SAFE-BY-DESIGN: AN EXAMPLE WITH POLYELECTROLYTE-SURFACTANT NANOMATERIALS

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ABSTRACT

Within the rapid development and innovation of nanotechnology, it is necessary to ensure its sustainable development and the safe use of nanomaterials (NMs). This is a fundamental issue when aiming to reduce the risks posed by NMs, which are already being released into the environment. Actually, the possibility of manipulating several characteristics of NM to enhance their functionalities may also constitute a key opportunity for a rational design of safer NMs. Following this rational, the present work aimed at assessing the influence of different functionalization of polyelectrolyte-surfactant NMs on its toxicity to aquatic biota. As these NMs building principle is based on the conjugation of two different compounds (polyelectrolytes and surfactants), allows a vast diversity in their design and also in their structural and functional properties. Therefore, meeting the goal a greener and sustainable nanotechnology, these materials constitute ideal raw material to promote a rational development of polyelectrolyte-surfactant NMs that by maintaining their functional properties will also exert lower adverse effects in the environment. To attain the main goal, ten variations of sodium polyacrylate/ dodecyltrimethylammonium (NaPA/DoTA) NMs were obtained by changing the number of repeating units (m; 25, 70, 2000, 3000 and 6000) of NaPA (NaPAm; which was always at a concentration of 10 mM), the counterion of the surfactant (bromide-DOTAB or chloride-DOTAC) and the concentration of DOTAC (3, 5 and 7 mM). The lethal and sublethal toxicity of these NMs was assessed by exposing *Vibrio fischeri, Raphidocelis subcapitata*, and *Daphnia magna* to serial concentrations of each NM. The following endpoints were evaluated: bioluminescence inhibition, growth rate and cumulative mortality, respectively. As well, the physical parameters of hydrodynamic size, index of polydispersity, and zeta potential were measured for the highest concentration tested of each NM. In general, the obtained results showed that the NMs composed of DOTAC at a concentration of 3 mM exerted the lowest toxicity to the three tested species, while those composed of NaPA with 2000 repeating units ($NaPA_{2000}$) were among the most toxic. Therefore, aiming a greener and sustainable development of nanotechnology, it is suggested that, for this type of NM, complexes formed by DOTAC (with the chloride counterion) at concentrations of 3 mM should be target to be developed.

Keywords: Nanomaterials, Ecotoxicity, Rational Design, Sustainable nanotechnology

1. Introduction

Nanotechnology is claimed to be a major innovative scientific and economic area aiming at investigate, develop, produce and apply materials and structures with nano dimensions (1). In fact, it enables the exploitation of novel physicochemical properties offered by such materials produced at nanometer scale-nanomaterials (NMs). These NMs can potentially benefit humankind, by empowering new approaches to a variety of industrial products and processes, medical devices or even water treatment and environmental remediation technologies (2).

However, in addition to these advantageous functionalities, the same NM-desirable properties may equip them with the potential to adversely affect biota, and, in fact, within the last decade several works reported toxic effects of diverse NM to a range of species and at different levels of biological organization (e.g. 3,4). Therefore, the release of NM into the environment may result in unforeseen deleterious effects, which demands for preventive actions. However, despite the recommendations of USA and European Union policies for a critical need of a risk assessment of NM, comprehensive knowledge on the toxic effects posed by NM to the environment is still poor (5-7), resulting in high levels of uncertainty in estimating risk. This knowledge gap is partly due to a poor and inadequate physical and chemical characterization of NM that is related to the lack of adequate analytical techniques for their detection, monitoring and control (8). Furthermore, the plethora of NM already being produced is large, exhibiting an infinite number of chemical compositions, sizes/shapes, surface coatings, which may greatly influence their ecotoxicity (9,10). Regardless of this fact, ecotoxicity data only exist for a very narrow range of NM; and the toxicity of most of the already produced NM remains unknown as well as uncertainties still persisting on the mechanisms involved in their fate, behavior and biological effects in the environment. Subsequently, numerous concerns arose considering the production of environmental friendly NM and national or international policy strategies have been projected and established to promote the sustainable development and innovation within nanotechnology. In the European Union, the EU2020 strategy launched a new paradigm by claiming "Safe innovation for a competitive and sustainable future" and the European Industry is expected to adopt such clear recommendations (11). Likewise, the Action Plan on Sustainable Industrial Policy of the European Commission targets improving the overall environmental performance of products and encourages EU industry to innovate towards the leadership in this field (12). These are fundamental issues when aiming to reduce the risks posed by NMs, which are already being produced and released into the environment. Actually, the possibility of manipulating several characteristics of NM to enhance their functionalities may constitute a key opportunity for a rational design of safer NMs, thus meeting the previously mentioned EU strategies. Following this rational, the present work aimed at assessing the influence of ten different functionalizations of a polyelectrolyte-surfactant NM on its toxicity to aquatic biota.

