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Scalable production of microbially mediated zinc sulfide nanoparticles and application to functional thin films

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ABSTRACT

A series of semiconducting zinc sulfide (ZnS) nanoparticles were scalably, reproducibly, controllably and economically synthesized with anaerobic metal-reducing *Thermoanaerobacter* species. These nanoparticles reduced partially oxidized sulfur sources to sulfides that extracellularly and thermodynamically incorporated with zinc ions to produce sparingly soluble ZnS nanoparticles with ~5 nm crystallites at yields of ~5 g l^{-1} $month^{-1}$. A predominant sphalerite formation was facilitated by rapid precipitation kinetics, a low cation/anion ratio and a higher zinc concentration compared to background to produce a naturally occurring hexagonal form at the low temperature, and/or water adsorption in aqueous conditions. The sphalerite ZnS nanoparticles exhibited narrow size distribution, high emission intensity and few native defects. Scale-up and emission tunability using copper doping were confirmed spectroscopically. Surface characterization was determined using Fourier transform infrared and X-ray photoelectron spectroscopies, which confirmed amino acid as proteins and bacterial fermentation end products not only maintaining a nano-dimensional average crystallite size, but also increasing aggregation. The application of ZnS nanoparticle ink to a functional thin film was successfully tested for potential future applications.

1. Introduction

Zinc sulfide (ZnS) nanoparticles (NPs) have been intensively investigated for a wide range of applications, including solid-state lighting [1], high-definition flat screen displays [2], targeted cancer imaging [3] and cadmium sulfide (CdS) buffer layer replacement in solar cells to alleviate the environmental concerns associated with cadmium [4]. Cubic sphalerite ZnS has a band gap of 3.6 eV, which is closer to that of CdS (2.4 eV) than the hexagonal wurtzite ZnS, which has a band gap of 3.9 eV. Current conventional synthesis routes for ZnS include chemical precipitation [5], electro-explosion of wire [6], pulsed laser deposition at 600 °C [4], ultrasonic spray pyrolysis at 700 °C with argon [7], and high-energy milling [8].

Metal sulfide NPs have also been produced using endogenous methods such as heavy metal tolerance and detoxification processes such as reaction with hydrogen sulfide (H_2S) [9]. ZnS NPs have been produced by aerotolerant sulfate-reducing bacteria of the family *Desulfobacteriaceae* from <1 ppm Zn in bulk solution at low temperature (~8 °C) in an abandoned mine [10]. Framboidal aggregates of sphalerite and polytypic wurtzite nanocrystallites associated with dissimilatory (bi)-sulfite reductase genes, a key enzyme in sulfate respiration, were found in peatlands [11]. Torres-Martínez et al. [12] reported synthesis of hexagonal ZnS (10H structure) and ZnS (2H structure) nanocrystals by the yeasts *Candida glabrata* and *Schizosaccharomyces pombe*. Da Costa et al. [13] reported inorganic synthesis using H_2S generated by *Desulfovibrio desulfuricans* and Zn^{2+} solution resulted in mainly sphalerite.

Microbial synthesis of metal sulfide NPs has been attracting attention as a novel green technology for low-cost, low-energy and scalable NP manufacturing not requiring organic solvents. Microbial methods using inexpensive oxidized reagents are more than 10-fold less expensive than using H_2S [14]. Microbial synthesis

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allows for scalable production of NPs of reproducible quality [15]. It appears that massive extracellular ZnS NP production facilitated by microbial activity could be a plausible alternative to traditional high-cost inorganic NP syntheses. Here, we preferred to use cultures of anaerobic thermophilic strains instead of classically considered mesophilic sulfate reducing bacteria, due to several advantages: these included fast reaction [16] and HS^- development at thermophilic temperature, free of intermediates or impurities such as cadmium sulfate hydroxide [14] and easy preparation for anaerobic conditions as compared to mesophile or psychrotolerant species, as well as a reduced likelihood of microbial contamination at thermophilic temperatures.

