Dispersion of ZrO\textsubscript{2} and Y\textsubscript{2}O\textsubscript{3} nanopowders in physiological suspensions

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Abstract. The dispersion of suspensions based on nanostructured powders (ZrO\textsubscript{2} and Y2O3) and the simplest physiological fluids (phosphate buffering saline, glucose solution, water) in terms of their use in ecotoxicological tests was studied. Using the scanning electron microscopy and laser diffraction, an increase in the size of particles and agglomerates in the suspensions in 2-11 times was shown. The particle size distribution in the suspensions was characterized by a single peak, but its magnitude and width varied ambiguously upon standing suspensions in time. It was shown experimentally that the average size of the dispersed phase could rapidly grow in the DW-suspension, could be stabilized in PBS-suspension and might decrease with time in Gl-suspension. By electro-acoustic method it was shown that the Zeta-potential on the solid/liquid boundary in the study varied over a wide range of values from $-200$ to $+200$, and the nature of change - a jump. This demonstrated the instability of the suspensions based on NP-ZrO2 and NP-Y2O3.

1. Introduction

Nanosized powders of metal oxides are widely used in electronics and optics (SiO\textsubscript{2}, Nd\textsubscript{2}O\textsubscript{3}, Eu\textsubscript{2}O\textsubscript{3}, Dy\textsubscript{2}O\textsubscript{3}, Au, Pt) \cite{1}, chemical and cosmetic industries (Al\textsubscript{2}O\textsubscript{3}, ZnO, TiO\textsubscript{2}, Ag, Pt) \cite{2}, water treatment technologies (Al\textsubscript{2}O\textsubscript{3}, Fe\textsubscript{2}O\textsubscript{3}), energetic (Dy\textsubscript{2}O\textsubscript{3}, Au), in agriculture and food industry (Fe\textsubscript{2}O\textsubscript{3}) \cite{3}.

The life cycle of nanopowders includes the manufacture, use and disposal of powders or products containing nanopowders. In this case, employees of nanotechnology industries and laboratories are primarily at risk, as they are exposed to nanoparticles \cite{4}. Intensively developing nanotech production of powdered materials requires consideration of safety and risk assessment of the impact of nanomaterials on humans and other living organisms.

Nanopowders possess properties that often radically differ from their counterparts in the form of macroscopic dispersions or solid phase \cite{2}. Small size and high physico-chemical activity of nanoparticles allow them to penetrate through biological membranes and bind to cells, tissues and organs easier than microsized particles. In contact with living organisms, nanoparticles can penetrate through cellular barriers, as well as to overcome the blood-brain barrier in the central nervous system, to circulate and accumulate in tissues and organs \cite{5,6}, thus causing toxic effects on organs and tissues.
According to several studies, even non-toxic by nature TiO$_2$, ZnO, Ag in nanoscale condition can cause a cytotoxic effect [9-12]. This phenomenon demonstrates that the development of methods assessing the risks associated with the production of nanopowders and the use of products containing nanoparticles is one of the leading trends of nanotechnology development [13].

2. Application of dispersion in the toxicological study
Identification of nanoparticles in the environment, determination of their composition, concentration, structure are influenced by a number of physico-chemical factors: the number of particles, their charge, size, size distribution, surface area, structure and shape, degree of agglomeration and element composition [7]. Therefore, toxicological study of nanomaterials in various environmental media has many analytical subtleties. One of the biggest problems in the study of nanomaterials is their tendency to aggregation [14]. In practice, to prevent aggregation or deposition of particles, dispersion is used.