2. Material and methods

2.1. Studied nanomaterials

Ten variations of sodium polyacrilic/dodecyltrimethylammonium (NaPA/DoTA) were here studied. These variations were obtained by changing the repeating units (m) of NaPA ($NaPA_m$, always at a concentration of 10mM) and the anion (bromide-DOTAB or chloride-DOTAC) and concentration of DOTA (3, 5 and 7 mM): $NaPA_{25}$10/DoTAC3; $NaPA_{70}$10/DoTAC3, $NaPA^{2000}$10/DoTAB5, NaPA200010/DoTAC3, $NaPA_{2000}$10/DoTAC5; $NaPA_{2000}$10/DoTAC7; $NaPA_{3000}$10/DoTAC3; $NaPA_{3000}$10/DoTAC5; $NaPA_{3000}$10/DoTAC7; and $NaPA_{6000}$10/DoTAC3. Stock suspensions were prepared, for each NM variation, in distilled water. To perform the toxicity assays, each stock suspension was diluted with the respective medium (distilled water, ASTM, or MBL, please see the section of Ecotoxicity assays) to obtain the tested concentrations.

The following physical parameters were measured, by light scattering, for the highest tested dilution of each variation of NaPA/DoTA, and for each media used in toxicity assays: hydrodynamic size, index of polydispersity, and zeta potential.

2.2. Ecotoxicity assays

Toxicity assays were carried out with the bacterium *Vibrio fisheri*, the microalgae *Raphidocelis subcapitata* and the cladoceran *Daphnia magna*, by exposing each of these three species to serial dilutions of the ten variations of NM.

To assess the influence of media composition in the toxicity of the ten variations of the NM, *Vibrio fisheri* was exposed to serial dilutions (8-0.3%) to of each variation suspended in distilled water, ASTM hardwater, and MBL medium, according to the 81.9% basic protocol of Azur

(1998). The effects on bioluminescence production were monitored after 5, 15 and 30 minutes of exposure.

For determining the toxicity of the NM variations to species belonging to different taxonomic groups assays with the freshwater microalgae *Raphidocelis subcapitata* and the cladoceran *Daphnia magna* were carried out. The effects of NaPA/DoTA variations in the growth rate of *R. subcapitata* were assessed in accordance with OECD guideline (OECD, 2006). Algae were exposed for 72h to serial dilutions (0.028 to 0.098%, dilution and control medium was MBL; Stein, 1973) of each NM variation and cell density was measured at the beginning and end of the assay. Regarding *D. magna*, the lethal effects of each NM variation were quantified by exposing neonates (< 24h old) of the cladoceran species to serial dilutions (0.027 to 0.013%, dilution medium was ASTM; ASTM, 1998) of each variation for 48h (OECD, 2004). After this exposure period the number of dead animals as counted. An organism was considered death when it remained immobile after 15 sec of gentle prodding.

2.3. Statistical analysis

The effective concentrations causing 50% (EC_{50}) of bioluminescence inhibition in *V. fischeri* were computed for each NM variation, at each observation time, by using the Software for MicrotoxOmni Azur (AZUR Environmental, 1998). The effective concentrations provoking 50% of growth inhibition in algae and 50% mortality in the cladoceran (EC_{50} and LC_{50}, respectively) were computed using a non-linear logistic model and Probit analyses, respectively.

3. Results

3.1. Physical parameters

All suspensions showed high stability and negative surface charge, in all tested media: the zeta potential values ranged within -88.3 and -45.8mV. Regarding the polydispersity index, values were below 0.2 for almost all NM variations, with the following exceptions: in distilled water - $NaPa_{3000}$10/DoTAC3; in ASTM medium - $NaPa_{6000}$10/DoTAC3, $NaPa_{2000}$10/DoTAC5, $NaPa_{2000}$10/DoTAB5; and in MBL medium - $NaPa_{70}$10/DoTAC3; $NaPa_{6000}$10/DoTAC3; $NaPa_{2000}$10/DoTAC7.

The NM-variation $NaPa_{6000}$10/DoTAC3 exhibited consistently the highest average size, in all tested media.

3.2. Ecotoxicity assays

Ecotoxicity assays revealed *Vibrio fisheri* to be less sensitive species to the ten variations of NaPa/DoTA NM.

