A STUDY OF SILVER NANOPARTICLE BEHAVIOR IN LIQUID MEDIA FOR ECOTOXICITY TESTS

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Abstract

The up to date increasing number of nanotechnology applications is linked with a serious danger of environmental pollution by nanomaterials. Consequently, the ecotoxicity testing of nanomaterials becomes an essential part of nanotechnology development. Classical ecotoxicity tests suffer with a number of complications when used for nanomaterials. In the case of aquatic toxicity tests, the nanoparticle agglomeration in the liquid media is a fundamental problem. The aim of this work is to evaluate the influence of important physico-chemical parameters (nanoparticle concentration and illumination conditions) on an agglomeration of 40 nm silver nanoparticles (AgNPs) in a liquid media prescribed for fish eco-toxicity test by the OECD 203 guideline (Medium 203).

Keywords: Silver, nanoparticles, aggregation, liquid media, UV-Vis spectroscopy

1. INTRODUCTION

An increasing amount of artificial products containing silver nanoparticles (drugs, antimicrobial agents, material coating, catalysts,…) results in its inevitable discharge into the environment. The toxicity of silver nanoparticles (AgNPs) is affected by intrinsic nanoparticle features such as particle size, surface chemistry, capping agents, but also by environmental factors such as pH, redox state, the presence of ligands, ionic strength and the ionic composition of the liquid medium [1].

Toxic effects of AgNPs have been studied under laboratory conditions on aquatic organisms. There have been documented effects such as developmental deformities, altered stress-related gene expression, respiratory stress, and nonspecific for many manufactured nanomaterials [2].

The fish acute toxicity test performed according to OECD guideline 203 suggests the application of good quality natural water or preferably reconstituted water (Medium 203) [3]. This cultivation medium is characterized by relatively high ionic strength, which is another important parameter influencing NPs aggregation and subsequently results of eco-toxicity tests.

The aim of this work is to evaluate the influence of nanoparticle concentration and light exposition on the agglomeration of AgNPs in a common liquid medium for an aquatic toxicity test (Medium 203).

2. MATERIALS AND METHODS

Primary AgNPs colloid was prepared from 10 mL of redistilled water, 10 mL of 5 mM silver nitrate, 10 mL of 0.025 M ammonia, 10 mL of 0.05 M sodium hydroxide and 10 mL of 0.05 M glucose. The reaction mixture was stirred at a room temperature for 15 min. The characterization of the particles was performed by dynamic light scattering (DLS) and atomic force microscopy (AFM). The hydrodynamic diameter of the spherical AgNPs ($D_{\text{H}}$) was 40 nm.

Medium 203 was prepared according to the OECD 203 guideline [3]. The resulting concentrations of salts were 2 mM CaCl\textsubscript{2}, 0.5 mM MgSO\textsubscript{4}, 0.77 mM NaHCO\textsubscript{3} and 0.075 mM KCl with the final ionic strength of 680 $\mu$S.cm$^{-1}$. 
The agglomeration of AgNPs was studied with the use of an UV-Vis spectroscopy in experimental colloids prepared by mixing of primary AgNPs colloid ($c_{Ag} = 1$ mM) with demineralized water or Medium 203 immediately prior to measurement. The final concentration of Ag in these colloids was verified by ICP-OES. A Perkin Elmer Lambda 12 employing a 3.5 mL cuvette with an optical path of 1 cm at laboratory temperature was employed in order to measure the UV-Vis spectra.

The illumination tests were carried out employing LED sources (417 and 740 nm, the light intensity 35 W/m$^2$ both) and a polychromatic light source (Hund FLQ 150 M, max. intensity 1600 W/m$^2$).

3. RESULTS AND DISCUSSION

3.1. UV-Vis spectra of AgNPs single particles and agglomerates in experimental colloids

Agglomeration of AgNPs in experimental colloids prepared by dilution of primary colloid with demineralized water or Medium 203 was studied with the use of UV-Vis. The spectra of AgNPs colloid diluted with demineralized water exhibit a single band with a maximum at 404 nm (Fig. 1a). This band corresponds to the surface plasmon resonance of the spherical single particles with a diameter of 40 nm [4]. The intensity of the band is correlated to the concentration of single AgNPs (Fig. 1a). The experimental colloids diluted with demineralized water revealed a high stability over a time scale of 80 min for the Ag concentration range 10 – 250 µM (Fig. 1a).

