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Structural and elemental characterization of traditional Indian Siddha formulation: *Thalagak karuppu*N. Kannan ^a, S. Balaji ^{a,*}, N.V. Anil Kumar ^b^a Department of Biotechnology, MIT, Manipal University, Manipal, India^b Department of Chemistry, MIT, Manipal University, Manipal, India

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ABSTRACT

Background: The traditional Indian medicine 'Siddha' uses metals, metalloids and minerals including toxic ones with no proven toxicity. *Thalagak karuppu* (TK) is remarkably stable over a century and used for treating *Suram* (Fever), *Kaasam* (Cough), *Elai* (Tuberculosis) and *Eraippu Erumal* (Bronchial Asthma). **Objective:** The present study addresses elemental and morphological characterization of therapeutic Siddha formulation: *Thalagak karuppu* (TK).

Materials and methods: TK was purchased from the Indian Medical Practitioners Co-operative Pharmacy and Stores (IMCOPS) Ltd, Chennai, Tamilnadu, India. The physicochemical properties were evaluated using UV–visible spectrophotometer, Fourier Transform Infrared Spectrometer (FTIR), Scanning Electron Microscope (SEM) with Energy Dispersive X-ray analysis (EDX), Zeta sizer and X-ray diffractometer (XRD).

Results: The mixed nature of arsenic was analyzed using UV–visible spectroscopy. The fingerprint region for arsenic derivatives was inferred from IR spectroscopy and X-ray diffraction patterns. The shape and size heterogeneity in the anisotropic mixture was observed in SEM images and the polydispersity was analyzed by Zeta sizer.

Conclusions: The structural, elemental and morphological analyses suggests that the arsenic may predominantly exist either as orpiment (As_2S_2) or realgar (As_2S_4) form. The possibility is less for the toxic arsenolite. Hence, the formulation may be considered safe.

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1. Introduction

Natural products are used extensively throughout the world, at least in 70% of drugs available in the global market. However, there is a great threat to biodiversity [1] due to overharvesting raw materials for herbal medicines and health care products. Since many medicinal plants are also in the endangered list, it is better to adapt mineral-based medicine practiced traditionally in India. An ancient system of traditional Indian medicine 'Siddha' uses metals, metalloids and minerals [2] that comprises toxic and heavy elements such as lead, mercury and arsenic. This raises the public concerns and alarms the large population on consumption of these traditional medicines [3–5]. Nevertheless, recently the inorganic arsenic

is accepted as the first line chemotherapeutic agent against certain hematopoietic cancers in the Western medicine [6]. The issues related to public concerns should be settled down scientifically by pinpointing its physicochemical properties and its biological interactions [7,8].

The usage of arsenicals in Indian system of medicine has a very long history of treating various diseases including gonorrhoea, epilepsy, syphilis, asthma, psoriasis, chronic fever, cancer, tuberculosis and other respiratory diseases [9]. Among the arsenicals: red, white and yellow, the Tamil word 'Thalagam' refers to 'yellow orpiment' and 'Karuppu' refers to 'black' as it marks the usage of toxic heavy metals or metalloids. *Thalagak karuppu* (TK) is remarkably stable over a century [10]. TK is included in the Essential Drugs List (EDL) of Siddha [11] for treating *Suram* (Fever), *Kaasam* (Cough), *Elai* (Tuberculosis) and *Eraippu Erumal* (Bronchial Asthma). TK is composed of *Thalagam* (Arsenic trisulphide) and snail's flesh. The preparatory procedure is as follows: the ingredients are mixed

* Corresponding author.

E-mail address: s.balaji@manipal.edu (S. Balaji).

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meticulously and grounded into fine powder using *Karuppu Kalvam* (Agate-black stone) mortar. The dried powder is spread in a clay pot and the mouth is sealed with another pot. Enough care is taken to seal it tightly with *Seelai* (a soil smeared cloth) rolled up to seven layers. Besides, any of the following sealing agent can also be used to fill the gap as per requirement; they are finely ground soft sand, wheat flour, black gram flour and lime with egg white. The set-up is then subjected to calcination process (referred as '*pudam*' in *Siddha*) [12]. *TK* is administered at the dose of 65–130 mg with honey twice a day after food [11]. The structural and elemental composition of this preparation is not well known. Hence, this is the first attempt to characterize *TK* formulation.

