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Properties of arsenic sulphide As_4S_4 nanoparticles prepared by high-energy milling

Peter Baláž [a,*], Anh V. Nguyen [b], Martin Fabián [a], Danka Cholujová [c], Michal Pastorek [c], Ján Sedlák [c], Zdenka Bujňáková [a]

[a] Institute of Geotechnics, Slovak Academy of Sciences, 043 53 Košice, Slovakia
[b] University of Queensland, Division of Chemical Engineering, Brisbane QLD 4072, Australia
[c] Cancer Research Institute, Slovak Academy of Sciences, 833 91 Bratislava, Slovakia

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ABSTRACT

In this study, the nanosized arsenic sulphide As_4S_4 particles have been prepared by mechanical activation in a planetary mill. The bulk and surface properties of the milled particles were characterized by XRD and XPS methods as well as by surface area and particle size distribution measurements. For assessment of arsenic sulphide biological activity, the viability tests of multiple myeloma cancer cells have been applied. The obtained results show the arsenic sulphide nanoparticles properly milled and modified by mechanical activation can be valuated as a potential drug for cancer treatment.

1. Introduction

Over the years, arsenic compounds have found application in the manufacture of cosmetics, foods, glass, insecticides, pigments, pyrotechnics, metallurgy as well as in medicine [1]. Arsenic sulphides such as arsenopyrite FeAsS, realgar As_4S_4 and orpigment As_2S_3 are of significant interest in minerals engineering because of their association with gold [2,3]. The interesting optical properties have been described for arsenic sulphides as a consequence of their sensibility to light exposure [4–9]. This sensitivity is pronounced in disordered solids and has found applications in optoelectronic materials [10,11]. Among these advanced materials arsenic sulphide As_4S_4 seemed to be the very promising material.

In 1878, the anticancer activity of arsenic compounds was reported from Boston hospital where the effect of Fowler´s solution (1% As_2O_3 in K_2CO_3) on the reduction of white blood cell counts in leukemia patients was described [12]. Today arsenic oxide As_2O_3 (brand name Trisenox) is applied in treatment of acute forms of leukemia [13–15]. However, its toxicity is high and a drug form posses severe side effects. The other forms of arsenic are also under consideration [12]. Arsenic sulphide As_4S_4 is much less toxic than arsenic oxide As_2O_3 (acute oral toxicity LD_{50} is 3.2 g/kg and 32 mg/kg for As_4S_4 and As_2O_3, respectively) and the mineral form of As_4S_4 (realgar) was frequently used in Chinese medicine for treatment of various non-cancer diseases [16]. However, its solubility is low and some type of pre-treatment is needed to enhance the solubility and/or to prepare nanosized particles with the better bioavailability and curring effect.

Mechanochemistry concerns with a broad spectrum of solids having applications in chemistry, agriculture, minerals engineering and materials science [2,17–20]. Several aspects of mechanochemistry like bulk disordering, polymorphous transformations and preparation of nanocrystalline powders by means of milling techniques have brought new impacts into pharmaceutical science [21–25]. Here, the increase in dissolution velocity and the better bioavailability play crucial role in the application of new drugs [26–29].

It is aim of this paper to examine the changes in solid state properties and anticancer effect of As_4S_4 arsenic sulphide particles prepared by high-energy milling.

2. Experimental

2.1. Materials

The investigation was carried out with arsenic sulphide sample (98% As_4S_4 in purity, Sigma-Aldrich, USA). The XRD analysis estimated arsenic sulphide As_4S_4 (JCPDS 48-1247). Small amounts of zinc have been estimated in the sample by EDX and XPS method.

2.2. Washing

In order to obtain the pure arsenic sulphide the washing procedure according to [30] has been applied. The sample (5–6 g) was suspended in

* Corresponding author.
E-mail address: balaz@saske.sk (P. Baláž).

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200 mL of distilled water at pH of about 6–7 for 5 min. Then several drops of HCl were added adjusting pH of the realgar suspension to pH about 4–4.5. The suspension was mixed for 15 min. The resultant supernatant was decanted and after filtration the solid was again poured into distilled water. The procedure was repeated until arsenic concentration in said supernatant is less than 0.5 mg/L. Then the remained sediment was washed with aqueous solution at pH about 6–7 and dried said powder of arsenic sulphide in vacuum drier at 105 °C for 1 h.

2.3. Milling

The high-energy milling of arsenic sulphide samples was performed in a planetary mill Pulverisette 6 (Fritsch, Germany). The following milling conditions were used: loading of the mill: 50 balls from tungsten carbide of 10 mm diameter; rotation speed of the planet carrier: 500 rpm; milling times: 1–20 min; milling in a wet mode with protective argon atmosphere; by milling 200 mL of 0.075% sodium dodecylsulphate as surfactant was applied; ball charge 370 g, sample charge 3 g; solid/liquid ratio 15 g/L (3 g arsenic sulphide + 200 mL liquid). 0.075% sodium dodecylsulphate solution had been prepared by dissolution of 0.75 g of dodecylsulphate in 1000 mL of water. From this volume 200 mL was applied for each milling experiment.

2.4. Characterization

The XRD measurements were performed by employing an X-ray diffractometer Rigaku Miniflex (Rigaku, Japan). The following measuring conditions were applied: 2Θ range 0–40°, Co radiation, scan speed: 2° 2 theta/min, scan step 0.2 theta 2 theta.

The specific surface area was determined by the low temperature nitrogen adsorption method in a Gemini 2360 sorption apparatus (Micromeritics, USA).

