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Influence of humic acid on the stability and bacterial toxicity of zinc oxide nanoparticles in water

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ABSTRACT

The present study investigated the stability of zinc oxide nanoparticles (ZnO NPs) by the adsorption of humic acid (HA) and the mechanism of adsorption. The effect of humic acid on NP toxicity was determined by *Escherichia coli* (ATCC 13534), *E. coli* (ATCC 25922), and *Pseudomonas putida* (MTCC 4910). The nanoparticles showed low zeta potential and were least stable in the absence of HA. However, the negative surface charge of the particles increased in the presence of HA (0–50 mg/L) that reduced the propensity of nanoparticles to aggregate in water. A decrease in absorbance of ZnO NPs at 375 nm (plasmon peak) was noted in the presence of HA by UV–visible spectrophotometric analysis. A blue shift towards 370 nm was noted when the concentration of HA was above 20 mg/L. The HA adsorbed ZnO NPs showed higher zeta potential (>−30 mV) and were highly stable. HA reduced the photocatalytic activity of ZnO and at the same time increased the photostability of ZnO.

1. Introduction

Nanoparticles, with varying sizes that range from 1 to 100 nm, have versatile applications in commercial products and industrial applications. Among them zinc oxide nanoparticles (ZnO NPs) are receiving increasing attention for a large variety of applications. ZnO NPs are widely used in toothpaste, cosmetics, sunscreens, textiles and skin lotions [20]. One of the major problems associated with the preparation of nanoparticles is its stability. The freshly prepared nanoparticles are quite unstable and the nanoparticles tend to aggregate in the aqueous solution, hence it loses its property. Hence it is inevitable to assess the stability of nanoparticles. The substance that can pose higher positive or negative zeta potential to nanoparticles could be used as a good capping agent [13,15].

Many factors are involved in the stability of nanoparticles, mainly pH and ionic strength. It was reported that the stability of nanoparticles could be enhanced by the presence of dissolved HA [2,5]. The HA provides enhanced physiological activity to nanoparticles [8] and it stabilizes nanoparticles by hydrophobic interactions and by hydrogen bonds [16]. The HA coated nanoparticles could be used for the detection of herbicides [3]. The present study focused on the stability of ZnO NPs in water due to the presence of HA. Consequently the study investigated the effect of humic acid on NP toxicity in three model bacterial systems, *Escherichia coli* (ATCC 13534), *E. coli* (ATCC 25922), and *Pseudomonas putida* (MTCC 4910).

2. Materials and Methods

2.1. Materials

HA was purchased from Sigma-Aldrich, USA. The nanoparticles were dispersed by using Vibra-Cell Ultrasonic Processor (Sonics, USA). Triplicates were maintained in all the experiments.

2.2. Preparation of HA

A stock solution of HA was prepared by hydrating 500 mg of HA in 1 L of nanopure water and the suspension was incubated in a rotary shaker at 150 rpm for 24 h. There after the solution was filtered using 0.1 μm syringe filter. Then the HA stock solution was stored at 4 °C for further use.

2.3. Preparation of ZnO NPs

The ZnO NPs are prepared by adding $ZnSO_4$ and NaOH in molar ratio of 1:4. $ZnSO_4$ solution of 0.1 M was mixed with 0.4 M NaOH solution under heat (80 °C) and magnetic stirring. There after the samples were centrifuged at 10,000 × *g* for 30 min. Pellet was collected and lyophilized.

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2.4. Characterization of ZnO NPs

The nanoparticles were characterized by using UV–visible spectrophotometer (Shimadzu UV — 1700, Japan) and high resolution transmission electron microscopy (TEM, Tecnai G-20). Size distribution of the particles was determined using particle size analyzer (Microtrac Blue Wave, Nikkiso, Japan). The surface area of ZnO NPs was determined using a BET surface area analyzer (Smart Sorb 93 Single point, Smart Instruments Co. Pvt. Ltd., Mumbai, India). For XRD analysis, lyophilized nanoparticles were coated on XRD grid and the spectra were recorded using Bruker AXS Diffractometer (D8 Focus, Germany) operated at the voltage of 40 KV using Cu Kα radiation. Energy dispersive X-Ray (EDX) spectroscopy was used for the elemental analysis of ZnO NPs.

2.5. UV–visible Spectral Studies

The fixed amount of ZnO NPs (50 mg/L) was interacted with different concentrations of HA (0–50 mg/L) for 4 h at 150 rpm. UV–visible spectra were recorded using a UV–visible spectrophotometer within a range of 200–600 nm.

2.6. Zeta Potential and Particle Size Measurement

A Brookhaven Zeta 90Plus analyzer (Brookhaven Instruments Corp., Holtsville, NY) was used to measure the zeta potential of ZnO NPs after interaction with HA. The particle size was measured by dynamic light scattering (DLS) method using 90Plus Particle Size Analyzer (Brookhaven Instruments Corp., NY).

