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## Factors Influencing the Stability of Aqueous Dispersions of Nanocrystalline Systems $ZrO_2$ - $Y_2O_3$ - $CeO_2$ , Optionally Modified with $Al_2O_3$ . Preparing for the Future *In-vivo* studies.

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### ABSTRACT

In this study the nanopowders of  $ZrO_2$ - $2Y_2O_3$ - $4CeO_2$  and  $ZrO_2$ - $2Y_2O_3$ - $4CeO_2$  + 3%  $Al_2O_3$  were investigated. Considering that  $ZrO_2$ -based ceramics are intended to be used for biomedical applications, their toxicological properties shall be tested. Characterization of such properties of nanoparticles as size,  $\zeta$ -potential, and degree of agglomeration in aqueous suspension and understanding the factors that affect these properties is imperative for toxicity investigations. Delivery of agglomerated nanoparticles in an *in vitro* and *in vivo* experiments can readily lead to an inaccurate assessment of nanoparticles toxicity. The agglomerated nanoparticle can be found less toxic than they actually are. The results from the analysis of these nanopowders are important for conduct the toxicity studies such, as preparing the stability dispersion for *in vitro* or *in vivo* experiments and for explanation of biological reactions.

**Keywords:**  $ZrO_2$ - $Y_2O_3$ - $CeO_2$ - $Al_2O_3$  nanoparticles of system, dispersions, agglomeration state,  $\zeta$ -potential, toxicity.

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## INTRODUCTION

Nanotechnologies are the rapidly developed production and application of nano-sized particles. The production of nano-sized powders (nanopowders) is one of the most successful nanotechnology branches; it attracts more attention as a prospective subject for industry application. Nanoparticles, or nanopowders, up to 100 nm are able to be lined independently in separate structures, which exhibit advanced catalytic, adsorptive and optical properties. Nanopowders have a set of physical, chemical, biological properties, which are strongly distinct in those of the same substance as solid phases or dispersions. In the nanopowders production, it should be recognized when and with what dimensions a nano-property changes, and may be used for unique materials production. Metal oxides make up 80% of all nanopowders produced and are characterized by a low sintering temperature, a high chemical activity and an extra energy. Now, we use nanopowders in different areas, and it makes the number of persons increase, which somehow touch with nanodispersed powders [1,2].

In this regard, a special attention should be paid to the impact of such materials on human health and environment [3]. Also, the analysis of latest epidemiological, clinical studies, and pre-clinical settings with ultrafine and nanoparticles showed that these particles may cause serious adverse health consequences [4-7]. The human organism is a complex biological system with different organization levels: from molecules and cells to tissues and organs. The organism is an open system, which carries out an exchange of materials and energy with the environment through biochemical reactions in the dynamic equilibrium. An entry of molecules or ions of toxicants from process or external environment into such a clearly coordinated biological system may not only reversibly or irreversibly break cellular biochemical processes, but also damage, destroy cells. As we know from the literature, nanoparticles, on the one hand, may reach derma (actually, the skin) through epidermis cells or in between, and, on the other hand, invade into deeper skin layers through perspiratory glands, hair bulbs or even nerve tactile corpuscles. In the healthy skin, epidermis provides an excellent protection against particles invasion. In addition, a small part may be absorbed by the gastrointestinal mucous coat and finally moved to system organs. In the blood flow, nanoparticles circulate through the whole body and accumulate in organs and tissues, including brain, liver, heart, kidneys, spleen, bone marrow, nervous and lymphatic system. While invading a cell, nanoparticles may damage its operation, cause adverse oxidation-reduction reactions, resulting even to death. Nanoparticles less than 10 nm in size are deposited in upper lungs. Particles 5-20 nm are deposited uniformly in the nose and pharynx, trachea and bronchioles, and finally in alveolus [8-12]. In [13], medical conditions of people exposed to nanoparticles of non-ferrous metals and those oxides were studied; rhinitis, pharyngitis, a predisposition of ARVI, bronchitis were observed. Some of above people showed changes in the nervous system, and vegetoendocrinic dystonia. Mixtures of metal powders may affect these organs more severe.

The study of nanomaterials properties for toxicological testing is a complicated enough process, since a wide range of materials properties should be considered, including size, size distribution, shape and other morphological characteristics, as well as particle size, surface charge, specific area and surface chemistry, and other physicochemical properties [14-17]. Existing synthesis methods are aimed at particles production with clearly sophisticated and well-controlled characteristics. However, nanoparticles properties and those behaviors in real biological tests are of great importance, since possible physicochemical changes, i.e. changes in the surface charge and agglomeration rate may happen directly in the nanoparticles solution. These changes may strongly affect the toxicological reactions observed [18,19].

Accordingly, the surface charge and hydrodynamic diameter of dispersed nanoparticles should be studied, since these properties may greatly affect, how the body would react to nanoparticles. The particle size controls its interaction with biological systems, including absorption, distribution, metabolism and clearance [20]. Surface characteristics are affected by the agglomeration in water medium and hydrodynamic diameter of the nanoparticle, which, in turn, may be modified through a small change in the particle surface charge. Absorption efficiency and internal nanoparticles movement are also affected by the surface charge. But the attention should be also paid to such by no means unimportant characteristics as dispersion stability, which may be controlled by the ion force change, pH level, surface charge and use of SAA.

