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Bioconcentration of ionic cadmium and cadmium selenide quantum dots in zebrafish larvae

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HIGHLIGHTS

- Wide characterization and properties definition of quantum dots.
- Monitoring of exposition conditions all throughout the bioconcentration experiment.
- Shorter and cheaper protocol for bioconcentration using zebrafish larvae instead adult fishes.
- Comparison of BCFs of ionic Cd and quantum dots.

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ABSTRACT

The concern related to the use of nanomaterials is growing nowadays, especially the risk associated with their emission or exposure. One type of nanomaterials that has attracted much attention is quantum dots (QDs). QDs incorporation in consumer goods increases the probability of their entering in the environment and then into living organisms and human. In order to evaluate their potential to be bioconcentrated, zebrafish larvae have been exposed to SeCd/ZnS QDs, after performing an exhaustive characterization of these nanoparticles under the assay conditions. These data were compared with those obtained when zebrafish larvae were exposed to ionic cadmium. Finally, distribution of ionic Cd and QDs in exposed zebrafish larvae have been evaluated by Laser Ablation ICP-MS.

1. Introduction

Cadmium is a biologically non-essential metal and usually toxic at very low concentrations. It is considered as a class I carcinogen by the International Agency of Research on Cancer (IARC, 1993). The main sources of cadmium in the environment are industrial processes, urban traffic, waste incinerators, solid waste such as plastics and batteries or as a contaminant of phosphate fertilizers (Choong et al., 2014). Its presence in the environment could be a serious issue, which could be aggravated owing to its accumulation in the food chain. Nanomaterials are present in the environment since several centuries (Petosa et al., 2010; Reibold et al., 2006), although their growing production due to their advantageous properties will conduce to an increase of their levels in the environment either in areas of production and in consumer goods. One type of nanomaterials which have attracted much attention in the last years is Quantum Dots (QDs). QDs are colloidal nanostructured materials composed by elements from the periodic groups II–VI, III–V, IV–VI, and within this group CdSe QDs are the most widely studied. Their special optical properties such as wide absorption band that allows multiple color QDs excitation with a unique source of light have favored their use as labels in bioanalysis. Some electronic applications, security inks or Light Emitting Diodes (LED), are under investigation but they have already demonstrated their great

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potential.

Their potential risk could be caused by the nanomaterial itself or by their free metallic components. Thus, the assessment of nanoparticles risk compared to their dissolved components is an area of interest, being rather complicated as each kind of nanoparticle has unique composition (core and ligands), and besides the possible degradation products generated will depend on the final application given to the nanomaterial. Nowadays, the European Union is limiting the use of cadmium and other metals in plastics or coatings (European Chemicals Agency, 2013). In general, adult fishes or mammalian models such as mice or rats are usually employed to evaluate *in vivo* effects of contaminants, but these studies are time consuming, present ethical issues and are expensive (Yong et al., 2013). The use of zebrafish larvae appears as an alternative model, since it represents the dynamic that occur *in vivo* in a complex organism (Dai et al., 2014; Kim, 2013).

In this work we present the results got in a bioconcentration assay based on the use of zebrafish larvae as an alternative to Test OECD 305, the official assay employed to test chemicals in adult fish to calculate the Bioconcentration Factors (BCF) (OECD, 2012). Previous papers by employing this alternative have demonstrated to be comparable with those obtained using OECD 305 guideline (Sanz-Landaluze et al., 2015). It implies a considerable reduction in exposure time to the chemical (from 28 days to 48–72 h), as well as the required amount of compounds under evaluation (Kim, 2013). Nevertheless, the determination of chemical concentration in larvae is a challenge since it requires highly sensitive analytical techniques owing to low sample amount (1 larvae ~ 0.44 mg). Zebrafish larvae were exposed following OECD 305 guidelines to CdSe/ZnS QDs and to ionic cadmium with the thought the potential event of Cd release from QDs, that is, considering the nanoparticles as a source of ionic cadmium (Liu et al., 2012). Prior to the bioconcentration study, it was necessary to perform an exhaustive nanomaterial characterization in the presence of larvae in order to provide as much information as possible about the evaluated nanoparticles either in the stock solution or in zebrafish exposure media.

2. Materials and methods

2.1. Chemicals

Analytical grade chemicals were used for all experiments. Ionic cadmium solution (1000 mg L^{-1} Cd, Fluka) was used to prepare the standard solutions to do daily calibrations. Sub boiled nitric acid (HNO_3 60% Scharlau, Barcelona, Spain), and hydrogen peroxide (H_2O_2 35%) were used to digest nanoparticles. Larvae exposure solution (ISO water) of similar composition to fresh river water was prepared as follows: 294 mg of $CaCl_2.2H_20$, 123.3 mg of $MgSO_4.7H_2O$, 63 mg of $NaHCO_3$ and 5.5 mg of KCl were diluted to 1 L in distilled water. Milli-Q Element ultrapure water (Millipore, Billerica, MA, USA) was employed for all reagent and standards dilutions.