Dispersion of solid particles is often achieved by the addition of solvents and surfactants (stabilizers), ultrasonic action and prolonged agitation. The main advantages and disadvantages of currently used methods for dispersing the nanoparticles are shown in table 1 [15]. Here it should be given more precise definition to the terms used in the paper – agglomerates and aggregates. So, the particles aggregates are the particular case of particles agglomerates but differ from the last with less force between the particles.

| №  | Method Name                  | Advantages                                                | Disadvantages                                                                 |
|----|------------------------------|-----------------------------------------------------------|-------------------------------------------------------------------------------|
| 1. | Without additional dispersion| No chemical additives                                      | Uncontrolled dispersion                                                      |
|    |                              | No additional toxicants                                    | Study of toxicity, behavior, activity of the agglomerates/aggregates         |
|    |                              | Rapid sample preparation                                   |                                                                              |
|    |                              | Close to the real conditions                               |                                                                              |
| 2. | Adding solvents and stabilizers| Rapid stabilization of suspensions                        | Possible toxicity of additives                                               |
|    |                              | Stability of suspensions                                   | Adding of toxic contaminants from additives                                  |
|    |                              |                                                          | Surplus supplements can alter the structure and influence the toxicity       |
|    |                              |                                                          | Limitations in obtaining the appropriate information about the real toxicity potential of an unknown nanomaterial |
|    |                              |                                                          | Need to control the concentration                                           |
| 3. | Sonication*                  | No chemical additives                                      | Need a time to process                                                       |
|    |                              | No additional toxicants                                    | Processing time affects the concentration of aggregates                      |
|    |                              | Not necessary to control additives                         | Processing can affect the shape and, consequently, the toxicity of the samples |
|    |                              |                                                          | Sound for natural samples in the presence of electron-donor promotes oxidation of the samples in solution |
|    |                              |                                                          | Unstable systems                                                            |
| 4. | Stirring and shaking         | Prolonged mixing                                           | Stirring can affect the shape and, consequently, the toxicity of the samples |
|    |                              |                                                          | Unstable systems                                                            |

*In this way “sonication” means particularly the water bath sonication.
Despite the shortcomings of the second method “Adding solvents and stabilizers”, it is the only one method that allows maintaining the dispersion of suspensions during the whole experiment. However, the nature of the solvent is one of the most important criteria for assessing its suitability. For example, tetrahydrofuran (THF), one of the most effective stabilizer of the fullerene, promotes solvation of the fullerene molecules in the water, as THF makes the system «solvent – water» less polar. This radically changes the behavior of C_{60}-nanoparticles in water. For example, when water fleas were exposed to THF-stabilized by and stabilized pre-mixing (2 months) fullerene suspensions, their lethal dose LD\textsubscript{50} for 48 hours reached 0.8 mg/l and 35 mg/l, respectively [16]. This suggests that the toxicity of the fullerene particles could increase in the presence of THF. So many researchers have questioned the use of THF as a non-toxic preservative. Chemical additives (amines and furans) are also invited to use as stabilizers, but the lack of experimental data leaves the question open [7,15].

Also one of the most common media for toxicological testing is phosphate buffering saline (PBS). PBS’s composition is very simple; its pH-value is very close to the blood serum pH. Moreover, PBS is not toxic to living organisms. However, experiments show that this medium is not always appropriate, because oxide and metal particles can be strongly agglomerated and can interact with the formation of oxide-hydroxide forms on the boundary of the particle/solution [17]. As a consequence, the use of stabilizers can uniquely affect the interpretation of physical and chemical reactivity of nanomaterials during the tests. What is more, the nanoparticles are not delivered in its original form to into tested organisms.

Initial experiments have shown that nanoparticles of metals and metal oxides demonstrate chemical stability and their dispersion can be well preserved in physiological solutions of organic nature. Therefore, the purpose of this study was to estimate the dispersity/stability of suspensions based on the metal oxide nanopowders and simple saline solution for their use in ecotoxicological tests in the further study.

3. Objects of research
Nanopowders of zirconium oxide (NP-ZrO\textsubscript{2}) and yttrium oxide (NP-Y\textsubscript{2}O\textsubscript{3}), obtained by plasma-chemical method, were taken for the study. These nanopowders are the promising materials for the manufacturing of functional and structural ceramic with the help of a dry compaction technique under the influence of an ultrasonic action [18]. In the Nano-Centre of Tomsk Polytechnic University (Russia, Tomsk) [http://portal.tpu.ru/departments/centre/nano/eng] a lab-scale production line of nanostructured ceramic has been working for more than 5 years. Presently, the Nano-Centre is introducing a line of nanopowder production (as a raw material for industry) using spray-drying methods of production. In the production process (obtaining, drying, packing, transport and use) nanopowders form an aerosol in the occupational area, which, somehow, makes contact with the staff.