The study of main characteristics of nanoparticles dispersions will give important information for further toxicity tests and interpretation of response biological reactions. Toxicological studies of Warheit and Shvedova [21, 22] showed graphically the use of unstable and agglomerated dispersions of nanoparticles,

which may further result in an approximate toxicity assessment and even in conclusions that may misinform. In some studies, effects of different dispersion media were showed while preparing nanoparticles dispersions for toxicological experiments. The hydrodynamic diameter of nanoparticles was found to exceed frequently the size of any particular particle while dispersing nanoparticles in water solutions. The understanding of stability and agglomerative behavior of nanoparticles in different solutions will promote these studies. Even if hydrodynamic particle dimensions in the solution is higher than its initial size, it is important to know in which state these particles are: agglomeration (with weak bonds between particles) or aggregation (rigid bonds between particles), since those appropriate biological reactions and their consequences will be different. The stability of nanoparticles dispersions and those abilities to the agglomeration may be considered in the context of forces, which may appear between particles: electrostatic, spatial and Van der Waals forces [23, 24].

**Work Objective**

The purpose is to develop a production method for stable dispersions of nanopowders used as bio-compatible materials for toxicological studies.

**STARTING MATERIALS**

In order to solve the original problem, powders  $ZrO_2-2Y_2O_3-4CeO_2$  and  $ZrO_2-2Y_2O_3-4CeO_2-3Al_2O_3$  were used as starting materials produced by the redeposition from inorganic precursors using the sol-gel technology. In order to synthesize nanopowders, yttrium nitrate  $Y(NO_3)_3 \cdot 6H_2O$ , zirconium oxychloride  $ZrOCl \cdot 8H_2O$ , cerous nitrate  $Ce(NO_3)_3 \cdot 6H_2O$ , aluminum nitrate  $Al(NO_3)_3 \cdot 9H_2O$ , ethanol, 25% ammonia water  $NH_4OH$  were used. Other chemical substances used in this study contain sodium chloride (NaCl), potassium chloride (KCl), hydrochloric acid (HCl), nitric acid ( $HNO_3$ ), sodium carboxymethylcellulose (Na-CMC), sodium-phosphate buffer (PBS).

**Methods**

$\zeta$ -potential titration, hydrodynamic diameter and surface charge ( $\zeta$ -potential) of powder nanoparticles dispersions of  $ZrO_2-2Y_2O_3-4CeO_2$  and  $ZrO_2-2Y_2O_3-4CeO_2-3Al_2O_3$  were measured by Zetasizer Nano ZS (Malvern Instruments Inc, UK) using the dynamic light scattering (DLS) and electrophoretic light scattering (ELS).

The stability of powder nanoparticle dispersions was measured by ICP-OES "Optima 5300DV" (The Perkin Elmer, USA) based on ICP-OES.

**RESULTS AND DISCUSSION**

Dry nanoparticles may exist in 2 forms: aggregated (a set of particles held tightly between themselves due to the agglomeration) and agglomerated (a set of particles held loosely between themselves by forces of van-der-Vaals). Once nanoparticles are aggregated in the solution, they may stay separate, or form agglomerates, or remain aggregated surrounded by the double electric layer.

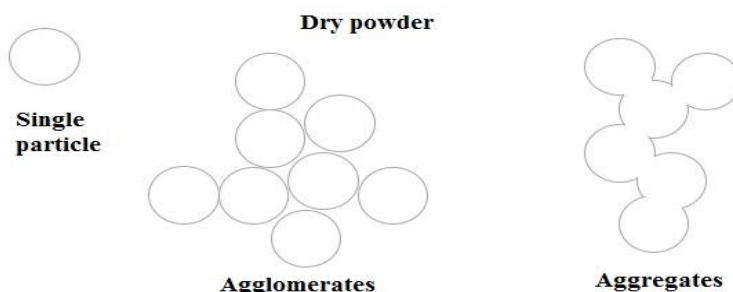


Figure 1: Differences between particle sizes in the dry state.

Fig. 1 showed a change in properties of dry powder nanoparticles when water is added. Agglomerated nanoparticles dissolved in water may be usually separated by passing the weaker attractive powers, while aggregated nanoparticles may not be separated with easy. Depending on purposes of the toxicological study

and the certain particle structure, those which may constitute a real danger for human body should be selected.

The agglomeration of nanoparticles and the dispersion incorrectly prepared – these both characteristics are a significant difficulty, which we may face in toxicological experiments related to nanomaterials. The agglomeration of nanoparticles and solution clusterization make trouble of the study of exactly nanosized particles in *in vitro* or *in vivo* examinations. When nanoparticles are agglomerated, the way of those deposition varies, due to the fact that the size of agglomerates is much more than the size of any particular particle. Actually, the use of agglomerated nanoparticles in *in vitro* or *in vivo* examinations may result in an approximate assessment of the specific nanoparticles toxicity. And further findings may bring to a conclusion that nanoparticles are less toxic than it is in practice.

Due to the agglomeration of particles and troubles in stable dispersions preparation, which impede the study of nanoparticles toxicity; more accurate methods of nanoparticles suspension preparation should be developed in the experimental setup. In order to solve problems of particles agglomeration and stable dispersions, a better method for the preparation of suspensions of nanosized particles should be developed. [25]

Interparticle interaction forces play important role when preparing stable nanopowders suspensions. A possibility to control and manipulate the interparticle interaction type is the first step for the preparation optimization of stable suspensions. Main interparticle forces in most nanoparticle systems are: van-der-Vaals, double layer (electrostatic) and spacial (polymer), as shown in Fig. 2.

