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Synthesis, characterization and toxicological evaluation of a core–shell copper oxide/polyaniline nanocomposite

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HIGHLIGHTS

- Synthesis of CuO NP coated with polyaniline (PANI) to form core–shell (CS) CuO/PANI.
- In acute tests, CuO NP and CS CuO/PANI were toxic to *D. magna* and *V. fischeri*.
- PANI presented no acute and chronic toxicity.
- To chronic exposition, CS CuO/PANI showed more toxic on reproduction parameter.

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ABSTRACT

The newest generation of copper oxide NPs (CuO NPs) is the CuO core–shell (CS), which has potential applications in several areas (e.g., electronics and paint) and is able to provide a greater service life due to its coating; however, its toxicity is not fully understood. The objective of this study was to synthesize, characterize and evaluate the aquatic toxicology of CuO NPs and CuO core–shells through acute and chronic toxicity tests with the freshwater microcrustaceans *Daphnia magna* and to evaluate its acute toxicity with the marine bioluminescent bacteria *Vibrio fischeri*. The NPs were synthesized by direct thermal decomposition after being coated as a CS with polyaniline (PANI). With respect to acute toxicity with *D. magna*, the CuO NPs and CS CuO/PANI presented EC_{50} values of 0.32 mg L^{-1} and 0.48 mg L^{-1}, respectively. For the tests with *V. fischeri*, the CuO NPs ($EC_{50-15min}$ = 7.79 mg L^{-1}) exhibited behavior similar to that of the CS CuO/PANI ($EC_{50-15min}$ = 9.05 mg L^{-1}) after 15 min of exposure. Regarding chronic toxicity, both forms showed a statistically significant effect ($p < 0.05$) on the growth and reproduction parameters. Based on the characterization and toxicity results, it can be concluded that both forms of CuO were toxic and presented similar behaviors during the acute tests; however, after 21 d of exposure, CS CuO/PANI showed higher toxicity to the reproduction parameter, highlighting the importance of a complete study of the NP to better understand its toxicity mechanism.

1. Introduction

In recent years, research in the field of nanomaterials (NMs) has led to the discovery that dramatically different properties may be present at this scale with respect to those of bulk materials (Curtis et al., 2006). Human and environmental exposures to NMs are inevitable because of their widespread use in many technological fields; consequently, nanotoxicology research is gaining greater attention. However, the variety of NMs and the applications developed for these materials continue to increase, whereas studies to characterize the effects of exposure and to identify the toxicological potential of NMs are lacking.

The use of NMs may lead to contamination of aquatic ecosystems by nanosized contaminants (Klaine et al., 2008). However, the ecotoxicological risks of NMs are difficult to evaluate because the mechanisms of toxicity of nanosized materials are still not fully understood. Several physicochemical properties of NMs that can be related to their toxicity have been identified through toxicological research, such as their size, shape, composition, aggregation and solubility, especially for NMs composed of toxic metals (Griffitt et al., 2008; Johnston et al., 2010).

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In the paint and textile industries, copper oxide nanoparticle (CuO NPs) are frequently used in a polymer-coated form, also called core–shell (CS), for their antimicrobial properties. The application of antifouling paints on the hulls of boats is a commonly used technique to reduce the growth and colonization of marine micro- and macro-organisms, which are responsible for a decrease in boat speed and an increase in fuel consumption (Almeida et al., 2007). Studies of polymer-coated CuO NPs have shown that, in some cases, the polymeric shell may potentiate the toxicity due to an effect known as the "Trojan horse." The polymeric shell can promote intracellular interactions, which increase interactions between the nanocomposites (NC) and cells, tissues or organs, thus increasing the toxicity of the NMs. An example of this effect was reported by Perreault et al. (2012). The effect of the polymer coating (poly(styrene-co-butyl acrylate) (PSBA) on CuO NP toxicity in the green alga *Chlamydomonas reinhardtii* enhanced the toxicological effects of the CuO NPs. Saison et al. (2010) investigated the toxic effect of CS CuO/PSBA NP on the green alga *C. reinhardtii* and observed changes in the algal population structure and in the primary photochemistry of photosystem II as well as the formation of reactive oxygen species (ROS). This study demonstrated the ability of CS CuO NPs to induce strong toxic effects in algae at the cellular level. Notably, neither the CuO NPs nor the polymeric shell alone induced such an effect. This result indicates that the morphological properties of NPs play an important role in the possible induction of a toxic effect in algae. The results from this study revealed that CuO NPs are more toxic to algal cellular systems when they are contained within an organic polymeric layer. Therefore, both the chemical nature of the NPs and their surface properties may determine the toxic effects.

