THE IMPACT OF NANOPARTICLE AGGREGATION IN LIQUID SOLUTION FOR TOXICOLOGICAL AND ECOTOXICOLOGICAL STUDIES

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Abstract. Given the widespread applications of nanotechnology products, the evaluation of the potential hazards related to human exposures and environmental release of nanoparticles has become an urgent need. The development of a safety database for nanoscale particles is evolving as new particles, materials and exposure scenarios are being developed.

Several studies have been focused on the importance of developing adequate physicochemical characterization of nanomaterials, in the relevant media being utilized, prior to undertaking experiments for *in vitro* and *in vivo* toxicity assessment.

In this work we utilize two optical techniques: Dynamic Light Scattering (DLS) and Time Resolved Fluorescence Polarization Anisotropy (TRFPA) for measuring particle size, size distribution and aggregation kinetics of nanoparticles in aqueous solution for toxicological and ecotoxicological studies. The application of these two optical methods gives a careful description of the aggregation status of nanoparticles and its evolution on different time-scale.

1. Introduction

Given the widespread applications of nanotechnology products, the evaluation of the potential hazards related to exposures to nanoscale particles has become an urgent need. Recently, several studies have been focused on the importance of developing adequate physicochemical characterization of nanomaterials prior to undertaking experiments for *in vitro* and *in vivo* toxicity assessment [1]. Particle size, size distribution, morphology, surface area are all important properties that

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must be accurately characterized in the relevant media being utilized for toxicity assessment. In fact, potential physicochemical changes can occur to nanoparticles properties while in solution and these changes may have a significant impact on the observed toxicological responses [2].

Currently, there are no well-defined techniques for the characterization of wet nanomaterials in aqueous or biological solutions [2, 3]. The use of multiple techniques should be attempted wherever possible to develop a more complete understanding of the system. Among the wide variety of available techniques for size measurements only few of them are suitable for physiological environments. Optical methods are the most desirable to this purpose due to their accuracy, flexibility and being not intrusive.

This study describes the setup and preliminary investigations devoted to carry out two nanotoxicological experiments.

2. Nanoparticles characterization

Surface area and pore size distribution of nanoparticle powders have been firstly characterized with BET technique [4]. Dynamic Light Scattering (DLS) and Time Resolved Fluorescence Polarization Anisotropy (TRFPA) have been employed for measuring particle size, size distribution and dispersion state of nanostructured materials in physiological media.

DLS measures Brownian motion by illuminating the particles with a laser and analyzing the intensity fluctuations of the scattered light. These temporal fluctuations are then related to the size of the particles.

TRFPA is a short-pulse laser technique that allows to resolve the fast rotational dynamics (in the picoseconds scale) of nanoparticles in water suspensions and, thus, to obtain nanoparticles' size by exploiting their self fluorescence.

2.1. *Evaluation of cancer effects on mice*

Mouse models of skin cancer provide an ideal *in vivo* model to identify skin carcinogens and to study the mechanisms involved on tumorigenesis. In particular, Car-S mice, phenotypically selected for susceptibility to two-stage skin carcinogenesis, are elective for studies of papilloma and squamous cell carcinoma (SCC) pathogenesis (Fig. 1).

The defense mechanisms of the organism against tumorigenesis is usually mediated by immunological responses or non-specific inflammatory reactions [5, 6]. Hereafter, the set up of a pilot study on the early inflammatory response potentially induced by multiwalled carbon nanotubes on the skin of Car-S mice is described. Mouse skin has been treated two times per day with Acetone/MWNT suspension for 5 days. A weak increment of epidermal cells inflammation (boxes) between sham ad treated groups is observed.

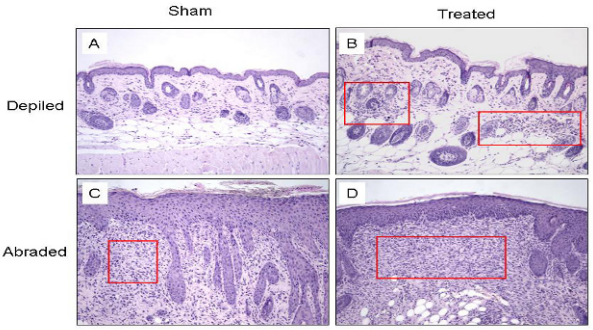

Figure 1. Figure show a different inflammatory degree in depilated or abraded (rows) Car-S mouse skin after MWNTs treatment compared with sham group (colons).

2.2. *Ecotoxicological effects*

Environmental impact of ZnO nanoparticles on aquatic systems has been deeply investigated [7], while little is known about its potential toxicity on soil and seawater. In this work, preliminary studies on potential toxicity of ZnO nanoparticles in soil and in seawater are reported. Toxicity test batteries with organisms belonging to different trophic levels and biological complexity (Table 1) have been executed. To assess if toxicity is attributable to ZnO nanoparticles or to soluble Zn^{2+} the same test batteries were carried out both on ZnO and $ZnCl_2$ contaminated soil and seawater.

Table 1. Ecotoxicity test batteries.

Soil test battery (standard method)	Ref	Seawater test battery (standard methods)	Ref
Plant germination and root inhibition test (*L. sativum*)	[8]	Bioluminescence acute test (*V. fischeri*)	[12]
Algae growth inhibition test (*S. capricornutum*)	[9]	Crustaceans acute test (*A. salina*)	[14]
Ostracod survival and growth inhibition test (*H. incongruens*)	[10, 11]	Algae growth inhibition test (*D. tertiolecta*)	[15]
Bioluminescence acute test (*V. fischeri*)	[12]		
Genotoxicity micronuclei test (*V. faba*)	[13]		

OECD Standard soil samples have been spiked with ZnO and $ZnCl_2$ at 0.029% w/w. Analogously, toxicity tests on seawater have been performed on ASTM Standard seawater samples spiked with ZnO and $ZnCl_2$ ranging from 0.03 to 9 mg/l. All the abovementioned concentrations are referred to the zinc content.

Results showed that ZnO NPs exerted toxic effects upon all test organisms. In particular, algae showed the highest sensitivity. A modest genotoxic effect was also observed. Soluble Zn^{2+} produced comparable effects except for *L. sativum* where a moderate biostimulation was observed. The highest NP toxicity was obtained with *V. fischeri*, while a moderate biostimulation was observed for algae. Crustaceans revealed as the less sensitive organisms. Soluble Zn^{2+} seems to be responsible of toxic effects.

3. Conclusions

DLS and TRFPA have been applied to the characterization of nanoparticles dispersions in two toxicity and ecotoxicity pilot studies. The application of these optical methods gives a careful description of the aggregation status of nanoparticles and should allow the preparation of suitable nanoparticles dispersions for experimental trials.

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