This study focused on (1) the investigation of the controlling factors in microbially facilitated ZnS NP synthesis; (2) the correlation of structure, size and optical properties of NPs with scale-up; (3) the doping effect of copper on ZnS NPs; and (4) the dispersion of bio-ZnS NPs for functional film deposition and other applications.

2. Materials and methods

2.1. Synthesis of ZnS NPs

ZnS NPs were extracellularly synthesized using the fast-growing, metal-reducing thermophilic bacteria, *Thermoanaerobacter*, X513 [17]. Media used in this study were modified from TOR-39 medium [16] containing the following ingredients ($g\,l^{-1}$): 2.5 $NaHCO_3$, 0.08 $CaCl_2{\cdot}2H_2O$, 1.0 NH_4Cl, 0.2 $MgCl_2{\cdot}6H_2O$, 1–10 NaCl, 7.2 hydroxyethylpiperazine-N′-2-ethanesulfonic acid (HEPES) buffer, 0.5 yeast extract, 0.1 ml of 0.1% resazurin, 10 ml of Oak Ridge National Laboratory (ORNL) trace minerals and 1 ml of ORNL vitamin solutions [18]. No exogenous electron carrier substance (i.e., anthraquinone disulfonate) or reducing agent (i.e., cysteine) was added to the anaerobic medium. The trace mineral solution contained ($mg\,l^{-1}$): 1500 nitrilotriacetic acid, 200 $FeCl_2{\cdot}4H_2O$, 100 $MgCl_2{\cdot}6H_2O$, 20 sodium tungstate, 100 $MnCl_2{\cdot}4H_2O$, 100 $CoCl_2{\cdot}6H_2O$, 1000 $CaCl_2{\cdot}2H_2O$, 50 $ZnCl_2$, 2 $CuCl_2{\cdot}2H_2O$, 5 H_3BO_3, 10 sodium molybdate, 1000 NaCl, 17 Na_2SeO_3, 24 $NiCl_2{\cdot}6H_2O$. The vitamin solution contained ($g\,l^{-1}$): 0.02 biotin, 0.02 folic acid, 0.1 B6 (pyridoxine) HCl, 0.05 B1 (thiamine) HCl, 0.05 B_2 (riboflavin), 0.05 nicotinic acid (niacin), 0.05 pantothenic acid, 0.001 B12 (cyanobalamine) crystalline, 0.05 PABA (P-aminobenzoic acid) and 0.05 lipoic acid (thioctic). Modified medium A was prepared by lowering sodium chloride (NaCl) from 10 $g\,l^{-1}$ to 2 $g\,l^{-1}$ and adding 15 mM 3-(N-Morpholino)propanesulfonic acid sodium salt (MOPS) buffer (titrated to pH 7.8), medium B modified from medium A by removing 30 mM sodium bicarbonate ($NaHCO_3$) and titrating with MOPS buffer (pH 7.8), and medium C modified from medium A by removing $NaHCO_3$ and titrating with 10 M sodium hydroxide (NaOH) to pH 8.2. Various optimized conditions allowed growth of X513 producing ZnS at the different final medium pH that may affect crystallite size (see Supplementary Table S.1).

The dissolved basal medium was boiled with N_2 gas purging and cooled with continuous N_2 purging. For small scale experiments 10 and 50 ml aliquots were dispensed into 35 ml pressure tubes and 165 ml serum bottles, respectively, with N_2 purging and had a final pH of ~8.0–8.2. Each bottle was sealed with a butyl rubber stopper (Bellco Glass, Inc.) and an aluminum crimp seal. To initiate the 24 l reaction, 12 l of growth medium was added to two 13.25 l glass carboys, equipped with ventilation ports to the headspace, and were autoclaved for 2 h. The carboys were cooled to 70 °C in the autoclave and were further cooled with continuous purging using N_2 gas through a 0.2 μm filter overnight and put into plastic containers which were connected to a water bath. Each 1-l medium was dispensed from carboys to 2-l culture bottles under N_2 gas purging.

