

Synthesis and Biological Activity of Engineered SiO₂ Nanomaterials

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Abstract – The multi-disciplinary use of nanomaterials in everyday products has led to their release into the environment, where they can pose a threat to living organisms, including microorganisms. Currently, the level of new nanomaterials exceeds the set of available information about their potential toxicity. Therefore, it is of the greatest importance and challenge for future research to carry out nanotoxicological studies in order to expand the knowledge about their biological activity. The aim of this study was to evaluate the potential toxic effect of newly synthesized nSiO₂ nanoparticles and the nCu/SiO₂ nanocomposite against selected microorganisms. For this purpose, traditional toxicological methods using reference bacteria *Escherichia coli* and *Bacillus subtilis* as well as Microbial Assay for Risk Assessment (MARA) were performed. The study showed that nSiO₂ had a greater antibacterial impact on reference strains than nCu/SiO₂. In addition to this, *B. subtilis* was more sensitive to nanomaterials than *E. coli*. Ecotoxicological MARA test showed that the tested nanomaterials exhibited low antibacterial activity and none of them had antifungal activity. SEM imaging indicated that both nanomaterials formed irregularly shaped agglomerates.

Keywords: Nanomaterials, Microorganisms, Toxicity, Risk assessment, Scanning electron microscope imaging

1. Introduction

Although naturally occurring nanomaterials have existed in nature for a very long time, products and modern technologies with ‘nano’ prefixes have only recently become very popular. Modern technological solutions at a nanometric scale and the huge potential for their implementation in various economic sectors have initiated a new era of the industrial revolution in the 21st century. Nowadays, there is an extensive production of different engineered nanomaterials, which are used in everyday products and many advanced technological processes. For this reason, there is a potential risk of their release and accumulation in various ecosystems. It is well documented that living organisms can adapt to the presence of nanoparticles of a natural origin, however, the impact of man-made nanomaterials on their functioning is relatively little known. Moreover, there is no agreed upon protocol for testing the potential impact of nanomaterials on the environment and living organisms. Hence, it is of the greatest importance to carry out case studies to learn and comprehend the biological activity of these structures. Since nanomaterial hazards cannot be categorised and toxicity testing methods are not standardized, an individual risk assessment by many authors is used [1, 2].

The objective of this study was to examine the biological activity of chemically synthesised nSiO₂ nanoparticles and the nCu/SiO₂ nanocomposite against two model bacteria species *Escherichia coli* and *Bacillus subtilis*. Also, to assess the antimicrobial activity of the nSiO₂ and nCu/SiO₂ against phylogenetically unrelated microorganisms, the Microbial Assay for Risk Assessment (MARA) was performed.

2. Materials and methods

2.1. Synthesis of SiO₂ nanomaterials

The nCu/SiO₂ were synthesized and made available by the Institute of Material Engineering, University of Silesia, Poland. Commercially available nSiO₂ were obtained from the US Research Nanomaterials Inc. (USA). For the chemical synthesis of the nCu/SiO₂, nSiO₂ were used as an oxide matrix for the attachment of the nCu. The nCu were prepared *via* a

chemical reduction method using $\text{Cu}(\text{CH}_3\text{COO})_2$ as Cu ions donor, ascorbic acid as the reducer and ammonia solution for the stabilization of synthesized nanoparticles. The synthesis of nCu/SiO₂ required prior introduction of prepared nCu to the aqueous solution of nSiO₂ with the addition of 10% NaOH. The suspension was mixed using a magnetic stirrer and then filtered through a polyethylene filter. The precipitate was washed with distilled water and ethanol, and dried at room temperature resulting in the formation of nanopowder [3].

2.2. Scanning electron microscopy

The morphology of nanomaterials was observed using a scanning electron microscope (SEM). Aqueous solutions of nanomaterials were transferred to graphite tapes, stained with technical gold and observed under a microscope (JEOL JSM-7100F with an accelerating voltage of 15 kV and a vacuum of $9.6 \cdot 10^{-5}$ Pa).