4. Determination of the composition and morphology of the powder particles
The phase composition of powders was determined by X-ray diffraction (XRD) method on the powder diffractometer XRD-7000 (Shimadzu). Recording X-ray was performed using CuK\textsubscript{α} radiation in the range of angles 2θ from 10 to 120°. To identify X-ray data it was used ICPDS (International Center for Diffraction Data) data.

Determining the characteristics of nanopowders microstructure surface was carried out with a scanning electron microscope (SEM) JSM-7500FA (JEOL), equipped with a microanalyzer EDS (JEOL), which allows to determine the elemental composition of the surface depth of 50 Å from boron to uranium.

5. Preparation of physiological solutions
To study the effect of the stabilizer nature, phosphate buffering saline (PBS) and glucose solution (Gl) were chosen. Distilled water was used for comparison. Chemical composition and pH values of the solutions are presented in table 2. Chemical vessels before preparing the solution were kept at least for 24 h in 10%-solution of HNO\textsubscript{3}, the vessels were cleaned twice in distilled water. On the base of
prepared solutions and chosen nanopowders the suspensions were prepared, the concentration of particles was 0.10-0.15 wt. %.

| №  | Name of the solution                  | Contents of chemicals, g/l                                      | pH    |
|----|--------------------------------------|-----------------------------------------------------------------|-------|
| 1  | Distilled water (DW)                 | ---                                                             | 7     |
| 2  | Phosphate buffering saline (PBS)     | 8.77 1.28 1.36 --- ---                                       | 6.4 – 7.2 |
| 3  | Glucose solution (Gl)                | ---                                                             | 5.5 – 6.5 |

PBS is the base of almost all biological fluids (serum, lung fluid, sweat, saliva, etc.); it is traditionally used for toxicological study; and it is easy to maintain pH in PBS. Gl-solution is a valuable source of easily digestible nourishing material by a human body. Furthermore, glucose is a component of blood and interstitial fluid. The selected media are not only nontoxic to living organisms, but also used for the therapeutic treatment (update and replenish fluids in diseases).

6. Determination of powder dispersion and dispersed phase in suspensions

The specific surface of dry powders (S) was determined using the low temperature adsorption of nitrogen (BET theory) with the instrument «SorbiPrep» (META, Russia). The accuracy of measurements was ± 0.20 m²/g. Assuming that the particles are spherical, average diameter of the particles was calculated with the experimental formula:

\[
d = \frac{6}{\rho \cdot S},
\]

where \(d\) - average particle’s diameter, m; \(\rho\) - material density, kg/m³; \(S\) - specific surface area of powder (BET data), m²/kg.

Particle size distribution in the suspensions was obtained using the particle analyzer SALD-7101 (Shimadzu) basing on the method of laser diffraction. The method of laser diffraction is based on the particle scattering light, since the scattering angle of light is universally proportional to the size of the particles. The measurement was carried out for suspensions of nanopowders volume of 220 ml for 60-90 minutes with an interval of 2-10 minutes. The experiment was carried out while shaking without ultrasound exposure, since ultrasound promotes fragmentation of agglomerates [15]. All vessels and tools used for the experiments were acid-cleaned (10%-HNO₃) and twice cleaned in distilled water. The experimental results are curves surround the particle size distribution in the range of 0.01-300 μm. The results were used to calculate the average diameter (\(D_{av}\)) as follows:

\[
D_{av} = \sum d \times \frac{q(\%)}{100}\%
\]

where \(d\) - particle size in dispersions, μm; \(q\) - number of the particles with diameter \(d\), %.

