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Stability studies of As_4S_4 nanosuspension prepared by wet milling in Poloxamer 407

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ABSTRACT

In this paper the stability of the arsenic sulfide (As_4S_4) nanosuspension prepared by wet milling in a circulation mill in the environment of copolymer Poloxamer 407 was studied. The obtained As_4S_4 particles in nanosuspension were of ~100 nm in size. The influence of temperature and UV irradiation on the changes in physical and/or chemical properties was followed. Long-term stability was observed via particle size distribution and zeta potential measurements. Influence of UV irradiation was studied via UV–vis spectroscopy (UV–vis), photoluminicsence (PL) technique and Fourier transform infrared spectroscopy (FTIR) measurements. The best stability of the nanosuspension (24 weeks) was achieved when stored at 4 °C and in the dark.

1. Introduction

The preparation of hydrophobic drugs in the form of nanosuspensions represents a challenging and potentially useful method for improving their bioavailability. Nanosuspensions are defined as liquid sub-micron colloidal dispersions of nanosized drug particles that are stabilized by a suitable polymer and/or surfactant (Rabinov, 2004). Several technologies have been developed for preparation of nanosuspensions (e.g., milling, high-pressure homogenization, impinging jet, electro-spraying, liquid-based methods and supercritical fluid processes) (Wang et al., 2013). Among these, wet media milling is considered an attractive approach that permits relatively easy scale-up with respect to industrial pharmaceutical nanosuspension production (Merisko-Liversidge and Liversidge, 2011). During the milling process, the drug crystals break into smaller particles, and thus fresh surfaces are continuously generated (Baláž, 2008).

In nanosuspensions, water-insoluble drugs become more soluble because of increasing the saturation solubility and the surface area available for dissolution. On the other hand, a nanosuspension is a thermodynamically unstable colloid dispersion system. A high surface area associated with the small size of the particles results in high interfacial tension, which in turn results in an increase in the free energy of the system. The high surface energy of nano-sized crystals results in particle size growth, a phenomenon known as Ostwald ripening (Boistelle and Astier, 1988). During this process, coarse particles grow at the expense of the redissolution of fine particles (Kim, 2004), which are more soluble than large ones and mass transfer occurs from the fine to coarse particles (Liu et al., 2011). To hinder this phenomenon and consequently aggregation, the electrostatic or steric stabilizers are needed.

Arsenic sulfide compounds have a long history of application in a traditional medicine (Wang, 2001). In recent years, they have been studied as promising drugs in cancer treatment (Wu and Ho, 2006; Ye et al., 2006; Baláž et al., 2009, 2012; Baláž and Sedlák, 2010; Wu et al., 2011; Yuan et al., 2013; Tian et al., 2014; Pastorek et al., 2014). In this paper the stability of the arsenic sulfide nanosuspension stabilized with Poloxamer 407 (PX407) was studied. PX407 is a nonionic surfactant composed of polyoxyethylene–polyoxypropylene triblock copolymers. It has a good solubilizing capacity and is considered a good medium for drug delivery systems with the least toxic properties of commercially available copolymers (Gilbert et al., 1986; Escobar-Chávez et al., 2006). It has been reported that both hydrophobic and hydrophilic properties of stabilizers are required for ensuring the appropriate stability. The driving force of the adsorption of stabilizers onto the surface of a hydrophobic drug is the hydrophobic moieties of the stabilizers. In the absence of adsorption, stabilization cannot occur, and no dispersed nanosuspensions can be obtained (Wang et al., 2013). The hydrophilic portion of the stabilizer tends to orient toward water providing an effective steric stabilization. In the case of PX407, central polyoxypropylene chain is hydrophobic (b) flanked by two hydrophilic chains of polyoxyethylene (a) (Fig. 1).

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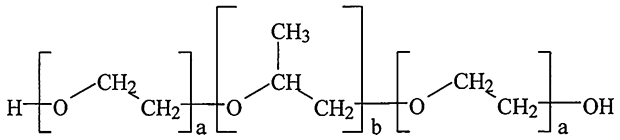

Fig. 1. Chemical structure of Poloxamer 407 (a-ethylene oxide portion, b-propylene oxide portion).

Although, the arsenic sulfides seem to be very promising anticancer agent, there is lack of information about stability of these nanosuspensions in the literature. This is the main aim of this study.

2. Materials and methods

2.1. Material

The investigation was carried out with arsenic sulfide (98%, Sigma–Aldrich). Poloxamer 407 (Sigma–Aldrich) was applied as a stabilizer.

