ISSN 2223-6775, Ukrainian Journal of Occupational Health, 2022, 18 (3), 197–203

UDC 546.82-022.532:[613.63+504.054]:57.08 https://doi.org/10.33573/ujoh2022.03.197

STABILIZATION OF DOPED TITANIUM-CONTAINING NANOMATERIALS IN THE FRAMEWORK OF SCREENING STUDIES OF POTENTIAL HAZARDS FOR WORKERS AND THE ENVIRONMENT

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Introduction. Doping with heavy metals can increase the initial toxicity of the nanomaterial, and cause adverse effects on environment and workers health.

The aim of the study – to analyze the dispersion of hydrosols of nanopowders of doped titanium-containing nanomaterials in different environments and to substantiate the feasibility of stabilizers for a series of screening studies of potential hazards.

Materials and methods of the study. The dimensionality of titanium dioxide complex doped with silver (TiO_2 + Ag nanocomposite, mass fraction of Ag ~ 4 %), titanium dioxide complex doped with silver (TiO_2 + Ag nanocomposite, mass fraction of Ag ~ 8 %), and titanium dioxide nanopowder (TiO_2) in different environments/stabilizers was estimated. The particle size was determined by dynamic light scattering using an Analysette 12 DynaSizer (Fritsch, Germany).

Results. Stabilization of titanium nanopowders with a glucose-citrate buffer makes it possible to obtain relatively stable hydrosols that can be used in screening studies using bovine spermatozoa as a test object. For studies of phyto- and antibacterial toxicity, it is advisable to use nanopowders in physiological solution.

Conclusions. In-depth investigations on the potentially toxic effects of additional doping of nanopowders with heavy metals on workers' health and environment are needed.

Key words: titanium-containing nanomaterials, doping, stabilization, screening, dispersion

Introduction

During the last decade, considerable attention of scientists has been focused on the unique properties of nanomaterials resulting from quantum size confinement and surface effects. In particular, quantum size confinement affects the energy structure and physical properties of doped (alloyed) nanomaterials and nanodevices. In turn, doping of nanomaterials provides a flexible way to tune the properties of materials while maintaining high surface areas. Doped nanomaterials are expected to make significant contributions to nanotechnology for practical applications in the fields of electronics, photonics, optics, medical sciences, homeland security, etc. [1]. The doping of various materials, especially semiconductors, is known as a powerful tool to improve the properties and find appropriate applications in industry, particularly in nanoelectronics and nanophotonics [2]. Luminescence phenomena are actively investigated in doped nanoparticles for radiation detection, in particular, in infrared detection and dosimetry. Luminescent nanoparticles in the near-infrared range are particularly promising for biological imaging, as higher image resolution can be obtained by using autofluorescence. Doped insulator nanomaterials, including carbon nanotubes,

ISSN 2223-6775, Ukrainian Journal of Occupational Health, 2022, 18 (3), 197–203

represent a new type of high-performance diagnostic luminescent material. As a new kind of biological markers, insulator nanoparticles are less toxic than semiconductor nanoparticles and are promising for the detection, diagnosis and treatment of cancer.

Hence, doping is a widely used method to modify nanoparticles to enhance their electrical, optical and biological activities [1]. For example, lanthanide-doped nanoparticles have been developed as a new class of luminescent nanomaterials. Unlike conventional bulk phosphors, nanoparticles provide a convenient platform for tunable optical emission and facilitate integration with other functional groups such as biological molecules. Therefore, such materials are very promising in bio- and information technologies, energy, etc. Impurities of doping (alloying) substances, such as Cu^{2+}, Mn^{2+}, Co^{2+}, Ni^{2+}, rare-earth and transition elements, play an important role in changing the electronic structure, in particular in terms of the possibilities of modulating the basic material. Also, doping can enhance the antimicrobial effect [3]. In particular, silver doped with polymeric chitosan and iron oxide provides high antimicrobial efficacy against *E. coli*, *B. subtilis* and *S. aureus bacteria*. In turn, titanium dioxide (TiO_2) doped with silver and nitrogen can enhance the antibacterial properties of TiO_2 nanoparticles against *E. coli* and *B. subtilis* when irradiated with fluorescent light after cultivation for 24 hours. In another study, TiO_2 nanoparticles doped with Ag also exhibited toxic effects against *E.coli* bacteria. The TiO_2 + Ag composite coating showed complete killing of methicillin-resistant *S. aureus* within 24 hours under all culture conditions.