2. Material and methods

2.1. Metal formulation

TK was purchased from the Indian Medical Practitioners Co-operative Pharmacy and Stores (IMCOPS) Ltd, Chennai, Tamil Nadu, India.

2.2. Qualitative test for sulphur

Qualitative detection of sulphur was performed by dissolving 10 mg of the sample with diluted HCl. On to this, a pinch of lead acetate was added, the black precipitate confirmed the presence of sulphur in the formulation.

2.3. UV–visible absorption spectrometer

The UV–vis spectra were recorded by a double beam spectrophotometer UV-1601 PC (Shimadzu, Japan) at a resolution of 1 nm. The spectra were recorded in the region between 200 and 800 nm.

2.4. Fourier transform infrared spectroscopy (FTIR)

The sample was prepared by pressed pellet technique [13]. The samples were mixed with KBr and pelletized for analysis. The FTIR spectra were recorded in the range of 4000–400 cm^{-1} .

2.5. X-ray diffraction (XRD)

The nature (crystalline/amorphous) of the formulation was analyzed with XRD. The XRD peaks of *TK* were recorded in the range of 5–100 (2θ) using $\text{CuK}\alpha$ radiation ($\lambda = 1.5418 \text{ \AA}$). All diffraction patterns were measured by XRD and compared with joint committee on powder diffraction standards (JCPDS) library data.

2.6. Microscopic and elemental characterization

A pinch of sample was sprinkled on a double side carbon tape and mounted on aluminum stubs that were placed inside an airtight chamber. The selected portion was analyzed through SEM (Carl Zeiss EVO 18 special edition microscopy) and EDX under ultra-high vacuum (Oxford instruments energy dispersive X-ray micro-analysis system) to examine morphological and elemental composition.

2.7. Size distribution

The particle size, distribution and zeta potential (surface charge) of *TK* formulation was determined using an electroacoustic environment, where the oscillation of charged particles in aqueous solution generates an alternate electric field to colloidal vibration current [14]. The size of the particles (Zetasizer, Malvern Instruments, UK) and its distribution (polydispersity index (PDI)) were measured.

3. Results and discussion

The *TK* formulation was sparingly soluble in solvents such as water, DMSO and ethanol due to its sulphur content as confirmed by qualitative test. When it is oxidized, it increases the solubility by interacting with hydroxyl groups of water molecules [6]. With this understanding, further analyses were quantitatively done to assess elemental composition as well as structural characterization.

3.1. Spectral and structural characterization of *TK*

The UV–visible spectroscopy is used to characterize metals and minerals. The UV–vis absorption spectrum of the *TK* formulation is shown in Fig. 1a. The formulation exhibits a characteristic peak at 290 nm. This confirms the presence of As. However, there are more distracted peaks, which were compared with standard reference spectra of National Institute of Standards and Technology (NIST) atomic spectra database (Fig. 1b) [15–18]. This further confirms the mixed nature of arsenic, such as As (I, II, III and IV). This almost matches with the published results of Karayunlu and Ay [19].

The FTIR spectrum of *TK* formulation is showed in Fig. 2. The presence of metal and metal salts in the formulation can be inferred from inter-atomic vibrations (in fingerprint region). The peaks were observed at 540, 802.23, 1033.77, 1643.24, and 3433.06 cm^{-1} , respectively. The finger print region was assigned as 540 S–S (disulfide), 802.23 As, As–H (C–H bend) and 1033.77 C=S [20]. The peaks at 3433.06, and 1643.24 were identified and assigned to –OH stretching and bending, respectively. This may be due to atmospheric