2.2. Preparation of nanosuspensions

The nanosuspensions were prepared in a laboratory circulation mill MiniCer (Netzsch, Germany). Five grams of arsenic sulfide were subjected to milling in the presence of 300 ml PX407 water solution (0.5%) for duration of 2 h at the milling speed 4000 rpm. The mill was loaded with yttrium stabilized ZrO_2 milling balls. The resulting nanoparticle suspension was filtered through a 0.22 μm sterile filter. After that, the nanosuspension was divided into two parts and stored in dessicator at laboratory temperature (21 ± 1 °C) or in refrigerator (4 °C). The solid phase was subjected to dissolution tests.

2.3. Dissolution experiments

The dissolution experiments were performed stirring 100 mg of solid phase into 100 ml of water (final pH 5.3) at 4, 21 and 36.5 °C for 120 min. Aliquots of the solution were withdrawn at appropriate time intervals to determine of the dissolved arsenic content by atomic absorption spectroscopy (SPECTRAA L40/FS, Varian, Australia).

2.4. UV irradiation

For UV irradiation, a 30 W mercury lamp emitting predominantly light at wavelength 254 nm was used. Six samples were irradiated in 2 ml acrylic cuvettes. The total time of irradiation was 6 h. Every hour one sample was taken out and the particle size distribution, FTIR, UV–vis and PL spectra were immediately measured.

2.5. Characterization of nanosuspensions

2.5.1. Particle size analysis

The particle size distribution was measured by photon cross-correlation spectroscopy using a Nanophox particle size analyzer (Sympatec, Germany). A portion of each nanosuspension was diluted with stabilizer containing solution to achieve a suitable concentration for measurement. This analysis was performed using a dispersant refractive index of 1.33. The measurements were repeated 4 times for each sample.

2.5.2. Zeta potential measurement

The zeta potential was measured using a Zetasizer Nano ZS (Malvern, Great Britain). The Zetasizer Nano measures the electrophoretic mobility of the particles, which is converted into the zeta potential by using the Helmholtz–Smoluchowski equation built into the Malvern Zetasizer software. The zeta potential was measured in the original dispersion medium. The measurements were repeated 3 times and the mean values are reported.

2.5.3. UV–vis spectroscopy

The optical spectra were recorded using a UV–vis spectrophotometer Helios Gamma (Thermo Electron Corporation, Great Britain) in the range 200–800 nm.

2.5.4. Photoluminiscence spectroscopy

The photoluminiscence (PL) spectra at room temperature were acquired at right angle on a photon counting spectrofluorometer PC1 (ISS, USA) with an excitation wavelength of 365 nm. A 300 W xenon lamp was used as the excitation source. The emission was collected in a 25 cm monochromator with a resolution of 0.1 nm equipped with a photomultiplier.

2.5.5. FTIR spectroscopy

The FTIR spectra were recorded using a Tensor 29 infrared spectrometer (Bruker, Germany) with TRANS and ATR method.

3. Results and discussion

3.1. Characterization of nanosuspension immediately after milling

As can be seen from Table 1 the average particle size (x_{50}) of the prepared nanosuspension was 115 nm and the largest particle size (x_{99}) did not exceed 170 nm. Zeta potential had the value −12.9 mV. The pH of the suspension was 5.3.

For consideration of the interaction between As_4S_4 and PX407, the FTIR spectroscopy was used. The characteristic peaks of PX407 were found at 2889, 1348 and 1103 cm^{-1} due to —C—H stretch, in plane —O—H bend and —C—O stretch, respectively (Fig. 2, black line). Since the —C—H bond stretching modes are sensitive to environmental and conformational change (Guo et al., 1999), the ~2800–3000 cm^{-1} region is employed to monitor changes after the reaction. The shift of —C—H bond stretching bands from 2889 cm^{-1} to 2863 cm^{-1} indicates more hydrophobic microenvironment in polyoxypropylene centre block of PX407 (Storm et al., 1995; Chen et al., 2006; Liu et al., 2010; Shou et al., 2011), resulted from the adsorption of PX407 molecules onto the surface of hydrophobic As_4S_4 nanoparticles (Fig. 2, red line). This mode of adsorption leaves the hydrophilic polyoxyethylene side-arms in a mobile state because they extend outwards from the particle surface. These side-arms provide stability to the particle suspension by a repulsion effect through a steric mechanism of stabilization, involving both enthalpic and entropic contribution (Moghimi et al., 1993; Li and Caldwell, 1996; Moghimi and Hunter, 2000). Besides the shift of —C—H stretch, no other shifts were observed after the adsorption process.

Table 1
Peoperties of As_4S_4 nanosuspension after milling.

Compound	Stabilizer	Properties		
		Particle size (nm)	Zeta potential (mV)	pH
Arsenic sulfide	0.5% PX407	x_{50} = 115 x_{99} = 170	−12.9	5.3