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Ultrasound irradiation of hydrothermally engineered sub-20 nm PbS nanoparticles and effect of their size on *Aspergillus* species morphology

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Experimental Section

Chemicals and instruments

Lead nitrate and thiourea were purchased from Sigma-Aldrich, while other organics for the synthesis of 2-(2-chlorophenylamino)benzoic acid (ArCOOH) were purchased from commercial suppliers. Elemental CHN data were obtained using an element analyzer "elementar Analysensysteme GmbH-vario EL III". The vibrational transitions data were detected on a Nicolet iS10 FTIR spectrophotometer and the nuclear magnetic resonance data were investigated relative to tetramethylsilane on a Bruker spectrometer (400 MHz) in DMSO-d_6. Thermal analysis data (TGA & DTA) of $PbL_2.H_2O$ were determined on a thermal analyzer "Shimadzu DTG 60-H" under the heating rate of 10 °C min^{-1} and airflow of 40 ml min^{-1}. An ultrasonic generator "Sonics Vibracell, SONICA-2200, USA" utilizing a frequency of 20 kHz and power output of 750 W was used for preparing *h,us*-PbS NPs. Investigation of the NPs' composition and packing system was done by employing a Philips PW 1710 X-ray diffractometer that works over the 2θ range of 20–90° with a step size of 0.06° and utilizes 40 kV, 40 mA and CuKα radiation (λ = 1.54060 Å). TEM images of *h*-PbS and *h,us*-PbS NPs for detection of their morphological shape and size were captured by a TECNAI G^2 spirit TWIN microscope utilizing 120 kV and VELETA camera. The NPs' porosity and surface area were determined at liquid nitrogen temperature via a NOVA 3000 (version 6.10) high speed gas sorption analyzer.

Synthesis of the nanoparticles precursor

A mixture that contained ArCOOH (1.98 g, 8 mmol), potassium hydroxide (448 mg, 8 mmol), lead nitrate (1.32 g, 4 mmol) and aqueous ethanol (50%, 200 ml) was stirred and refluxed for an hour. The product was filtered, washed with boiling water and ethanol and dried over anhydrous calcium chloride. $(ArCOO)_2Pb{\cdot}H_2O$ (yield = 2.13 g, 74 %): Anal. Calcd. (Found) for $PbC_{26}H_{20}N_2O_5Cl_2$ (MW = 718.55 g/mol), C = 43.46 (42.97) %, H = 2.81 (2.40) % and N = 3.90 (4.17) %. FT-IR (KBr, cm^{-1}) = 3439 υ_{water}(OH), 1610 υ_{asym}(COO) and 1485 υ_{sym}(COO). Molar conductance (DMF, $\Omega^{-1}cm^2mol^{-1}$) = 5.21.

Synthesis of h-PbS

A suspension of $(ArCOO)_2Pb{\cdot}H_2O$ (2.0 g, 2.78 mmol) in water (150 ml) was mixed with thiourea (2.12 g, 27.86 mmol), and the mixture was stirred for 10 min before transferring it into a 200 ml Teflon-lined stainless-steel autoclave. The autoclave was then transferred to a preheated oven at 160 °C and left at this temperature for 24 hours. Afterwards, the autoclave was cooled to the room temperature, the product was centrifuged, washed thrice with distilled water and ethanol and dried over anhydrous calcium chloride.

Synthesis of h,us-PbS NPs

A suspension of *h*-PbS (400 mg, 1.67 mmol) in milliliters of water (50 ml) was exposed to high power ultrasonication for fifteen minutes, while an ice bath was used to keep the medium temperature below 60 °C. The obtained nanoparticles were collected by centrifugation, washed thrice with distilled water and ethanol and dried over calcium chloride.

Microorganisms and inoculums preparation

Five *Aspergillus* species namely; *A. flavus* Link (Af-10), *A. fumigatus* Fresenius (Af-43), *A. niger* van Tieghem (An-14), *A. oryzae* (Ahlburg) Cohn (Af-45) and *A. terreus* Thom (At-40) were isolated on Czapek's dextrose agar medium from outdoors of an Assiut Governorate public hospital, Egypt. The isolates were identified according to their morphological and microscopic properties and maintained at 4±1 °C in the same medium. However, preceding the experiment, the isolates were re-cultured on Czapek's plats and incubated at 28±1 °C for three days.

Effect of h-PbS and h,us-PbS NPs on growth of Aspergillus species

Czapek's agar medium was prepared and autoclaved, and then chloramphenicol fortified with 0.22 mm sterile membrane was added. Cycloheximide, *h*-PbS NPs and *h,us*-PbS NPs (100 µg ml^{-1}) were added to the medium before vortexing using ZX vortex (VELP Scientifica, UK) for 5 minutes. Control samples contained no PbS NPs and cycloheximide. All treatments were poured into sterilized petri dishes and left for solidification in sterilized conditions. *Aspergillus* agar disk (5 mm and three days old) was added in the center of each plate. All plates were incubated at 28±1 °C for one week and the growth was estimated by measuring the extended growth distance of the growing mycelia in all plate directions. All experiments were carried out with three replicates and data are expressed in mm.

Microscopic features of Aspergillus species exposed to h-PbS and h,us-PbS NPs

The *Aspergillus* species in all treatments were examined morphologically for color and growth pattern changes and, using Olympus microscope CX41, Japan, for mycelia, phialid, vesicle and conidia abnormalities. The cultures were washed with alcohol and blue stained by lactophenol cotton blue before estimation by Olympus system for image analysis.