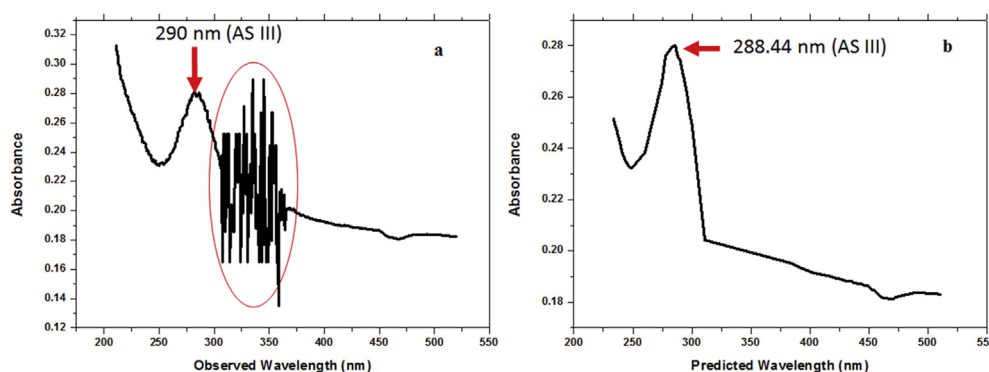


Fig. 1. The observed (a) and predicted (b) UV–visible spectra of *TK* formulation.

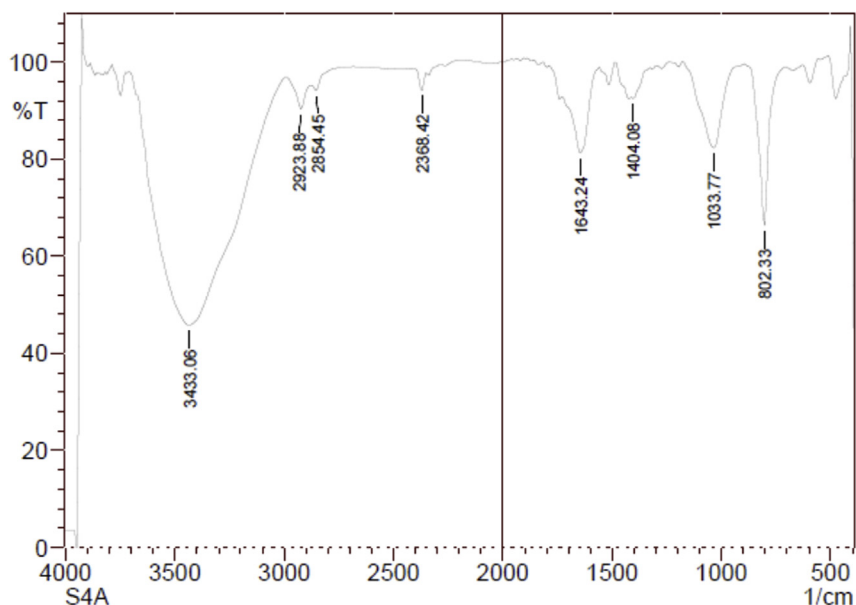


Fig. 2. Infrared transmission spectra of TK formulation.

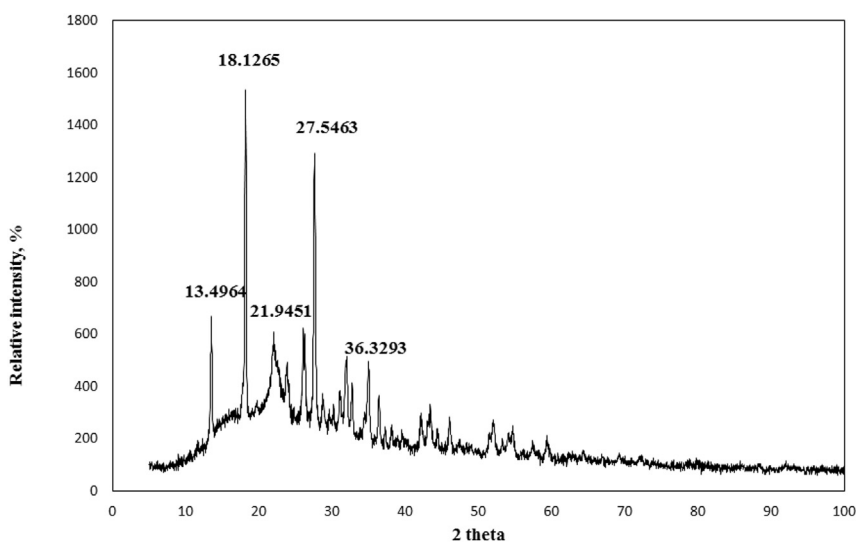


Fig. 3. The XRD pattern of TK formulation.

water molecules (moisture content) during sample handling. The observed peaks correspond to inorganic metal and its salt [13]. There are no other organic compounds present [21,22]. This ascertains the purity of the sample during *pudam* (calcination) process.