Incubation was initiated with inoculation of 10 mM of glucose, 5–10 mM of a sulfur source such as thiosulfate or sulfite and 2 vol.% of a mid-logarithmic growth phase X513 culture as final concentration in duplicates up to 1 l scale. After cell growth and the development of H_2S ions (HS^-), aliquots of zinc chloride ($ZnCl_2$, reagent grade ⩾ 98%) were dosed: either a single dose of the target amount (5 or 10 mM) or 5–10 discrete pulsed doses every day with a 10–20% fraction of target amount using syringes and needles, and incubated at 65 °C until the termination of the experiment. The precipitate was recovered by centrifugation (8000*g*, 10 min) and washed more than three times with deionized water (see Supplementary Fig. S.1) and stored as wet condensed samples or freeze-dried solid samples.

2.2. Characterization

After the termination of experiments, tubes or bottles were removed from the incubator and transferred to an anaerobic chamber (Coy Laboratory Products, Inc., Ann Arbor, MI). The final pH and Eh of growth medium were measured using a combination of pH electrode (Orion 815600) and Eh electrode (Tiod 9180BN) with Orion EA-920 Expandable ion analyzer (ThermoOrion, Beverly, MA). Cell densities were determined by using a Thoma cell counting chamber (Blaubrand, Wertheim, Germany) with an Axioskop2 Plus microscope (Zeiss, Thornwood, NY) with phase-contrast illumination.

The concentrations of dissolved hydrogen sulfide species including H_2S and HS^- were measured by colorimetry [19]. H_2S and CO_2 gases in the headspace were collected using a gas-tight syringe and analyzed using a gas chromatograph (Agilent 5850, Agilent Technologies, USA) equipped with a thermal conductivity detector as described previously [20]. Elemental analysis was performed on acid-digested samples by inversely coupled plasma mass spectroscopy (ELAN 6100, Perkin Elmer SCIEX, Waltham, MA).

The ZnS NPs were mixed with methanol, and the slurry was applied to a silica zero background plate for X-ray diffraction (XRD) analysis. The phase identification was conducted using an X-ray diffractometer (X'pert PRO, PANanalytical, Natick, Massachusetts) equipped with Cu-Kα radiation at 45 kV/40 mA between 10° and 70° 2θ or Mo-Kα radiation at 60 kV/45 mA between 5° and 35° 2θ with 1.5° $2\theta\,min^{-1}$. Average crystallite size (ACS) was determined from XRD results using the Scherrer equation in the JADE software package (Material Data Inc.). Transmission electron microscopy (TEM; FX 2000, JEOL, Japan) was used to study the morphology and grain size of the precipitated NPs.

The ZnS NP suspension was extracted using needle and syringe and diluted with anaerobic deionized water for fluorescence (FL) measurement. Measurement was immediately conducted using a JY-Horiba fluorometer at 275 nm excitation. Diffusive reflectance spectra were measured using a Spectralon fluoropolymer as a 100% reflectance standard. The measurements were done using the Praying Mantis Accessory (Harrick Scientific) with a Cary 5000 spectrophotometer (Agilent). Raman spectra were measured using a confocal micro-Raman system (Renishaw RM1000) in back reflection configuration and a 632.8 nm helium neon (HeNe) laser as the excitation source. The excitation and collection of the scattered light was through a 50 × objective.

Photoluminescence (PL) properties of solid NP powders were evaluated using a 325 nm helium cadmium (HeCd) excitation source. The spectral dependence of the monochromator and photomultiplier tubes was determined using a National Institute of Standards and Technology-calibrated tungsten lamp. Fourier transform infrared (FTIR) analysis of the samples, deposited on a zinc selenide window, was accomplished using a Nicolet Magna-IR 760 spectrophotometer at 4 cm^{-1} resolution. X-ray photoelectron spectroscopy (XPS) data were acquired by using the K-Alpha XPS system (Thermo Fisher Scientific) equipped with a monochromated Al K_α source ($h\upsilon$ = 1486.6 eV).