2.3. Potential toxicity of nanomaterials

Antibacterial activity of nSiO₂ and nCu/SiO₂ was studied against two bacteria species *E. coli* (ATTC[®] 25922[™]) and *B. subtilis* (ATTC[®] 6633[™]) using a traditional plate method [3]. For this purpose, bacteria were grown in a Luria-Bertani medium (LB) for 3-4 h (37°C, 130 rpm) to reach the mid-exponential growth phase. Next, bacterial cells were inoculated into fresh LB (OD₆₀₀ = 0.1) and appropriate nanomaterials in the concentration range of 10 to 1500 mg L⁻¹ were added. Bacterial cultures with and without the addition of nSiO₂ and nCu/SiO₂ (control samples) were incubated for 24 h (37°C, 130 rpm). After this time, 10-fold dilution series were made from each culture in 0.85% saline solution and transferred (100 µl) on LB agar plates. After 24 hours of incubation at 37°C, the grown single colonies were counted. Microbial inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were determined based on 99% and 100% inhibition of bacteria growth, respectively, in comparison with their growth in a control sample. In contrast, the half maximal inhibitory concentration (IC₅₀) was determined using the Prism 5 program (GraphPad Software, USA).

2.4. Microbial Assay for Risk Assessment (MARA)

The MARA test was used to assess the toxicity of nSiO₂ and nCu/SiO₂ on 11 different microbial strains from diverse environmental niches. A MARA plate was independently prepared for each type of nanomaterial with final concentrations of 5 to 1000 mg L⁻¹. After an 18-hour incubation, the MARA plates were scanned using a HP PrecisionScan Pro scanner and analysed using the MARA program. Assessment of the overall toxicity of nanomaterials was expressed as a microbial toxic concentration (MTC, mg L⁻¹).

3. Results and discussion

Scanning electron microscopy imaging of the nSiO₂ and nCu/SiO₂ structure showed that both nanomaterials tended to form irregularly shaped agglomerates (Fig. 1). This tendency to form larger structures may affect their biological properties and bioavailability. The determined MIC, MBC and IC₅₀ for nSiO₂ and nCu/SiO₂ against *E. coli* and *B. subtilis* are presented in Table 1. The results clearly indicated that nSiO₂ was characterised by higher antibacterial activity than nCu/SiO₂. The smallest bactericidal activity of nCu/SiO₂ was recorded for *E. coli*. The MIC, MBC and IC₅₀ values confirmed the greater sensitivity of *B. subtilis* to nCu/SiO₂ than *E. coli*, however, the addition of nSiO₂ to both bacterial cultures had a similar effect. A comparable effect was observed by Palza et al. [4], who tested the biocidal action of spherical SiO₂ and Cu/SiO₂ on *E. coli* cells. Their antibacterial character was determined by the MIC and MBC values, which were 1500 and 1650 mg L⁻¹ for Cu/SiO₂, respectively, and >5000 mg L⁻¹ for SiO₂. Here, the MIC and MBC values for nCu/SiO₂ against *E. coli* corresponded to the values >1000 mg L⁻¹. Interestingly, the MIC and MBC concentrations of nSiO₂ in this work were 10-fold smaller in comparison with the obtained values by [4]. The stronger antimicrobial effect of nSiO₂ may be associated with the irregular shape of agglomerates, which increases their biological reactivity. In another study, Adams et al. [5] reported 99% and 48% growth inhibition of *B. subtilis* and *E. coli* after the exposure to 5000 mg L⁻¹ of SiO₂. Moreover, *B. subtilis* was more sensitive to nanoparticles than *E. coli*.