The crystalline nature and grain size were investigated through XRD. The crystallite size was calculated from the full width half maximum data of the XRD peaks. The average grain size is found to be 30.11 nm using Scherrer formula, $D = 0.9\lambda/\beta \cos\theta$, where D is the particle size, λ the wavelength, β , θ are the half-width of X-ray diffraction lines and half diffraction angle of 2θ . The diffraction pattern of the formulation is shown in Fig. 3. The diffraction pattern were compared with the JCPDS data (Table 1). The peaks observed (2θ) at 18.12, 22.54 and 36.32 are signatures of As_2S_3 as per JCPDS data (No: 19-0084). The peak which appears at 18, corresponds to orpiment (As_2S_3) [23]. The peaks (2θ) at 13.49, 21.94, 27.54, 28.75, and 32.69 were compared with JCPDS data and found to be As (rhombohedral) with average intensity of 90 (arbitrary unit). The

peaks (2θ) observed at 18.12, 26.04, 31.01, 42.06, 46.01, and 54.62 were identified as As_2S_3 , (monoclinic). The appearance of peak at 26.04 (2θ) corresponds to arsenolite, As_2O_3 (JCPDS No: 02-0530).

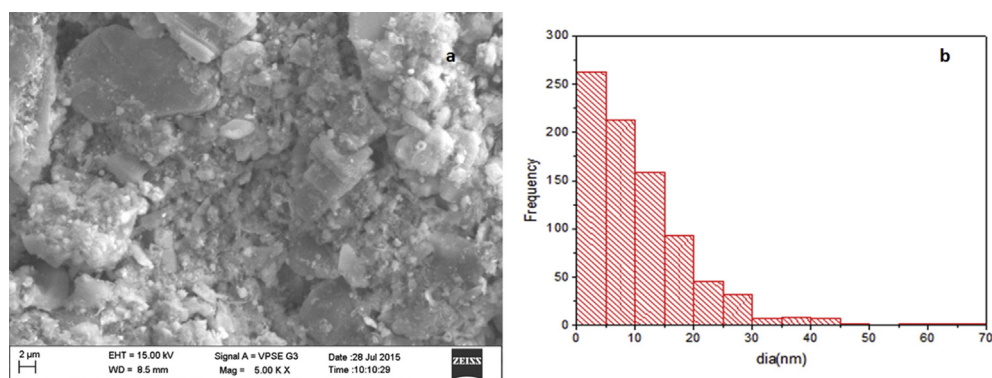
3.2. Morphological and elemental characterization of TK

The morphological and elemental composition were analyzed using SEM and EDX. Nanoparticles were observed as heterogeneous mixture of size ranging between 0.5 and 60 nm (refer Fig. 4B). This may enhance permeability and efficacy. They were anisotropic and distributed as spherical to plate-like structures (Fig. 4a). The plate-like structures are of interest due to high surface to volume ratios when compared to spherical structures. Moreover, the shape anisotropy plays an important role in their chemical reactivity [24]. The appearance of pores inside the platelets may indicate aggregation of tiny particles [25]. The pores also support a corrugated layered structure of orpiment [26].

Table 1

The validation of experimental data with JCPDS database.