Commercial QDs were purchased from Sigma (Saint Louis, USA). The first type *QDs-C*, (CdSe/ZnS alloyed quantum dots –COOH functionalized 1 mg.L^{-1} in water) was employed as received without further modifications. The second type *QDs-P*, (Lumidot CdSe/ZnS core–shell type quantum dots, 5 mg L^{-1} in Toluene) were prepared following a reported procedure in order to make them compatible in aqueous media (Fernandez-Arguelles et al., 2007). In both cases, QDs concentration, as stated by the manufacturer, was 1000 mg L^{-1} without further specifications. They were stored in the dark at 4 °C until use. QDs suspensions with ISO water were prepared at the desired concentration through the corresponding dilutions for the bioconcentration experiments.

2.2. Instrumentation

Cadmium extraction from larvae: A Vibra cell VCx130 focused ultrasonic probe (USP) (Connecticut, USA) equipped with a 3 mm diameter titanium microtip and fitted with a high frequency generator of 130 W at a frequency of 20 KHz was used for sample treatment. A centrifuge model FVL-2400 N from Combi-Spin (Boeco, Germany) was used for sample centrifugation.

Ultrafiltration: Ionic components released from QDs degradation can be evaluated by ultrafiltration using appropriate centrifugal filters (10 KDa, Amicon, Millipore). Three aliquots of exposure solution were filtrated, centrifuged, and measured by ICP-MS.

Fluorescence measurements: Fluorescence spectra from the QDs exposure solutions were recorded using a Varian Cary Eclipse (Varian Iberica) luminescence spectrophotometer equipped with a Xenon discharge lamp using a fixed excitation wavelength of 480 nm. All measurements were carried out using conventional 1 cm quartz luminescence cuvettes (Hellma, Germany). Quantum Yields (QY) were measured according to a comparative method (Lackowicz, 2006), employing a well characterized standard sample with a known fluorescence QY value (Rhodamine 6G 95% in ethanol).

UV–Vis Detector: QDs core diameter was estimated by UV–Vis spectrophotometry (Hewlett Packard 8453) according to Pens's equation (Yu et al., 2003) based on calculations from absorbance and fluorescence data. Also making use of this empirical equation the concentration of nanoparticles (molar concentration) in solution was estimated applying Lambert–Beer Law.

Dynamic Light Scattering (DLS): Measurement of hydrodynamic diameters of nanoparticles were performed using a Zetasizer Nano-ZS (Malvern Instruments, United Kingdom) equipped with a 633 nm laser filter. Hydrodynamic diameter measurements were carried out in zebrafish media at QDs concentration of 3 mg L^{-1} Cd (0.125 μM Cd).

Transmission Electron Microscopy (TEM): TEM images were obtained on a JEOL JEM 2100 (Tokio, Japan) equipped with a micro analysis system coupled with Energy Dispersive X-ray analyzer (EDXS). Samples were prepared by placing several drops of QDs solutions onto a copper TEM grid and then allowed to air-dry.

Inductively coupled plasma mass spectrometer (ICP-MS): ICP-MS HP-7700 Plus (Agilent Technologies, Analytical System, Tokyo, Japan) was employed to determine cadmium content. Ions monitoring at m/z ^{111}Cd, ^{114}Cd, and ^{115}In as internal standard, were selected for data collection. Experimental parameters have been summarized in Table S1a (Supporting Information).

Laser ablation coupled to ICP-MS (LA/ICP-MS): Spatial distribution on was performed by elemental mapping of cadmium in zebrafish larvae, using a KGW-Yb crystal infrared femtosecond laser (ALFAMET, Novalase Sa, Amplitude Systemes, France) coupled to ICP-MS (Perkin Elmer Sciex ELAN 6100). The frozen zebrafish larvae were placed over a polycarbonate plate previously covered with a thin gold layer to be employed as internal standard. ^{111}Cd, ^{112}Cd, ^{197}Au and ^{13}C isotopes were monitored. Data obtained were exported for further treatment for imaging processing using PAMAL (Plataforme d'Analyse des Métaux traces par Ablation Laser) (Sarrat et al., 2011; Gholap et al., 2010; Barst et al., 2011). Experimental parameters have been summarized in Table S1b (Supporting Information).

2.3. Zebrafish larvae exposure

Zebrafish larvae were obtained from wild type adult zebrafish bred and maintained in AZTI Zebrafish Facility (EU-10-BI) under standard conditions. All the experimental procedures were approved by the Regional Animal Ethics Committee. The OECD