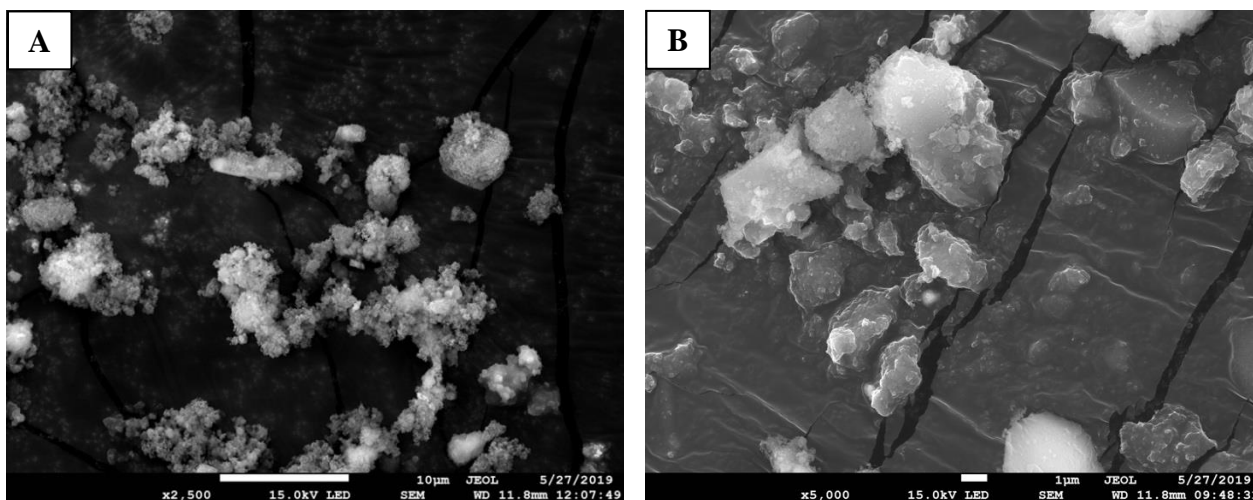


Fig. 1: Scanning electron micrographs of nSiO₂ (A) and nCu/SiO₂ (B).

Table 1: The values of MIC, MBC and IC₅₀ for nSiO₂ and nCu/SiO₂ against *E. coli* and *B. subtilis*.

Type of nanomaterial	<i>Escherichia coli</i>			<i>Bacillus subtilis</i>		
	MIC	MBC	IC ₅₀	MIC	MBC	IC ₅₀
	mg L⁻¹					
nSiO ₂	500	500	90,22	200	500	131,79
nCu/SiO ₂	>1000	>1000	>1000	525	600	503,22

In order to gain more knowledge about the potential toxicity of tested nanomaterials against 11 other microorganisms, the MARA test was performed (Table 2). Both nSiO₂ and nCu/SiO₂ exhibited low antimicrobial activity (MTC > 1000 mg L⁻¹) in relation to most tested strains. The exception was *Staphylococcus warneri* (8), for which a high toxicity of nSiO₂ (MTC = 435 mg L⁻¹) was recorded. Strains *Brevundimonas diminuta* (2) and *S. warneri* (8) were susceptible to the toxic effects of nCu/SiO₂, however, nSiO₂ proved to have a twice stronger antibacterial effect on *S. warneri* (8) than nCu/SiO₂. This indicates a similar trend to those obtained for model *E. coli* and *B. subtilis*. It is also worth emphasizing that none of the tested nanomaterials, even at the highest concentration of 1000 mg L⁻¹, showed no antifungal activity towards *Pichia anomala* (11). There are currently relatively few literature reports on the use of the MARA test to assess the toxicity of nanomaterials. An example is a study by Santos et al. [6] who reported that Er₂O₃ and Ho₂O₃ (MTC 67 - 69 mg L⁻¹) were lethal to tested microorganisms. The lethal concentration of these nanomaterials was much lower than those tested in this study, therefore, each nanomaterial characterised by its own unique toxic profile should be analysed individually. Here, the tested nanomaterials demonstrated a smaller biocidal activity than the ones studied by [6], presumably due to their tendency to agglomerate.

Table 2: The MTC values of nSiO₂ and nCu/SiO₂ in relation to MARA microorganisms.

Type of nanomaterial	MTC [mg L ⁻¹]											Average MTC
	1	2	3	4	5	6	7	8	9	10	11	
nSiO ₂	>max	>max	>max	>max	>max	>max	>max	435	>max	>max	>max	>max
nCu/SiO ₂	>max	847	>max	>max	>max	>max	>max	920	>max	>max	>max	>max

1 - *Microbacterium* spp., 2 - *Brevundimonas diminuta*, 3 - *Citrobacter freundii*, 4 - *Comamonas testosteroni*, 5 - *Enterococcus casseliflavus*, 6 - *Delftia acidovorans*, 7 - *Kurthia gibsonii*, 8 - *Staphylococcus warneri*, 9 - *Pseudomonas aurantiaca*, 10 - *Serratia rubidaea*, 11 - *Pichia anomala*.

4. Conclusion

The obtained MIC, MBC and IC₅₀ values indicated that the impact of nSiO₂ and nCu/SiO₂ on the growth of *E. coli* and *B. subtilis* depended on their concentration and the species of microorganism. Despite the formation of agglomerates, the tested nanomaterials exhibited antibacterial activity, with nSiO₂ having greater bactericidal activity than nCu/SiO₂. Furthermore, the MARA test indicated that nSiO₂ and nCu/SiO₂ did not have antifungal activity. Undoubtedly, the obtained results confirmed a diverse impact of nanomaterials on microorganisms, however, further research is needed to explain their antimicrobial activity through multiple mechanisms including damage to the membrane and bacterial cell wall, damage to DNA, proteins and internal components, release of ions as well as oxidative stress. Detailed characteristics of these nanomaterials will enable their practical use in public nanotech products.

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