Thus, doped titanium-containing nanomaterials, in particular, titanium dioxide nanopowder doped with silver, can contribute to the destruction of pathogenic microorganisms and be used in disinfectants. At the same time, it should be noted that doping with heavy metals can increase the initial toxicity of the nanomaterial, and therefore – cause environmental pollution and adversely affect the health of workers. It should also be noted that a limited number of studies show that some nanomaterials may have ecotoxicological effects [4]. The toxicological behaviour of nanomaterials in direct contact with cells depends on their chemical composition, amount, solubility, shape, area and charge. The impact may depend on how long the nanoparticles remain intact and how they can accumulate in the biosystem. Impurities generated during the production of nanomaterials also affect their toxicity.

At the same time, as at the beginning of the development of nanotechnology, the development and implementation of new materials are significantly ahead of biomedical research on the potential hazardous effects of nanomaterials on the human body and the environment. In its turn, the implementation of these studies has a number of obstacles and complications associated with the aggregate state of the substance: a significant number of new materials are presented in the form of nanopowders, which raises the issue of obtaining stable hydrosols of nanopowders for experimental studies *in vitro* and the choice of stabilizers that do not affect the toxicity and biological properties of the starting material.

The aim of the study – to analyze the dispersibility of hydrosols of nanopowders of doped titanium-containing nanomaterials in different environments and to substantiate the feasibility of certain stabilizers for a series of screening studies of potential hazards *in vitro*.

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Materials and methods of the study

The size of titanium dioxide complex doped with silver (TiO_2 nanocomposite + Ag, mass fraction Ag ~ 4 %), titanium dioxide complex doped with silver (TiO_2 nanocomposite + Ag, mass fraction Ag ~ 8 %) and titanium dioxide nanopowder (TiO_2) synthesized by thermal decomposition in different media/stabilizers was evaluated. The particle size was determined by dynamic light scattering using an Analysette 12 DynaSizer (Fritsch, Germany).

Results of the study and their discussion

Titanium dioxide nanoparticles (TiO_2) are used in the manufacture of a wide range of products, which can ultimately create an additional burden on ecosystems. It is also known that skin exposed to UV light can be a significant target for photosensitized damage by commercial TiO_2 nanoproducts at concentrations of 0.2–3.0 mg/ml [5].

Our own experimental studies have shown that TiO_2 nanocomposite + Ag (mass fraction of Ag ~ 4 mass.%), and TiO_2 nanopowder at concentrations of 3 mg/ml initiate pathological changes in bovine spermatozoa, which are markers of oxidative stress (abnormalities of the head, middle part and tail, as well as the absence of acrosome, etc.), while the pathological effect of TiO_2 + Ag (4 %) nanocomposite is more pronounced [6].

Experimental studies *in vitro* have shown that under the influence of TiO_2 at a concentration of 30 μg/ml on peripheral blood mononuclear cells we observe a statistically significant increase in the production of IL-1. On the contrary, TiO_2 + Ag in concentrations of 30 μg/ml is able to increase the functional activity of peripheral blood mononuclear cells by the production of pro-inflammatory cytokines IL-1, IL-6, TNF-α and IL-4 production in volunteer donors, which indicates a potential impact on the formation of chronic inflammation and allergic reactions in the relevant category of nanomanufacturing workers [7]. The results of the study of the impact of titanium-containing nanomaterials on honey bees (Apismellifera) are also of interest, since it is believed that people who ate bee products (pollen, propolis, etc.) are exposed to nanoparticles. The LC_{50} values evaluated over 96 hours were 5.865 mg/L for TiO_2 and 312.845 mg/L for TiO_2 + Ag. The concentration group that had the highest contribution in terms of mortality rate was 100 mg/L for TiO_2, compared to 10 mg/L for Ag-TiO_2. The toxic effect of TiO_2 and TiO_2 + Ag nanoparticles increased with increasing concentration and exposure time [8].

Usually, titanium-containing nanomaterials are presented in the form of nanopowders, which raises the issue of their conversion into stable hydrosols for *in vitro* and *in vivo* studies and, accordingly, the search for an adequate stabilizer. To stabilize nanomaterials in solutions, organic and inorganic compounds are used to obtain colloidal solutions of varying degrees of stability (polythiocyanohydroquinone, low molecular weight polyvinylpyrrolidone, human serum albumin, polysaccharides, sodium citrate, etc. The toxicity of the composition is determined not only by the toxicity of the active ingredient (e.g., metal nanoparticles), but also by stabilizing or other auxiliary components that can affect the biological activity of the resulting solution, as well as increase its toxicity. In particular, a method of stabilization of silver nanoparticles with sodium citrate is known, the disadvantage of which is a wide distribution of the obtained nanoparticles in size, low stability during storage, as well as contamination of the final ash with oxidation products of citrate anion, in particular acetondicarboxylic and itaconic acids [10]. There is also a known

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method of stabilizing nanocrystalline cerium dioxide with citric and polyacrylic acids, which are added directly during the synthesis and adsorbed on the surface of cerium dioxide nanoparticles and prevent their agglomeration during the synthesis process [11]. The disadvantage of this method is its complexity and laboriousness. In turn, the possibility of using polyhydroxyl compounds (primarily carbohydrates) as a stabilizer of nanoparticles has been revealed [12]. Glucose-citrate buffer (glucose (4 g), sodium citrate (1 g) in 100 ml of distilled water) is used in a rapid method for determining the toxicity of nanomaterials in *in vitro* solutions using bovine spermatozoa as a test object for defrosting sperm, as well as a control solution. Thus, when bovine spermatozoa are used as a test object, stabilization of metal nanopowders with glucose-citrate buffer (glucose and sodium citrate ratio 4:1) is the optimal solution, as it allows to obtain stable hydrosols (Table 1).