Conducting polymers are promising materials because of their potential applications in multidisciplinary areas, such as electronics, thermoelectrics, electromagnetics, chemicals, membranes, and sensors (Bhadra et al., 2009). Among the conducting polymers, polyaniline (PANI) is one of the most studied polymers due to its excellent environmental stability, ease of synthesis, low cost of the monomer, redox properties, large conductivity range, and the different redox states that can be obtained: the fully reduced form, leucoemeraldine (yellow); the half-oxidized form, emeraldine (blue); and the fully oxidized form, pernigraniline (purple) (de Albuquerque et al., 2004). PANI can also be doped/dedoped by protonation, forming the so-called salt forms. The emeraldine salt (green) is the most conductive form of PANI (Salvatierra et al., 2010). Some studies describe the PANI CS structure synthesized with Fe_3O_4 (Deng et al., 2003; Lee et al., 2007) and SiO_2 (Jeong et al., 2011). However, few studies are found that describe the toxicity of PANI. The cellular toxicity of PANI was evaluated in nanodiamond-PANI under *in vitro* conditions using human embryonic kidney cells, and this study indicated that at low concentrations, the material can be used for bioapplications without a negative influence on the life processes of cells (Villalba et al., 2012). In another study, Yslas et al. (2012) assessed the ecotoxicity and teratogenic effect of PANI nanofibers in *Rhinella arenarum* larvae and embryos under controlled laboratory conditions. The results showed a low risk potential for *R. arenarum* after acute exposure to PANI nanofibers.

In this study, we synthesized, characterized and evaluated the acute and chronic toxicities of the NMs CuO NPs, PANI and CS CuO/PANI (NC) by exposing the freshwater microcrustacean *Daphnia magna* and the bioluminescent bacteria *Vibrio fischeri* to these NMs. Microcrustaceans and bacteria are good representatives of different trophic levels, and they are widely used in toxicity tests and as bioindicators in toxicology studies with NPs. The conducting polymeric shell may reduce toxicity and chemical/physical degradation and facilitate manipulation. These NMs were characterized using X-ray diffraction (XRD), transmission electron microscopy (TEM), inductively coupled plasma mass spectrometry (ICP-MS), speciation of metallic ions and zeta potential (ζ) measurements.

2. Materials and methods

2.1. Materials

The following materials were used to synthesized the PANI and NMs: 37% hydrochloric acid (HCl) and double-distilled nitric acid (HNO_3) from Merck® (Darmstadt, Germany); aniline (ANI) from Sigma–Aldrich®(St. Louis, MO, United States); ammonium persulfate ($(NH4)_2S_2O_8$), ammonium hydroxide (NH_4OH), copper sulfate hydrate ($CuSO_4.5H_2O$), sodium carbonate (Na_2CO_3), sodium dodecyl sulfate (Na-SDS), and super-pure hydrogen peroxide (H_2O_2) from Vetec® (Duque de Caxias, RJ, Brazil); and ethanol and acetone from Nuclear®(Diadema, SP, Brazil). All chemicals were of reagent grade and were used as received.

2.2. PANI synthesis

To 100 mL of HCl (15 mmol, in water) at 0 °C (ice bath) was added ANI (26.7 mmol), and then 100 mL of $(NH4)_2S_2O_8$ (6.7 mmol, in water) was slowly added over the course of 10–15 min. After stirring at 0 °C for an additional 3 h, the solids that formed (PANI salt) were collected by filtration and were thoroughly washed with ethanol, acetone and water to yield a dark-greenish solid after drying at 50 °C for 48 h. The PANI salt was deprotonated with a 0.1 M NH_4OH solution and stirred for 24 h. Then, the product was washed several times with water and ethanol and dried at 50 °C for 24 h. A dark blue powder was obtained.

2.3. CuO NP synthesis

CuO NPs were prepared via a direct thermal decomposition method with a few modifications (Das et al., 2013). The precursor was synthesized by adding 100 mL of a 60 mmol Na_2CO_3 solution to 100 mL of a 50 mmol $CuSO_4.5H_2O$ solution, and the mixture was ultrasonicated in a ultrasonic cell disruptor (Unique – 100 W, 99% maximum power) for 60 min at 60 °C. The resulting precipitate was separated by centrifugation and washed several times with warm distilled water to remove any possible ions remaining in the final product. Then, the precipitate was transferred to a silica crucible and placed in an oven at 70 °C for 12 h. Finally, it was placed in a preheated muffle furnace at 600 °C for decomposition. After 2 h, the silica crucible was removed from the furnace and allowed to cool to room temperature, and the resulting dark brown powder was ground and sieved.

2.4. CS CuO/PANI synthesis

CuO NPs were dispersed in 50 mL of distilled water containing 40 mg of Na-SDS under ultrasonication for 20 min, followed by stirring overnight at room temperature. Subsequently, ANI monomer and 0.5 mL of a 1 M HCl solution (as a dopant) were successively added to the suspension. The ratio between the CuO NPs and ANI was 3:1 (m/m). $(NH_4)_2S_2O_8$ (114 mg in 5 mL of distilled water) was added to the system as an oxidant to initiate polymerization. After stirring the mixture in an ice/water bath for 3 h, the CS CuO/PANI were recovered by centrifugation and rinsed several times with water and ethanol. The samples were dried at 50 °C for 24 h. Finally, the CS CuO/PANI was deprotonated with a 0.1 M NH_4OH solution and stirred for 24 h. The product was then washed several times with water and dried at 50 °C for 24 h.