the EDX results we can interpret that the atomic ratio of Hg to S is 1:1.

HRTEM analysis indicated that the particles of rasasindur have spherical shape nanostructures. Particle size distribution shows that most of the particles are in the range of 8–16 nm range. From the high resolution HRTEM image (Fig. 2 (b)), the lattice spacing have been determined to be 0.337 ± 0.005 nm, 0.332 ± 0.005 nm which corresponds to (101) plane of HgS. The lattice parameters are a = 0.415 ± 0.005 nm and c = 0.949 ± 0.005 nm corresponding to Hexagonal crystal system, Space group-P3221 (PCPDF No. 060256).

From the diffraction pattern (Fig. 2 (c)), the interplanar distance has been calculated to be 0.281 nm, 0.231 nm and 0.171 nm which

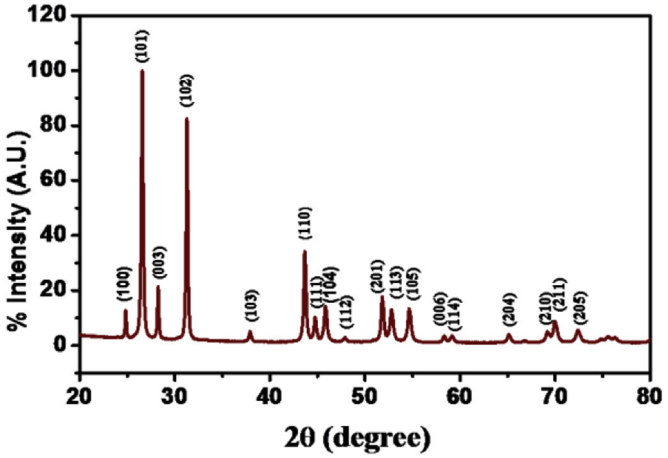

Fig. 1. Powder X-Ray Diffraction patterns of rasasindur.