The frontier of the Environmental Analytical Nanotechnology

Single Nanoparticle Detection by ICP-Mass Spectrometry

Cell Toxicity and Genotoxic Assays

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The use of engineered nanoparticles (ENPs) is rapidly increasing and it is inevitable that they are released in the environment, where their fate and behaviour are largely unknown. Nowadays, the lack of reliable methods to determine ENPs identity, concentrations and characteristics in complex environmental samples at environmentally relevant concentrations, is one of the largest gaps in environmental nanosciences. Single particle detection using ICP-MS can be considered as one of the challenging analytical approaches necessary to assess the environmental impact of the release of ENPs into the environment.

In single particle detection, when one nanoparticle is introduced into the ICP, the atoms of the analyte produce a flash of gaseous ions in the plasma, which are measured as a single pulse by the detector. The number of counts of this single pulse is related to the quantity of analyte atoms in the nanoparticle, and the frequency of the pulses is proportional to the number concentration of nanoparticles. Adequate time resolutions are required to ensure that each pulse correspond to just one nanoparticle. On the other hand, the analyte in dissolved forms will produce pulses of averaged constant intensity.

The different behaviours of dissolved silver and silver nanoparticles under ICP-MS single particle detection conditions have been used to differentiate directly between both forms of silver. A methodological approach based on single particle detection using ICP-MS for identification, characterization and determination of mass and number concentration of silver nanoparticles in aqueous samples will be presented.

The recommended minimum physical and chemical parameters for characterizing nanomaterials in toxicological studies should include [2]: Particle size/size distribution, agglomeration state/aggregation, shape, overall composition, surface composition, purity, surface area, surface chemistry and surface charge. In addition, some overarching considerations should be taken into account: (i) Stability—how do material properties change with time storage, handling, preparation, delivery...? including the material release through dissolution. (ii) Context/media—how do nanomaterial properties change in different media? [3]

In vitro cell-based assay systems are important tools in the field of nanotoxicology. The considerations mentioned above with respect to knowing how the nanomaterial behaves in the test system are especially important for *in vitro* systems because nanoparticles can exhibit strong interactions with culture media. The formulation of the culture medium with respect to serum concentration, pH, and other factors can influence the behavior of nanoparticles and their response in the cellular system.

We are going to describe the effects of a commercial nano silver product described in pharmacopeia as strong antiseptic, has been extensively characterized and its toxicity in hepatic human cells has been studied. Furthermore, silver nanoparticles and other forms of soluble silver have been identified and characterized in different culture media.

The nanomaterial is a granulated powder with a total silver content of around 70%. It contains metallic silver nanoparticles below 20 nm, but small amounts of Ag(I) are also present. Toxicity test were performed with the human hepatocellular carcinoma cell line HepG2. Cell viability and genotoxicity were

evaluated by the Neutral Red Uptake Cytotoxicity Test and the Alkaline Single-Cell Gel Electrophoresis (Comet Assay), respectively. Cell viability test showed that the product has no effect up to 15 mg/L. At higher concentrations, the antiseptic drug reduces the cell viability significantly, showing a dose-response effect. IC_{50} for product on HepG2 is 35-37 mg/L. The drug is moderately genotoxic at 10 and 20 mg/L, whereas it is highly genotoxic from 40 mg/L.
The stability and behavior of silver components in this material was studied in the culture media DMEM (Dulbecco Modified Eagle's medium) and RPMI (Roswell Park Memorial Institute medium) supplemented with fetal bovine serum, used along the toxicity tets. Asymmetric Flow Field Flow Fractionation (AsFlFFF) coupled to UV-Visible and ICP-MS detection was used for characterization of silver nanoparticles and Ag(I) species in these media. Nanoparticle aggregation as well as dissolution and complexation of Ag(I) with serum proteins were observed. Analytical methodology as well as detailed results will be presented.

References

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