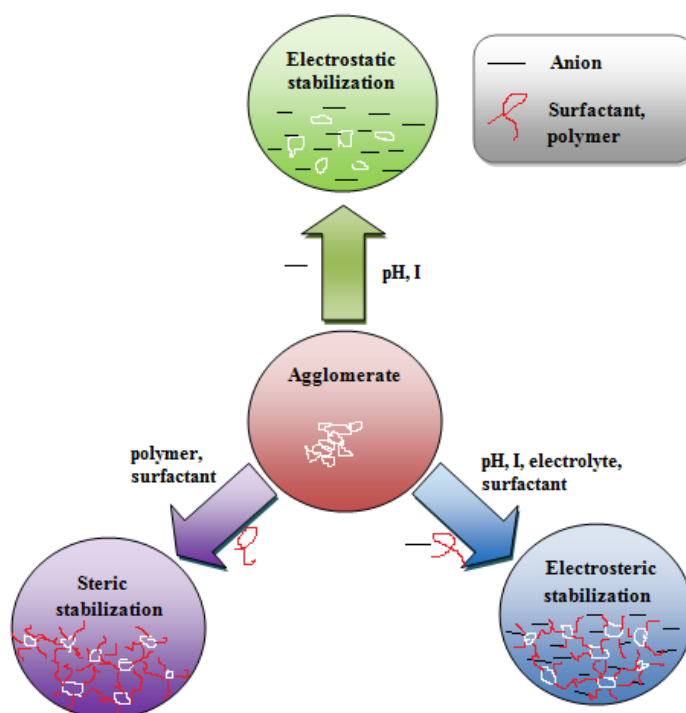


Figure 2: Diagram of main mechanisms for the stabilization of nanoparticle dispersions, with assumption that the surface of nanoparticles is positively charged.

The prevention of aggregation (combination) of primary disperse particles is possible due to three factors, affecting the stability of disperse systems:

- Kinetic,
- Electric,
- Structural-mechanical.

### **Kinetic stability factor**

A prerequisite for a coalescence of two particles of the dispersion phase is those approximation, sufficient for the demonstration of attractive forces. If the collision frequency of colloid particles is small, the dispersion system may be stable. This may happen with a very low concentration of dispersion particles (for example, in some aerosols) or with a very high viscosity of the dispersion medium, for example in those systems where both dispersion phase and medium are solids.

Most stable dispersion systems, except the dispersion phase and medium, contain a third component, which is the dispersion stabilizer. The stabilizer may be both ions and molecules; with this regard there are two ways of dispersion systems stabilization: electric and molecular-adsorptive (structural-mechanical). Both are based on the adsorption, but in case of the electric variant, an ion adsorption takes place on the surface of colloid particles, and in case of the molecular-structural – an adsorption of molecules.

### **Electric stabilization of dispersion systems**

The electric stabilization of dispersion systems is related to the double electric layer on the phase boundary. This stabilization is largely aimed at the production of stable aerosols and suspensions in the polar medium, for example, in water. All colloid particles have the same charge in any aerosol. But the colloid micelle is electrically neutral as a whole due to the double electric layer. Therefore, any electrostatic repulsion appears between colloid particles (электрический фактор устойчивости) only if they are close enough, when their ion atmospheres are overlapped. The potential energy of electrostatic repulsion is the higher, the more diffusion parts of the double electric layer of colloid particles are overlapped, i.e. the less the distance between them and the higher the value of the double electric layer.

Except the electrostatic repulsion, intermolecular attraction forces, of which dispersion forces are of the greatest importance, appear between colloid particles, like between molecules of any substance.

The potential interaction energy ( $U$ ) between colloid particles is an algebraic sum of the potential electrostatic repulsion energy ( $U_e$ ) and the potential dispersion attraction energy ( $U_m$ ) between them:  $U = U_e + U_m$

If repulsion forces prevail over attraction forces, the dispersion system is stable. To the contrary, if attraction forces appear to be higher, colloid particles coalesce colliding in Brownian motion into larger aggregates and then sedimenting. The colloid solution coagulates, i.e. segregates into a coagulate (sediment) and a dispersion medium.

Thus, when stabilizing dispersion systems, they achieve that repulsion forces between colloid particles prevailed over intermolecular attraction forces. In case of the electric stabilization, it is achieved by maintaining a relatively high electric potential of the colloid particle grain (referred to as  $\zeta$ -potential). Colloid particles without any or with a small electric charge coagulate easy and quickly. The electric stabilization of particles may be achieved by adding the electrolyte into the sol. A quantity of the electrolyte added is then of great importance, since the electrolyte, on the one hand, may stabilize the sol, on the other hand, its excessive addition may result in a coagulation of sols.

### **Molecular-adsorptive (steric) stabilization of dispersion systems**

The molecular-adsorptive stabilization of dispersion systems is of great importance for the stability of dispersions both in aqueous, and in nonaqueous media. Dispersion systems in nonaqueous media are less stable in principle, than those in aqueous media. In a nonpolar and nonaqueous dispersion medium, dispersion phase particles are lack of the electric charge. There is no electric factor of the stabilization. Attractive interaction forces only exist between dispersion particles. Any weakening of such forces, which results in a stabilization of dispersion systems, may happen due to adsorption layers around colloid particles from molecules of the dispersion medium and substances resolved therein. Such layers weak the attractive interaction of dispersion phase particles and form a structural-mechanical barrier against their attraction.

The stabilization of dispersion systems through the salvation of the dispersion phase by dispersion

medium molecules is possible both in polar, and nonpolar media. However, the stabilization of dispersion systems is much more effective if superficially active substances (SAS) and high-molecular compounds, which are adsorbed on phase boundaries, are added. Adsorptive layers of SAS and high-molecular compounds, with elasticity and mechanical strength, prevent surely any coalescence of dispersion particles.

This mechanism is of great importance when producing highly stable colloidal solutions and suspensions not only in nonaqueous, but also in aqueous media. Alkali metal soaps, proteins, amyllum are used for the structural-mechanical stabilization of dispersions in aqueous media, and alkali-earth metal soaps, resins, rubber – in nonaqueous media. Such substances are referred to as protective colloids.

The study of toxicity of such powders is an important part of investigations, since this nanopowders is supposed to be used in medical applications. As mentioned above, a stable dispersion is to be prepared for *in vivo* experiments. Based on the above, we tried to prepare a stable suspension of nanosized powders of  $ZrO_2-2Y_2O_3-4CeO_2$  and  $ZrO_2-2Y_2O_3-4CeO_2-3Al_2O_3$ , using different stabilization techniques (Fig 2).