7. Determination of electrokinetic properties of the suspension

Zeta-potential at the solid/liquid boundary in suspensions were determined with the electro-acoustic spectrometer Zeta-ASP (Matec Applied Sciences, USA). The method is based on electro-acoustic effects arising as a result of transmission of ultrasound through a Newtonian liquid, dispersion medium or a porous body. According to the method, the sensor analyzer sends short pulses of high
frequency (pulse duration 30 µs), to the sample located in the space around the electrode sensor, with the pulse frequency of 0.5-3.5 MHz, the amplitude of 100-600 V.

The measurements were carried out for 20-90 minutes at intervals of 1 minute in 200 ml of suspension. Each experiment was repeated twice, vessels were acid-cleaned at least for 24 h (10%-HNO₃).

8. Results and discussion

X-ray data of the NP-ZrO₂ and NP-Y₂O₃ nanopowders are demonstrated in figure 1. It shows that the nanopowder of NP-ZrO₂ was stabilized with small amounts of yttrium oxide, which is a common practice in the use of this nanopowder for the manufacture of ceramics.

According to SEM the particles of dry NP-ZrO₂ nanopowder form the agglomerates with the size of 3-6 µm (figure 2a). Individual particles (size 100-500 nm) are spherical in shape, but inside they are hollow (figure 2b) with a wall thickness of 20-30 nm (figure 2b). According to the forecasts particles of such a form can be carriers of substances due to the presence of the internal cavity. Agglomerates of dry nanopowder NP-Y₂O₃ are blocks with dimensions of 0.5...0.8 × 0.8...2.0 µm (figure 3a). Particles constituting the agglomerates are in the form of grains with a size of 20-250 nm (figure 3b).

According to the BET specific surface S = 8.10 m²/g

![Figure 1](image1.png)

Figure 1. X-ray data of the nanopowders of ZrO₂ and Y₂O₃

![Figure 2](image2.png)

Figure 2. The morphology of the initial ZrO₂ powder: a) agglomerates of the powder, and b) the individual particles. According to the BET specific surface S = 8.10 m²/g

![Figure 3](image3.png)

Figure 3. The morphology of the initial Y₂O₃ powder: a) agglomerates of the powder, and b) the individual particles. According to the BET specific surface S = 10.45 m²/g
Data of the dispersion obtained with SEM are confirmed by the BET-data: surface average particle
diameter as a rough approximation for NP-ZrO$_2$ (density 6.02 g/cm$^3$) $d_{av} = 123$ nm, and for NP- Y$_2$O$_3$
(density 4.84 g/cm$^3$) $d_{av} = 118$ nm.

Stability of studied suspensions may be evaluated with the dispersion changing (the size
distribution and/or average size) and the Zeta-potential at the boundary of the dispersed phase and the
dispersion medium. To analyze the dispersion of nanopowders in the suspension based on the simplest
physiological solutions, the experimental data on the amount of dry agglomerates (SEM) and the data
on the agglomerates size of the dispersed phase (the method of laser diffraction) were compared.

According to the method of laser diffraction, the particles distribution is characterized with a single
peak in all the suspensions. It is important to note that, despite the fact that the number of peaks does
not change over time, the peak value can vary ambiguously upon testing the suspensions. Thus, for the
both powders the value of the peak is gradually changing in the Gl-suspensions: for NP-ZrO$_2$ the peak
value (the value of q, the wt.%), increases in time from 11 to 19 wt.% while the maximum particle size
is significantly reduced from 45 to 31 µm (Figure 4a). While for the sample NP-Y$_2$O$_3$, magnitude of
the peak decreases from 13 to 10 wt.%, the peak itself is expanding, and the maximum particle size
increases from 47 to 70 µm (Figure 4b). Apparently, the opposite trend to a dispersion change can be
related to particle shapes and, consequently, to agglomerate forms.

![Graph](image1)

**Figure 4.** The particles size distribution in the Gl-suspension: a) the suspension of the ZrO$_2$
nanopowder, b) the suspension of the Y$_2$O$_3$ nanopowder (method of laser diffraction)

The dispersion of powders in PBS- and DW-suspensions varies ambiguously, with the clear trend
to a change observed neither for the magnitude of the peak, nor for the maximum aggregates.