Table 1: Toxicity rank for the ten tested variations of NaPa/DoTA NM.

V. fisheri ($H_2O_{dist.}$) 30min	*V. fisheri* (ASTM) 30 min	*V. fisheri* (MBL) 30 min	*R. subcapitata*	*D. magna*
$NaPa_{2000}$ 10/DoTAC5	$NaPa_{3000}$ 10/DoTAC7	$NaPa_{2000}$ 10/DoTAC7	$NaPa_{2000}$ 10/DoTAB5	$NaPa_{2000}$ 10/DoTAC7
$NaPa_{2000}$ 10/DoTAC7	$NaPa_{2000}$ 10/DoTAC7	$NaPa_{2000}$ 10/DoTAB5	$NaPa_{3000}$ 10/DoTAC7	$NaPa_{2000}$ 10/DoTAB5
$NaPa_{2000}$ 10/DoTAC3	$NaPa_{2000}$ 10/DoTAC5	$NaPa_{3000}$ 10/DoTAC7	$NaPa_{3000}$ 10/DoTAC5	$NaPa_{3000}$ 10/DoTAC5
$NaPa_{3000}$ 10/DoTAC5	$NaPa_{3000}$ 10/DoTAC5	$NaPa_{3000}$ 10/DoTAC5	$NaPa_{3000}$ 10/DoTAC3	$NaPa_{2000}$ 10/DoTAC5
$NaPa_{3000}$ 10/DoTAC7	$NaPa_{2000}$ 10/DoTAB5	$NaPa_{2000}$ 10/DoTAC5	$NaPa_{2000}$ 10/DoTAC7	$NaPa_{3000}$ 10/DoTAC3
$NaPa_{70}$ 10/DoTAC3	$NaPa_{25}$ 10/DoTAC3	$NaPa_{3000}$ 10/DoTAC3	$NaPa_{2000}$ 10/DoTAC3	$NaPa_{2000}$ 10/DoTAC3
$NaPa_{25}$ 10/DoTAC3	$NaPa_{2000}$ 10/DoTAC3	$NaPa_{25}$ 10/DoTAC3	$NaPa_{2000}$ 10/DoTAC5	$NaPa_{70}$ 10/DoTAC3
$NaPa_{2000}$ 10/DoTAB5	$NaPa_{70}$ 10/DoTAC3	$NaPa_{6000}$ 10/DoTAC3	$NaPa_{6000}$ 10/DoTAC3	$NaPa_{3000}$ 10/DoTAC7
$NaPa_{6000}$ 10/DoTAC3	$NaPa_{3000}$ 10/DoTAC3	$NaPa_{2000}$ 10/DoTAC3	$NaPa_{25}$ 10/DoTAC3	$NaPa_{6000}$ 10/DoTAC3
$NaPa_{3000}$ 10/DoTAC3	$NaPa_{6000}$ 10/DoTAC3	$NaPa_{70}$ 10/DoTAC3	$NaPa_{70}$ 10/DoTAC3	$NaPa_{25}$ 10/DoTAC3

For V. *fisheri* the ecoxicity of the ten NM variations did not varied much with exposure time. However, media type (distilled water, ASTM, and MBL) influenced the adverse effects caused by the NM-variations. In general, for distilled H_2O and ASTM, NM with higher number of NaPa repetitions were less toxic, while for MBL medium, specifically for $DOTAC_3$, a higher number of NaPa repetitions caused lower toxicity (Table 1).

The assays carried out with the microalgae *R. subcapitata* and the cladoceran *D. magna* revealed the NM variation holding the bromide anion ($NaPa_{2000}10$/DoTAB5) to be the most toxic for these two species. Furthermore, $NaPa_{\mathbf{25}}10$/DoTAC3, $NaPa_{\mathbf{70}}10$/DoTAC3 and $NaPa_{\mathbf{6000}}10$/DoTAC3 were the least toxic for *R. subcapitata* while $NaPa_{\mathbf{25}}10$/DoTAC3, $NaPa_{\mathbf{6000}}10$/DoTAC3 and $NaPa_{\mathbf{3000}}10$/DoTAC7 were the least toxic for *D. magna* (Table 1).

4. Conclusions

The variation $NaPa_{6000}10$/DoTAC3 exhibited the highest size in the three aqueous media, which may be related with the higher number of NaPA repetitions. In general, the variations holding the anion chloride and a concentration of 3mM of DoTA were the less toxic. Therefore, is suggested that variations with DoTAC3 should be targeted by industry aiming at a greener and sustainable nanotechnology.

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