A carboxy-methyl-cellulose is generally used when studying the toxicity of substances in *in vivo* experiments. It is used as a liquid, whereby substances to be investigated are introduced into the stomach of the testee animal, also we can use such a polymer as the stabilizer.

Fig 3. pH-dependent  $\zeta$ -potential titration of the dispersion of nanopowders dissolved in distilled water (MQ) and in carboxy-methyl-cellulose (CMC) solution: a – suspension of the nano-sized powder  $ZrO_2-2Y_2O_3-4CeO_2$ ; b - suspension of the nano-sized powder  $ZrO_2-2Y_2O_3-4CeO_2-3Al_2O_3$ .

The importance of  $\zeta$  -potential is known to be that it may be related to the stability of colloid dispersions.  $\zeta$  -potential determines the rate and nature of interaction between dispersion system particles. The pH-dependent  $\zeta$ -potential titration was therefore performed to determine  $\zeta$ -potential and zero point of charge depending on pH.

Two cases were considered in the experiment: 1) nanopowders dissolved in distilled water (MiliQ water) and 2) nanopowders dissolved in 0,002%, 0,1% and 0,5% carboxy-methyl-cellulose (CMC) solution. The concentration of nanopowders was 0,5 g/l (500 ppm).

As described on Fig. 3a, zero point of charge lies in the range pH=4.2 for the powder free of the aluminum oxide dissolved in MQ. This dispersion may be considered as theoretically stable in the range pH>8, since at  $\zeta$ -potential >  $\pm 30$  mV particles repulse from each other, that determines the dispersion stability, at lower than  $\pm 30$  mV – a metastable state, and at low  $\zeta$  -potential (lower than  $\pm 10$  mV) particles flocculate quickly (deposit as flocks), it characterizes the unstable dispersion.

In the next experiment, the nanopowder dissolved in carboxy-methyl-cellulose (CMC) has the zero point of charge out of the diagram region, i.e. in the range pH<3. When adding CMC as the stabilizer,  $\zeta$  -potential of nanopowder dispersion lies in the range -30 mV. Resulting data are indicative of the fact that the obtained dispersion of nanoparticles is stable in theory.

While analyzing the nanopowder with aluminum oxide in the same manner, the zero point of charge is shifted to the range pH=7 for the powder dissolved in MQ as shown in the diagram of Fig. 3b. A potentiometric curve of nanoparticles dispersion lies in the range of unstable and metastable states.

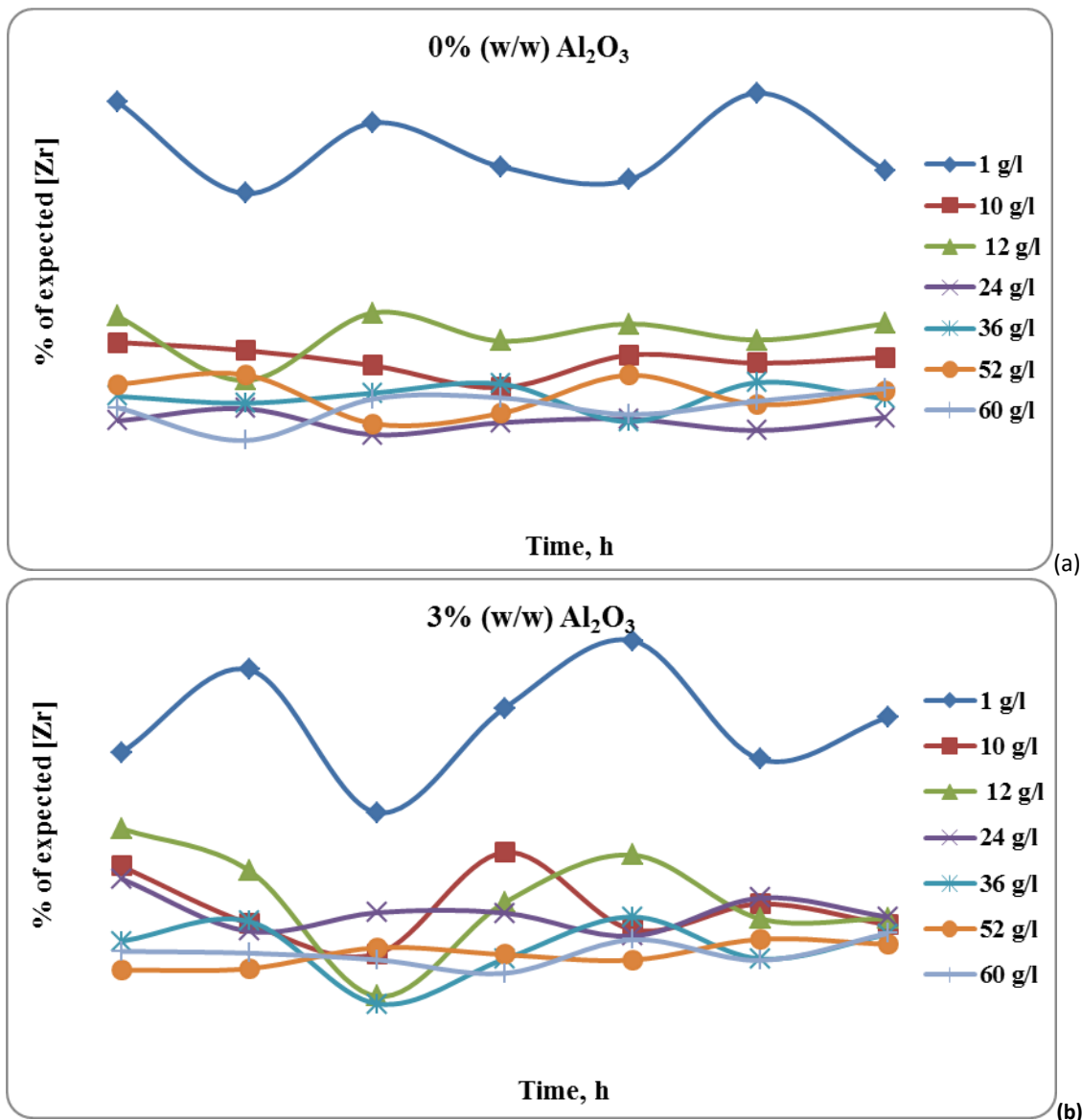
We have a metastable dispersion for the same dispersion of nanoparticles, but it is stabilized by the solution of carboxy-methyl-cellulose in the range pH=3 to pH=4.5, and in the range pH>4.5 we have an electrostatically stable dispersion. If there is an aluminum oxide in the powder, the nature of curve showed no alterations.