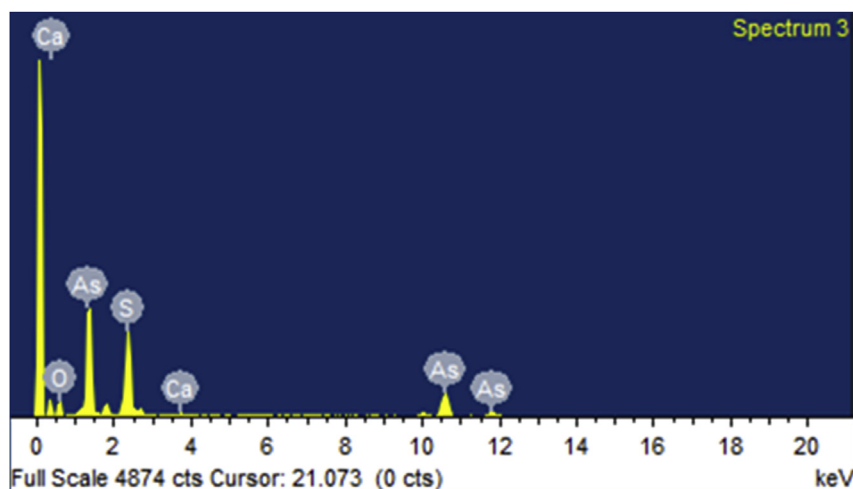
2-theta (deg)	Predicted value	Int-f	Face	Compound	Size (nm)	System	JCPDS PDF number
13.49	14.75	100	(012)	As	35.34	Rhombhohedral	01-1019
18.12	18.12	100	(020)	As ₂ S ₃	45.30	Monoclinic	19-0084
21.94	21.73, 21.85	20, 90	(110)	As	5.035	Rhombhohedral	01-1019, 02-0872
26.04	26.44	90	(232)	As ₂ O ₃	38.10	Monoclinic	02-0530
26.27	26.27	8	(202)	As	53.17	Rhombhohedral	01-1019
27.54	27.70	30	(211)	As ₂ S ₃	31.91	Monoclinic	19-0084
28.75	28.67	60	(101)	As	30.67	Rhombhohedral	03-0749
31.00	30.94	20	(400)	As ₂ S ₃	30.35	Monoclinic	19-0084
31.91	31.64	30	(202)	As	22.43	Rhombhohedral	02-0872
32.69	32.05, 32.27	100,100	(012), (004)	As	34.75	Rhombhohedral	03-0749, 05-0632
42.06	42.22	10	(311)	As ₂ S ₃	25.71	Monoclinic	19-0084
43.33	43.15	30	(217)	As	13.64	Rhombhohedral	02-0872
44.34	44.13	60	(104)	As	53.83	Rhombhohedral	03-0749
46.01	46.09	5	(212)	As ₂ S ₃	30.73	Monoclinic	19-0084
52.03	52.29	50	(223)	As	13.28	Rhombhohedral	03-0754
54.62	54.31	30	(441)	As ₂ S ₃	23.98	Monoclinic	19-0084
57.35	57.95	50	(116)	As	32.85	Rhombhohedral	03-0754

**Fig. 4.** The SEM monograph (a) and the histogram of particle size distribution (b) of TK formulation.

The selected portion of the EDX spectrum reveals the presence of As (46.65%), S (29.02%), O (23.41%) and Ca (0.92%) (Fig. 5). The results suggest that the formulation may contain As, S and O. The proportion of sulphur is more than oxygen to bind with As. Although arsenic may form covalent bonds with oxygen as well as sulfur, the atom % suggests that the formulation may contain predominantly connected to sulfur atoms, either as orpiment (As₂S₃)

or realgar (As₂S₄), and less likely As₂O₃. The nature of bonding and atomic arrangements of both orpiment and realgar may be related to their stability, as they are stable over a wide range of temperature [27].

The Zeta-potential measurements were performed to study colloidal stability of TK formulation (Fig. 6A). The measured average size of the formulation was found to be 821.8 nm and the

**Fig. 5.** The EDX spectra of TK formulation.

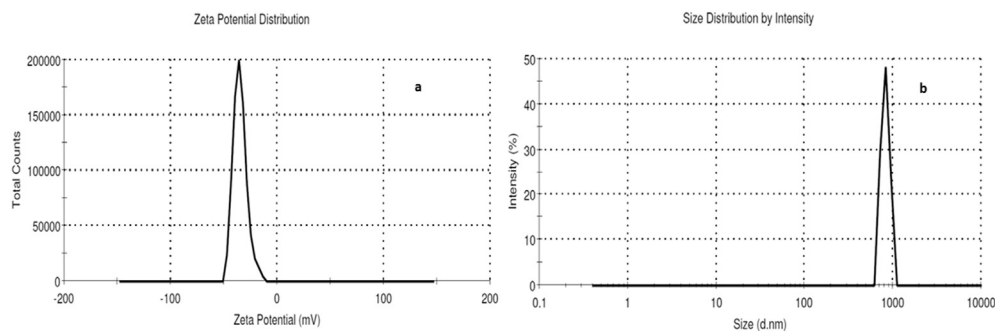


Fig. 6. Zeta potential (a) and size distribution (b) of TK formulation.

hydrodynamic diameter of approximately 87 nm (Fig. 6B). This is in agreement with the reported realgar nanoparticles with a difference in size [25]. PDI of the formulation 0.99, showed a relative variance in the particle size distribution (PDI > 0.7) [28]. This also confirms that the shape and size heterogeneity due to mixed nature of arsenic (as in the SEM images, Fig. 4A).