2.7. Effect of pH and NaCl Concentration on Adsorption

The effect of pH on the adsorption of HA onto ZnO NPs was studied by interacting HA with ZnO NPs for 2 h at different pH (4–10). The ionic strength of the interaction sample was maintained at 0.75 mM NaCl. The interaction was performed at 200 rpm in room temperature (28 ± 2 °C). After 2 h of interaction, the nanoparticles were obtained as a pellet by centrifugation at 12,000 ×*g* for 30 min and the supernatants were collected. The amount of humic acid in the solution was determined by measuring the absorbance at 420 nm by using a UV–visible spectrophotometer. HA solution devoid of ZnO NPs was used as control. The amount of HA adsorbed on ZnO NPs was determined by subtracting the concentrations of HA contained in the samples from control. Similar methodology was adopted to evaluate the effect of NaCl concentrations (0.05–1.5 M) on the adsorption of HA onto ZnO NPs.

2.8. Effect of Humic Acid on NP Toxicity

The effect of humic acid on NP toxicity was evaluated by interacting different concentrations of NPs with *E. coli* (ATCC 13534), *E. coli* (ATCC 25922), and *P. putida* (MTCC 4910) bacterial cells in the presence of HA. 10 mL of log phase cultures were centrifuged at 5000 ×*g* for 10 min. The pellet was suspended in a solution that contains ZnO NPs (0.1, 0.5 and 1 μg/mL) with and without HA (50 mg/L), and the cell number was adjusted to 1×10^8 CFU/mL. After 4 h of interaction, the cultures were plated on nutrient agar plates. The number of colony forming units was examined after 24 h of incubation. The same procedure was adopted in the control experiment (without ZnO NPs). The NP concentration was selected on the environmental relevance. Six replicates were kept for each concentration.

2.9. Adsorption Isotherms

Adsorption isotherm studies were performed to investigate the affinity of ZnO NPs for HA. Here a fixed concentration of ZnO NPs (20 mg/L) was interacted with varying concentrations of HA (0–50 mg/L) for 2 h in a rotary shaker at 200 rpm and pH 7. The ionic strength of the interaction sample was maintained at 0.75 mM NaCl. The interaction was performed at room temperature (25 ± 2 °C) and pH 7. Then the samples were centrifuged at 12,000 ×*g* for 30 min and the supernatants were collected. The amount of HA left in the supernatant was quantified by measuring the absorbance at 420 nm using UV–visible spectrophotometer. The amount of HA adsorbed on ZnO NPs at equilibrium q_e (mg/L) was calculated as follows:

$$q_e = \frac{(C_0 - C_e)V}{W} \quad (1)$$

where C_0 and C_e (mg/L) are the initial and equilibrium concentrations of HA respectively, V is the volume of the interaction solution in L and W is the concentration of ZnO NPs used in mg/L.

2.10. Adsorption Kinetics

The adsorption kinetics can provide a valuable data for understanding the mechanism of adsorption process and can predict the rate of removal of a substance from aqueous solutions. Here 20 mg/L of ZnO NPs was interacted with 100 mg/L of HA at pH 7 to evaluate the kinetics of adsorption process. A small aliquot of the interaction sample was removed at regular time intervals and centrifuged and the concentrations of HA left in the supernatants were determined. The amount of humic acid q_t adsorbed at time t was calculated as follows:

$$q_t = \frac{(C_0 - {}^0C_t)V}{W} \quad (2)$$

where C_0 and C_t (mg/L) are the amount of HA at initial and at time t respectively, V (L) is the total volume used in the interaction and W is the concentration of ZnO NPs (mg/L) used for the interaction process.

2.11. Evaluation of Photocatalytic Property

The photocatalytic property of ZnO capped with HA (ZnO-HA) was evaluated by interacting 10 mg/L of MB solution containing 5 mg/L of NPs. The solution was exposed to a 500 W Xenon lamp (Oriel instruments), placed 30 cm above the dishes. The photocatalytic efficiency was evaluated based on the degradation of MB and it was monitored by UV–vis spectrophotometer at 10 min time intervals. The characteristic absorption of MB was monitored at 665 nm. According to the Beer–Lambert Law, the concentration of MB is directly proportional to the absorbance. Hence the degradation efficiency can be calculated by the following equation:

$$R = \left(\frac{C_0 - C_t}{C_0}\right) \times 100 \quad (3)$$

where C_0 and C_t are the absorbance of MB at time 0 and t, respectively. In order to evaluate the photostability of ZnO-HA, the photocatalytic experiment was repeated continuously for 6 cycles by complete transformation of NPs from each cycle.

The rate of •OH formation during photocatalytic degradation under visible light was evaluated by the photoluminescence technique [23]. The excitation wavelength and the scanning speed were adjusted to 332 nm and 1200 nm/min respectively. After visible light irradiation, the solution was filtrated to measure the photoluminescence intensity at 456 nm.

3. Results and Discussion

3.1. Characterization of ZnO NPs

UV–visible spectroscopy is used for the preliminary characterization of ZnO NPs and it showed an absorption maximum at 376 nm (Fig. 1a). TEM was used to analyze the size and morphology of ZnO NPs and is