Resulting data make it possible to figure out in what range pH we have to work out for the production of stable dispersions. It is arguable that the addition of 3%  $Al_2O_3$  affects  $\zeta$  -potential and zero point of charge, and these results should be considered for the stabilization of dispersion using both electrostatic, and steric techniques.

**Structural-mechanical (spacial) stabilization**

Then, the stability of dispersions was studied depending on the concentration of powder in dispersion. Based on the above results of  $\zeta$ -potential titration, the following parameters were selected: concentration of carboxy-methyl-cellulose 0.5% and level of pH=7.

Fig. 4 shows results of dispersion stability test. For this experiment, concentrations used for toxicological mouse studies in vivo were selected. Since it is in our interests to study the dispersion stability, which may be used in toxicological investigations.



**Figure 4: Stability of dispersions as a function of powder concentration: a – suspension of nanosized powder  $ZrO_2-2Y_2O_3-4CeO_2$ ; b – suspension of nanosized powder  $ZrO_2-2Y_2O_3-4CeO_2-3Al_2O_3$**

Based on resulting tests for dispersion stability, we may see that in both cases: without an additive and with an aluminum additive, the dispersion is not stable (Fig. 4a and 4b). This test has been performed for 6 hours, where data of dispersion were taken every hour. And diagrams (Fig. 4) show the expected powder quantity as a function of time.

The dispersion with powder concentration 1 g/l is the most stable – about 20% of expected quantity in case of the powder dispersion without aluminum oxide (Fig. 4a). And about 25% of expected quantity in

case of the powder dispersion with aluminum oxide additive (Fig. 4b). Dispersions with concentrations 10 d/l, 12 g/l, 24 g/l, 36 g/l, 52 g/l and 60 g/l – only 10% and 15% of expected quantity for the powder without additive and with aluminum oxide additive, respectively (Fig. 4a and 4b).

Based on resulting data, it has been decided to perform further tests of dispersions with powder concentration 0.5 g/l (500 ppm). Since such high concentrations (over 1 g/l) result in strong interparticle interactions. The particles are in so small distance from each other, that they aggregate and precipitate quickly, leading to an unstable dispersion even in the presence of stabilizer (carboxy-methyl-cellulose). Perhaps, if a stabilization mechanism of dispersions with low concentrations is found, it will be possible to develop stable dispersions with higher concentrations in future.

Fig. 5 and 6 show results of tests for the dispersion stability using different types of stabilizers carboxy-methyl-cellulose (CMC) и bovine serum albumin (BSA), respectively.

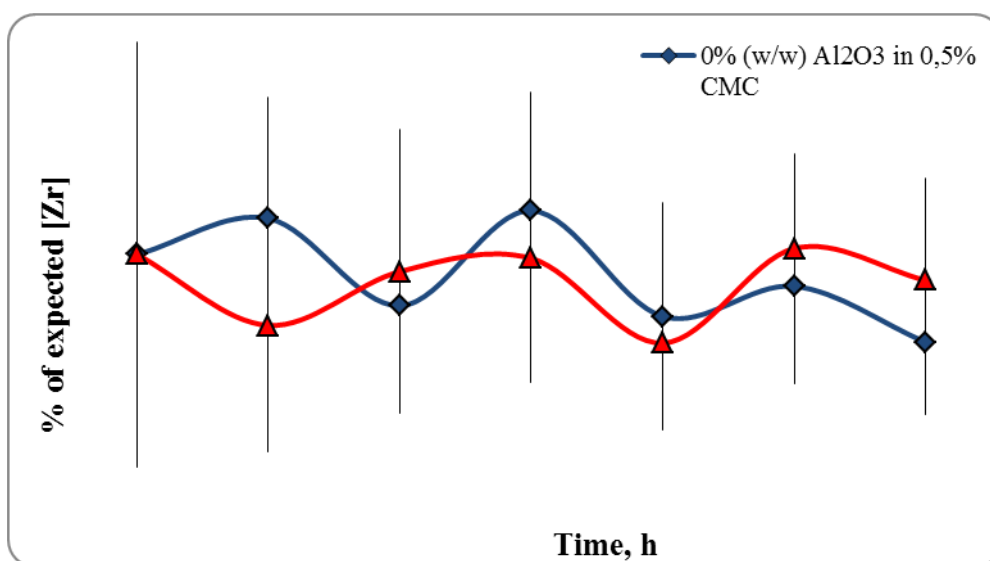


Figure 5: The study of stability of powder dispersions stabilized by carboxy-methyl-cellulose (CMC)

According to the diagram in Fig. 5, it appears that there are no strong differences in the stability between the powder with and without aluminum oxide. Even when the powder concentration 0.5 g/l and pH=7 is used, dispersions are not stable enough, since it is just about 25% of expected quantity. It means that these dispersions are stable just by a quarter.