The structural, elemental and morphological analyses suggest that arsenic may predominantly exist either orpiment (As_2S_3) or realgar (As_2S_4). The possibility is less for the toxic arsenolite (As_2O_3) as inferred from EDX spectrum. The oxidized form is probably due to calcination. However, it is not clear, whether arsenolite exists as individual particles or in an alloy form along with orpiment or realgar? It is presumed that realgar and orpiment forms the core with arsenolite on the surface. However, further studies are required in this direction to confirm.

4. Conclusion

The characterized traditional therapeutic formulation is a complex mixture of arsenic compounds. The size and shape heterogeneity was observed for the mixture (as confirmed by polydispersity index). The formulation may be considered as safe because it contains very less oxide form. The main limitation of the study is there is no proper control specimen(s). Hence, this is a major challenge for metal mixtures, as they vary from batch-to-batch. Moreover, there are no additional specimens available in the market for ensuring the elemental composition. Further studies on standardization are required to confirm safety and efficacy.

Conflict of interest

There are none. This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

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References

[1] IUCN red list of threatened species. Cambridge, UK: International Union for Conservation of Nature and Natural Resources; 2007. www.iucnredlist.org.

- [2] Barathi S, Amuthan A. *Siddhars and Siddha Medicine: the forgotten Indian saints and their medical knowledge*. University News; 2013. p. 41–53. ISSN 0566-2257 51.
- [3] Ernst E. Toxic heavy metals and undeclared drugs in Asian herbal medicines. *Trends Pharmacol Sci* 2002;23:136–9.
- [4] Cooper K, Noller B, Connell D, Yu J, Sadler R, Olszowy H, et al. Public health risks from heavy metals and metalloids. *J Toxicol Environ Health A* 2007;70:1694–9.
- [5] Balaz P, Fabian M, Pastorek M, Cholujova D, Sedlak J. Mechanochemical preparation and anticancer effect of realgar As_4S_4 nanoparticles. *Mater Lett* 2009;63:1542–4.
- [6] Miller Jr WH, Schipper HM, Lee JS, Singer J, Waxman S. Mechanisms of action of arsenic trioxide. *Cancer Res* 2002;62:3893–903.
- [7] Saper RB, Kales SN, Paquin J, Burns MJ, Eisenberg DM, Davis RB, et al. Heavy metal content of ayurvedic herbal medicine products. *J Am Med Assoc* 2004;2868–73.
- [8] Mitra A, Chakraborty S, Auddy B, Tripathi P, Sen S, Saha AV, et al. Evaluation of chemical constituents and free radical scavenging activity of *Swarna bhasma* (gold ash), an Ayurvedic drug. *J Ethnopharmacol* 2002;80:147–53.
- [9] Panda AK, Hazra J. Arsenical compounds in Ayurveda medicine: a prospective analysis. *Int J Res Ayur Pharm* 2012;3:772–6.
- [10] Muthaliar KNK, Uttamarayan KS. *Siddha Pharmacopoeia*, 1987. Parinilayam, Chennai, pp. 160.
- [11] Essential drugs list-Siddha. New Delhi: Department of AYUSH, Ministry of Health & Family Welfare, Government of India; March 2013, ISBN 978-93-81458-11-2. p. 10. www.indianmedicine.nic.in.
- [12] Thiyagarajan R. Introduction, apparatus, drugs, nine gem stones. In: Anandan Anaivari R, Thulasimani M, editors. *Siddha materia medica (mineral & animal section)*. 1st ed., vol. 1. Chennai: Dept of Indian Medicine and Homeopathy; 2008. p. 24–7. 54, 56, 63, 373–374.