On the other hand, bovine spermatozoa cannot be used as a test object for studies of antibacterial activity and phytotoxicity. It is also obviously inappropriate to use glucose-citrate buffer as a stabilizer, which is a favorable environment for spermatozoa, but not for microorganisms and plants.

Studies of the dispersibility of titanium-containing nanomaterials have shown that in physiological solution the particles of TiO_2 nanocomposite + Ag (4 %) at a concentration of 3 mg/ml have a diameter comparable to that in glucose-citrate buffer (with a ratio of glucose and sodium citrate 4:1) (Table 2). However, the obtained hydrosols are unstable, as they significantly agglomerate on the second day with an average particle diameter of about several thousand nanometers. Thus, the obtained hydrosols should be used in experimental studies *in vivo* and *in vitro* immediately after preparation. Also, the use of FicollPaque Premium as a medium for nanopowders – a sterile solution of polysaccharose 400 with sodium diatrizoate with a density of 1.077 g/ml for *in vitro* biomedical studies – demonstrated rapid agglomeration of nanoparticles (Table 2).

Taking into account the above, in-depth studies of the impact of additional doping of nanopowders with heavy metals on the body of workers in the field of nanotechnology and environmental objects are necessary. It also seems expedient to use microorganisms (in particular, sanitary-indicative ones) as a test object in screening studies of titanium-containing nanomaterials.

Table 1

Dispersion of TiO_2 + Ag (4 %) nanocomposite in glucose-citrate buffer over time

Number n/a	Environment/stabilizer	Day	Aerodynamic diameter, nm
1	Glucose-citrate buffer (glucose : sodium citrate: 4:1)	I	48.65
2	Glucose-citrate buffer (glucose : sodium citrate: 4:1)	II	53.37
3	Glucose-citrate buffer (glucose : sodium citrate: 4:1)	III	52.12
4	Glucose-citrate buffer (glucose : sodium citrate: 4:4)	I	134.93
5	Glucose-citrate buffer (glucose : sodium citrate: 4:4)	II	195.04
6	Glucose-citrate buffer (glucose : sodium citrate: 4:4)	III	234.49

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Table 2

Dispersion of titanium-containing nanomaterials in different environments

Number n/a	Nanomaterial	Environment/stabilizer	Aerodynamic diameter, nm
1	TiO_2	Glucose-citrate buffer	46.84
2	TiO_2	Physiological solution	Particle size cannot be measured due to rapid agglomeration
3	TiO_2	FicollPaque Premium (polysaccharose with sodium diatriazole)	128.86
4	TiO_2 + Ag (4 %)	Glucose-citrate buffer	48.8
5	TiO_2 + Ag (4 %)	Physiological solution	48.32
6	TiO_2 + Ag (4 %)	FicollPaque Premium (polysaccharose with sodium diatriazole)	328.8
7	TiO_2 + Ag (8 %)	Glucose-citrate buffer	80.8
8	TiO_2 + Ag (8 %)	Physiological solution	98.08
9	TiO_2 + Ag (8 %)	FicollPaque Premium (poly sucrose)	314.25

Conclusions

1. Stabilization of titanium-containing nanopowders with glucose-citrate buffer allows to obtain relatively stable hydrosols that can be used in screening studies using bovine sperm as a test object.
2. For the study of phyto- and antibacterial toxicity of nanomaterials, it is advisable to use nanopowders in physiological saline, which can be used during the first day. The use of a sterile solution of polysaccharose with sodium diatriazole as a stabilizer of powdered titanium-containing nanomaterials is impractical due to the rapid agglomeration of nanoparticles.
3. In-depth studies of the potential toxic effects of additional doping of nanopowders with heavy metals on the body of workers in the field of nanotechnology and the environment are needed.

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ISSN 2223-6775, Ukrainian Journal of Occupational Health, 2022, 18 (3), 197–203

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Information on the sources of funding for the study: the research was carried out on the topic «Development of predictors of potential hazard of industrial aerosols of different dispersion», state registration number 0122U000674.

Received: August 17, 2022
Accepted for publication: September 15, 2022

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