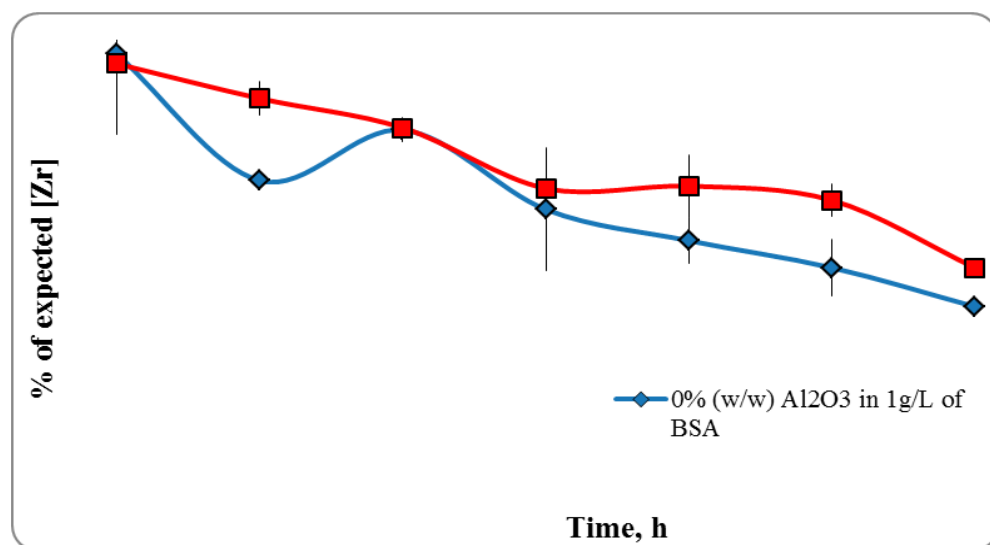


Figure 6: The study of stability of powder dispersions stabilized by bovine serum albumin (BSA)



In order to study the stabilizer in more detail, a decision was made – to use proteins as a stabilizer. Fig. 6 shows a diagram of stability tests for dispersions stabilized by bovine serum albumin, which indicated that the stability was about 30% of expected quantity at the beginning of test, but then it decreased hour after hour and achieves 15% of expected quantity at the end.

While comparing test results for stability of dispersions stabilized using the structural-mechanical stabilization (Fig. 5 and 6), it is arguable that carboxy-methyl-cellulose results in a relatively stable dispersion (about 25% of expected quantity) for a long time. While proteins may stabilize the dispersion system only in first hours, then particles aggregate, and dispersion becomes unstable.

**Electrostatic stabilization**

As indicated above, one of production types of the stable dispersion is the electrostatic stabilization of dispersion systems using electrolytes. As a preliminary, it was studied how these dispersion systems are affected by salt, and how soon particles will sediment in the dispersion system.