- [13] Unni K, Karalam BS, Vijayasankar AV. Scientific basis for the preparation and characterization of iron based traditional drug *Annabhedhi sindooram*: a materialistic approach. *Int J Res Ayur Pharm* 2013;4:149–52.
- [14] Dukhin AS, Goetz PJ. *Ultrasound for characterizing colloids: particle sizing, zeta potential, rheology*. New York: Elsevier Science; 2002.
- [15] Meggers WF, Shenstone AG, Moore CE. First spectrum of arsenic. *J Res Natl Bur Stand (US)* 1950;45:346–56. and NIST ASD Team. NIST Atomic Spectra Database (ver. 5.2), [Online]. National Institute of Standards and Technology, Gaithersburg, MD. Available: <http://physics.nist.gov/asd>, 2014.
- [16] Rao KR. The third spark spectrum of arsenic (As IV). *Proc R Soc Lond Ser A* 1932;134:604–13. and NIST ASD Team. NIST Atomic Spectra Database (ver. 5.2), [Online]. National Institute of Standards and Technology, Gaithersburg, MD. Available: <http://physics.nist.gov/asd>, 2014.
- [17] Li H, Andrew KL. First spark spectrum of arsenic. *J Opt Soc Am* 1971;61:96–109. and NIST ASD Team. NIST Atomic Spectra Database (ver. 5.2), [Online]. National Institute of Standards and Technology, Gaithersburg, MD. Available: <http://physics.nist.gov/asd>, 2014.
- [18] Lang RJ. On the spectra of doubly ionized arsenic, antimony and Bismuth (As III, Sb III, Bi III). *Phys Rev* 1928;32:737–45. and NIST ASD Team. NIST Atomic Spectra Database (ver.5.2), [Online]. National Institute of Standards and Technology, Gaithersburg, MD. Available: <http://physics.nist.gov/asd>, 2014.
- [19] Karayunlu S, Ay U. Spectrophotometric determination of total inorganic arsenic with hexamethylene ammonium-hexamethylene dithiocarbamate in nonionic triton X-100 micellar media. *J Anal Chem* 2010;65:244–8.
- [20] Brown CL, Bushell G, Whitehouse MW, Agrawal DS, Tupe SG, Paknikar KM, et al. Nanogold-pharmaceutics. *Gold Bull* 2007;40:245–50.
- [21] Iovu MS, Shutov SD, Andriesh AM, Kamitsos EI, Varsamis CPE, Furniss D, et al. Spectroscopic studies of bulk As_2S_3 glasses and amorphous films doped with Dy, Sm and Mn. *J Optoelectron Adv Mater* 2001;3:443–54.
- [22] Chen P, Yan L, Wang Q, Li H. Arsenic precipitation in the bioleaching of realgar using *Acidithiobacillus ferrooxidans*. *J Appl Chem* 2013:424253–8.
- [23] Bujnakova Z, Balaz P, Makreski P, Jovanovski G, Caplovcova M, Caplovic L, et al. Arsenic sulfide nanoparticles prepared by milling: properties, free-volume characterization and anti-cancer effects. *J Mater Sci* 2015;50:1973–85.

- [24] Yin Y, Erdonmez C, Aloni S, Alivisatos AP. Faceting of nanocrystals during chemical transformation: from solid silver spheres to hollow gold octahedral. *J Am Chem Soc* 2006;128:12671–3.
- [25] An YL, Nie F, Wang ZY, Zhang DS. Preparation and characterization of realgar nanoparticles and their inhibitory effect on rat glioma cells. *Inter J Nanomed* 2011;6:3187–94.
- [26] Mullen DJE, Nowacki W. Refinement of crystal-structures of realgar, AsS and orpiment, As₂S₃. *Z Fur Kristallogr* 1972;136:48–65.
- [27] Nordstrom DK, Archer DG. Arsenic thermodynamic data and environmental geochemistry. In: Welch AH, Stollenwerk KG, editors. *Arsenic in groundwater: geochemistry and occurrence*. Boston: Kluwer Academic Publishers; 2003. p. 1–25.
- [28] Nidhin M, Indumathy R, Sreeram KJ, Nair BU. Synthesis of iron oxide nanoparticles of narrow size distribution on polysaccharide templates. *Bull Mater Sci* 2008;31:93–6.