The time-resolved UV-Vis spectra of AgNPs in Medium 203 exhibit a new band with maxima 620 – 760 nm associated with the growth of the agglomerates. The band maxima are shifted to higher values of wavelengths in correspondence with the increase in the agglomerate diameter [4]. The agglomeration process is exhibited on UV-Vis spectra by decreasing the intensity of the single AgNPs band and an increase in the intensity as well as the wavelength maxima of the agglomerate band for the AgNPs colloids based on Medium 203 (Fig. 1b). The concentration of single AgNPs in the experimental colloid was determined by deconvolution of the enveloping curve (Fig. 1c).

![Fig. 1 a. UV-Vis spectra of AgNPs in demineralized water, b. UV-Vis spectra of AgNPs in Medium 203 ($c_{Ag} 50$ µM), c. Illustration of deconvolution of envelope curve](image-url)
3.2. The influence of AgNPs concentration in Medium 203 on particle agglomeration

The agglomeration process was described by a relative concentration of single particles related to the total AgNPs concentration (c/c₀). The actual concentrations of single AgNPs (c) were determined by employing the above-mentioned procedure of UV-Vis spectra deconvolution. The c/c₀ values are decreasing for all of the studied Ag concentration (Fig. 2) during the experimental period. The increased concentration of AgNPs in Medium 203 led to the acceleration of the agglomeration process and an increase in the agglomeration extent. The agglomeration process was not detected in the demineralized water (Figure 1a).

3.3. The influence of illumination of experimental colloids on particle agglomeration

The effect of light exposition on the agglomeration process was studied employing the following light sources:

- 417 nm – close to the maximum absorption of single particles (light intensity 35 W/m²);
- 740 nm - close to the maximum absorption of agglomerates (light intensity 35 W/m²);
- polychromatic light – model of natural light condition (maximum light intensity 1600 W/m²).

Fig. 3 illustrates the effect of different light conditions on AgNPs agglomeration in colloid with an initial concentration of single AgNPs c₀=50 µM. The rate of actual and initial concentrations of single AgNPs (c/c₀) was used for a description of the agglomeration process. The time-dependences of the relative single particle concentration clearly indicates the acceleration of agglomeration rates and the extent in order dark<740 nm<417 nm < polychromatic light illumination.
The value of time when relative values $c/c_0$ reach 0.5 ($\tau_{1/2}$) and value $c/c_0$ in 80 min ($c^{80\text{min}}/c_0$) were used for quantification of the rate and the extent of the agglomeration process under different conditions (Tab. 1). The above-mentioned order (dark < 740 nm < 417 nm < polychromatic light illumination) of the agglomeration rate and extent is a general trend independent from the concentration in a range of 50 – 250 µM (Tab. 1). It could be tentatively assigned to the efficiency of the energy addition of to the AgNPs. The added energy induces the erosion of the AgNPs sheltering the double layer and accelerates the agglomeration.

This hypothesis is in sound correlation with the result of the experiment with polychromatic light intensity (400 – 1600 W/m$^2$). The higher intensity induced a higher agglomeration rate and extent (Tab. 1).

<table>
<thead>
<tr>
<th>c (µM)</th>
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<th>250</th>
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<tr>
<td></td>
<td>$\tau_{1/2}$</td>
<td>$c^{80\text{min}}/c_0$</td>
<td>$\tau_{1/2}$</td>
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<tr>
<td>Dark</td>
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<td>26.5</td>
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4. CONCLUSION

UV-Vis spectroscopy was used for a description of time dependent behavior of AgNPs colloids in demineralized water and Media 203. The system based on demineralized water exhibits a high time stability with the, rate of AgNPs agglomeration in Medium 203 being strongly dependent on the colloid concentration.

The agglomeration rates and extents were tested with respect to the colloid illumination condition with blue light (417 nm - single particles absorption), red light (740 nm – agglomerates absorption) and with polychromatic light. The illumination effected the agglomeration rate and extent in the trend of polychromatic light > blue light > red light > dark reference.
Obtained results indicate the potentially high influence of tested parameters (AgNPs concentration and light irradiation) on results of aquatic toxicity tests. Consequently, the test design of classical aquatic toxicity test should be modified to respect the importance of these parameters.

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LITERATURE
[3] OECD guideline for testing chemicals No 203 - Fish acute toxicity test.