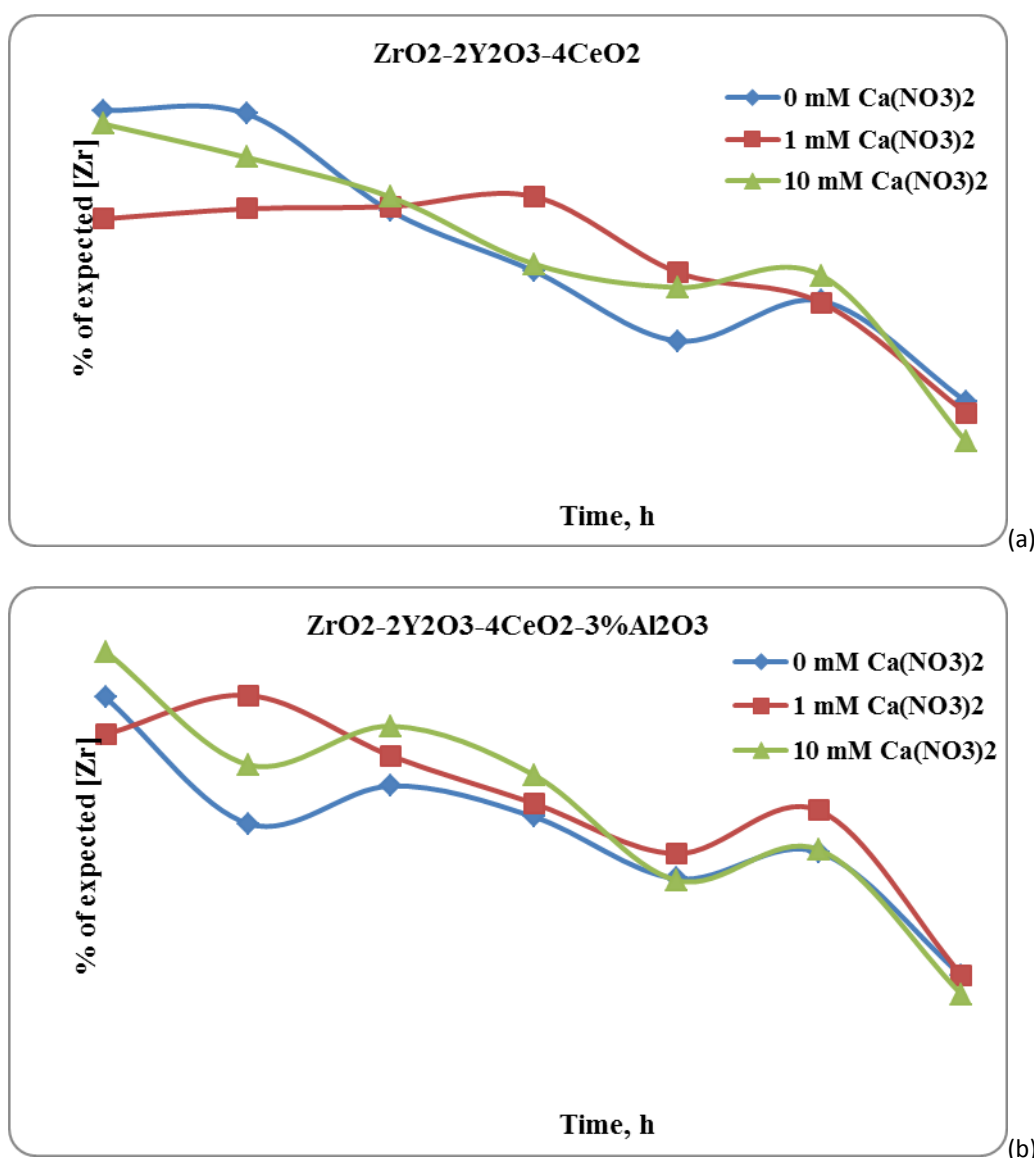


Fig. 7. The study of stability of powder dispersions with and without calcium nitrate salt: a – suspension of nanosized powder ZrO<sub>2</sub>-2Y<sub>2</sub>O<sub>3</sub>-4CeO<sub>2</sub>; b – suspension of nanosized powder ZrO<sub>2</sub>-2Y<sub>2</sub>O<sub>3</sub>-4CeO<sub>2</sub>-3Al<sub>2</sub>O<sub>3</sub>

Fig. 7 shows a dependence diagram of dispersion stability from time and quantity of the calcium nitrate salt. As it may be noticed, the nature of curves does not change as a function of presence or absence of

aluminum oxide additive. But it may be also noted that the dispersion system with oxide aluminum additive (Fig. 7b) is more stable at the beginning of tests, than without this additive (Fig. 7a). The presence of calcium nitrate salt also does not change the sedimentation rate of particles; therefore, we may conclude that this dispersion system itself is unstable.

Fig. 8 shows results of the study how stability of dispersions change as a function of the electrolyte quantity. For this study, it is decided to decrease the powder concentration to 0,05 g/l and make it in the range pH=2, since previous tests indicated that this area is stable both for powder free of any additive, and that with aluminum oxide.

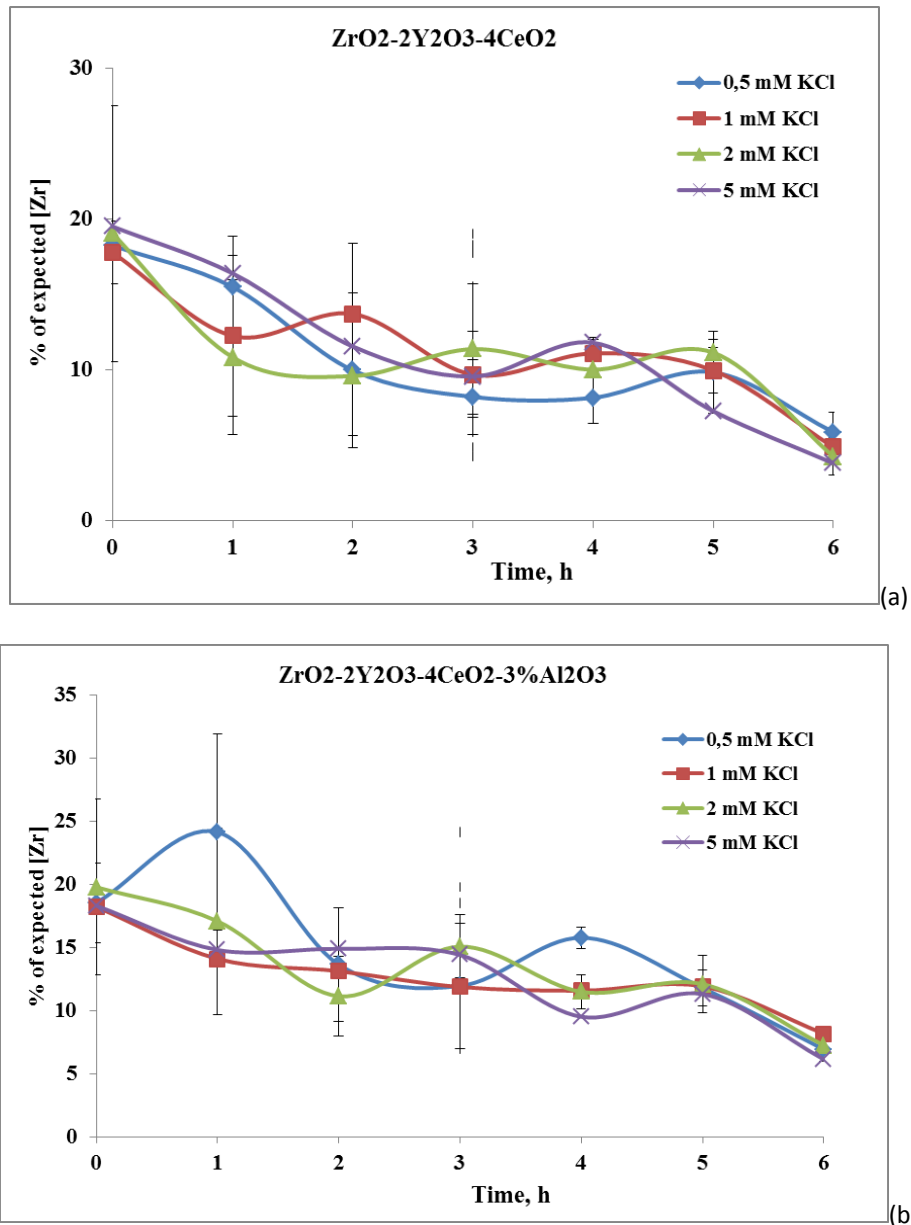


Figure 8: The study of stability of powder dispersions as a function of the electrolyte quantity: a – suspension of nanosized powder ZrO<sub>2</sub>-2Y<sub>2</sub>O<sub>3</sub>-4CeO<sub>2</sub>; b - suspension of nanosized powder ZrO<sub>2</sub>-2Y<sub>2</sub>O<sub>3</sub>-4CeO<sub>2</sub>-3Al<sub>2</sub>O<sub>3</sub>

Both diagrams (Fig. 8a and 8b) show that any change in the electrolyte quantity does not affect the stability of dispersion system. The nature of curves is similar both in the system free of additive, and in that with aluminum oxide. And the highest stability, which may be achieved only at 20% of expected quantity, and just when the dispersion is prepared, since particles start agglomerating and sedimenting after an hour.