The particle size distribution was measured by the method of photon cross correlation spectroscopy on particle sizer Nanophox (Sympatec, Germany).

The XPS spectra were collected using a Kratos Axis Ultra X-ray photoelectron spectrometer (Shimadzu, Japan) with Kα excitation from Al anode operated at 15 kV and 10 mA.

Suspensions of the arsenic sulphide nanoparticles for testing their biological activity have been prepared by filtration of wet milled samples through 0.22 μm filter. For testing in vitro, human multiple myeloma cell lines (OPM1, RPM1-LR5) were cultured in RPM1 1640 medium supplemented with 10% fetal calf serum as suspension cell cultures. All cells were maintained in 6-cm dishes in a humidified incubator at 37 °C with 5% CO_2. Effect of milled As_4S_4 (serial dilutions) on survival of cells was determined by a MTT assay [31]. The number of viable cells is proportional to the content of MTT metabolite-formazan. The absorbance of DMSO dissolved formazan crystals was measured at 540 nm and 690 nm in a microplate reader (Dynatech Lab Inc., Chantilly, VA, USA). The concentration of arsenic that inhibited cell survival to 50% (IC_{50}) was determined by Calcusyn software. For cytofluorimetric analysis of mitochondrial transmembrane potential cells (5×10^5) were incubated in 400 μl of PBS, 0.2% BSA containing 4 μM of JC-1 (from a 7.7 mM stock in DMSO) for 30 min at 37 °C. After 30 min incubation in the dark at 37 °C, the cells were measured using a flow cytometer. Data acquisition was done on Coulter Epics Altra flow cytometer equipped with 488 nm excitation laser. For each analysis, 1×10^4 cells were acquired as described [32]. Data were analysed with FCS software (DeNovo Software).

3. Results and discussion

3.1. Surface changes

The comminution of As_4S_4 particles by high-energy milling is accompanied by an increase in their number by generation of fresh surfaces. The dependance of the specific adsorption area increase and percentage of particles less than 10 μm on the time of mechanical activation (wet milling) is represented in Fig. 1. The traces show that the rate of new surface area and small particle formation are similar and limited by time of milling. After the first stage where increase in values of S_A and Q_{10} is sharp ($t_M \leq 3$ min), the second stage issteady. Here, the formation of micrometer particles as well as new surface area formation is manifested. The samples were milled in presence of surfactant and it is a reason why a standstill was observed with increasing milling time instead of decrease of S_A and Q_{10} values as obtained in our previous work where dry milling has been applied [33].

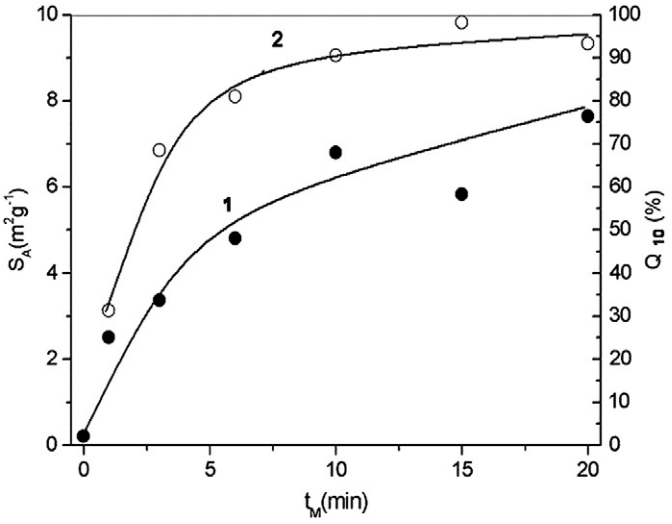

Fig. 1. Specific surface area, S_A (1) and percentage of As_4S_4 particles less than 10 μm, Q_{10} (2) vs. milling time, t_M.

The size distribution of arsenic sulphide nanoparticles is given in Fig. 2. The non-milled sample shows broad poly-modal distribution of

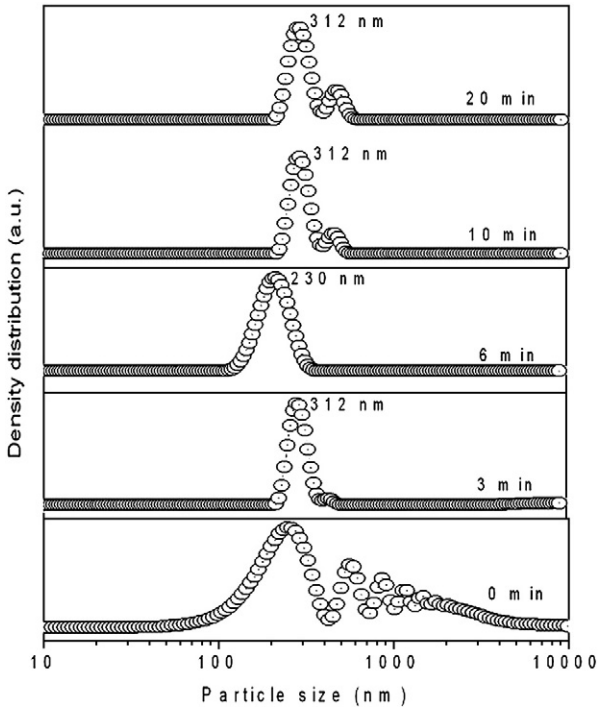

Fig. 2. Size distribution of milled As_4S_4 nanoparticles (milling time is given on curves).