Data on changes in the calculated average particle size ($D_{av}$) in the suspensions are shown in
figure 5. Comparison with the initial size of the powders agglomerates, determined by SEM, reveals
that the average size of dry aggregates (3-6 µm) for NP-ZrO$_2$ under suspension stirring slightly
increased in the PBS- and DW-suspension - up to 7.5-8.0 µm, and when released into the Gl-solution
$D_{av}$ it increased almost in 2 times - up to 12-13 µm (figure 5a). However, after a 30-minute exposure
in the DW-suspension $D_{av}$ is intensively growing; in PBS-suspension $D_{av}$ is stabilized, and in Gl-
suspension it is dropping slightly. The opposite is typical for NP-Y$_2$O$_3$: the size of dry aggregates
increases significantly in all the solutions: from 2 µm to 13 µm (Gl-suspension), 17 µm (DW-
suspension) and up to 18-19 µm (for PBS-suspension) (figure 5b). Moreover, within an hour
aggregates size of this sample tends to increase, and in the Gl-solution to increase significantly.
Surface properties of nanoparticles determine their behavior in suspensions. Thus, in the formation of suspensions based on nanopowders the particles will be characterized by coagulation or aggregative stability, as evidenced by the experimental results using the method of laser diffraction. However, traditionally the stability of colloids is characterized by the electrokinetic potential or Zeta-potential that arises in a diffuse layer of counterions on the particle/fluid boundary. Since Zeta-potential is proportional to the charge of colloidal particles, the aggregative stability of the soles increases as its size grows [7].

Electro-acoustic method, used for the determining Zeta-potential of colloidal solutions and suspensions, has revealed that the Zeta-potential of suspensions changes in a large range of values from -200 to +200, with jump pattern of change (figure 6). On the one hand, these data may indicate that in the suspensions on the basis of the studied nanopowders it is difficult to reach equilibrium, so the measurement of Zeta-potential in these conditions may be not reasonable. On the other hand, the measured potential can carry the information about the processes of adsorption and desorption, that can explain the abrupt change in Zeta-potential difference that occurs between the dispersed particles and the disperse medium with their mutual displacement. However, changing of the Zeta-potential in such a large interval testifies the instability of the suspensions based on NP-ZrO$_2$ and NP-Y$_2$O$_3$ and chosen physiological solutions.

**Figure 5.** Change in the average size of agglomerates $D_{av}$ in the suspensions on the basis of:

a) ZrO$_2$ nanopowder, b) Y$_2$O$_3$ nanopowder.

**Figure 6.** Zeta-potential of suspensions at the solid/liquid boundary in the suspensions of:

a) ZrO$_2$ nanopowder, b) Y$_2$O$_3$ nanopowder.
9. Conclusion
The experimental data about the state of the dispersed phase in suspensions formed by nanopowders of zirconia dioxide and yttrium oxide in the simplest physiological fluids are ambiguous. Obtained by the scanning electron microscopy and laser diffraction data indicate that Gl-suspension of the dispersed phase can be characterized by a desire to monodispersity. Therefore, it is possible to predict the most probable size of the particles in the solutions for its application in ecotoxicological tests. While for water inorganic solutions, probability of obtaining stable suspensions is very small, that according to a restriction of their use for research in aqueous objects.

Electro-acoustic method confirmed that the magnitude of Zeta-potential in the suspensions based on NP-ZrO$_2$ and NP-Y$_2$O$_3$ varied over a wide range (-200 / +200 mV) and had a jump character. It is likely that in the ecotoxicological tests, which evaluated the effect of dose and particle size on the toxic effect, electrophoretic values will not affect because they change too quickly. But to interpret the physical, chemical and biochemical processes, the magnitude of Zeta-potential is an important magnitude, so the interpretation of phenomena occurring on these particles may be limited by the impossibility of achieving a balance in the forming systems.

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