Upon completion of our study, we investigated the stability of dispersions as a function of powder quantity, at concentration less than 1 g/l. Based on previous results, it is decided to stabilize by potassium chloride electrolyte in quantity 5 mM and operate with dispersion in the range pH=2. On diagrams (Fig. 9a and 9b), you can see that the dispersion system with concentration 0.75 g/l (750 ppm) is the most stable; at this concentration the powder sediments in the first hour. But the use of concentration 0.25 g/l (250 ppm) and 0.5 g/l (50 ppm) also resulted in a stable dispersion.

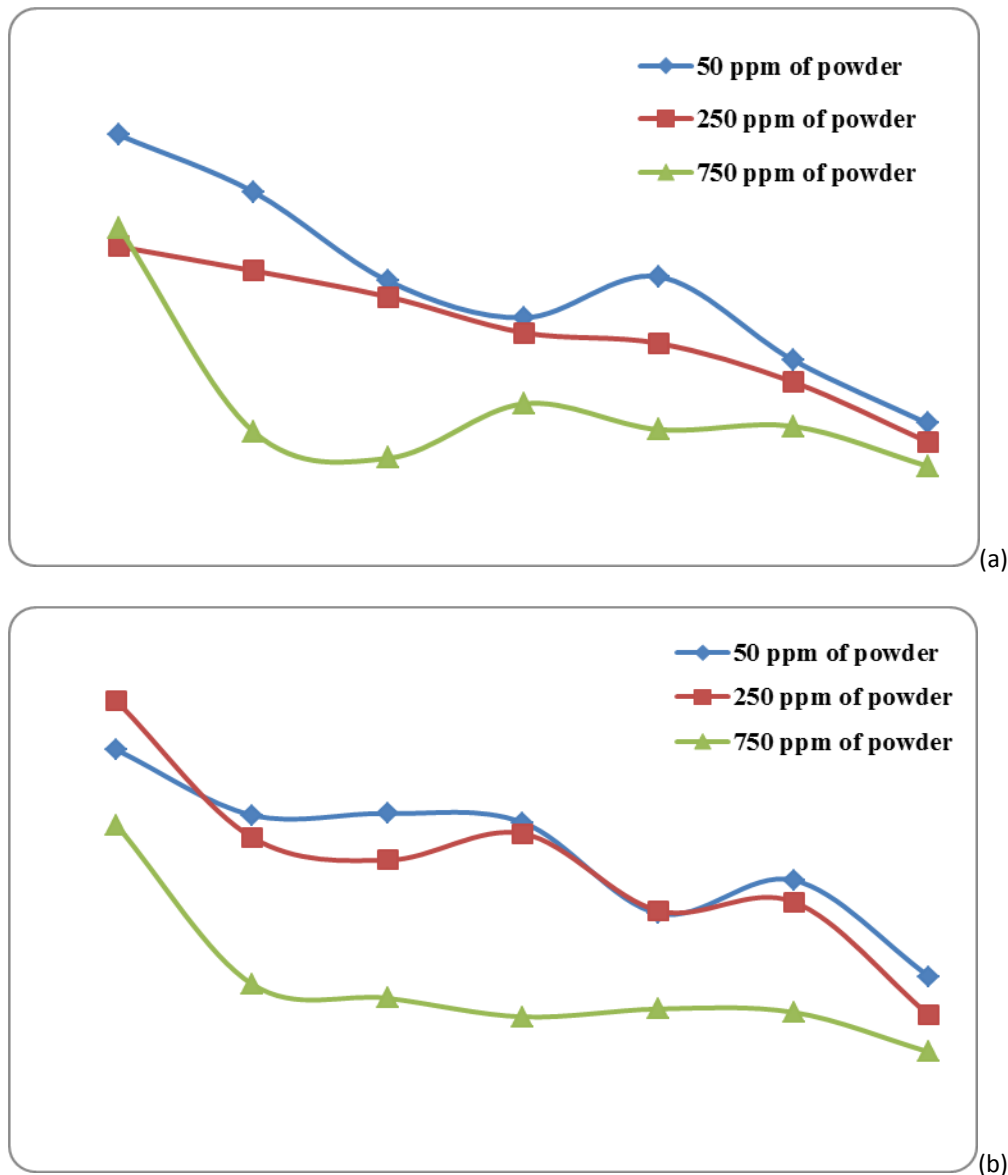


Figure 9: The study of stability powder dispersions as a function of powder concentration, stabilized by potassium chloride electrolyte: a – suspension of nanosized powder  $ZrO_2-2Y_2O_3-4CeO_2$ ; b - suspension of nanosized powder  $ZrO_2-2Y_2O_3-4CeO_2-3Al_2O_3$

#### SUMMARY

- A production technique for suspensions of nanosized particles was developed for the study of nanoparticles toxicology.
- The measurement of  $\zeta$ -potential was shown to be the forecast technique of dispersion stability.
- This study may help in production of low-concentrated dispersions, so dispersions with higher concentrations may be developed in future.

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