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RESEARCH PAPER

Solubility of nano-sized metal oxides evaluated by using in vitro simulated lung and gastrointestinal fluids: implication for health risks

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Abstract The solubility of nano-sized metal oxides (nZnO, nCuO, $nTiO_2$, $nCeO_2$, and nFe_3O_4, 17–42 nm) and some non-nano-mineral powders (ZnO, $ZnSiO_3$, ZnS, and CuO) were evaluated by using gastrointestinal solubility bioavailability research consortium (SBRC), in vitro gastrointestinal (IVG) method, pulmonary artificial lysosomal fluid (ALF), and Gamble solution method, respectively. It is found that these nano-sized metal oxides aggregated more or less when suspending in the simulated biological fluids analyzed by dynamic light scattering (2 mg L^{-1}) and UV-Vis spectrometry (100 mg L^{-1}). The aggregation and sedimentation of nano-metal oxides in a simulated biofluid are influenced by its surface property and the ingredient of the liquid. The dissolution in fluids may decrease the aggregating radius of a nano-metal oxide. In return, the aggregative effect can influence the solubility of metal elements and result in their weakened bioaccessibility. The suspending stability was consistent in the order of nFe_3O_4 < nCuO < $nTiO_2$ < $nCeO_2$ < nZnO in all the simulated biological fluids. Nano-ZnO and nCuO showed higher gastrointestinal and pulmonary bioaccessibility than nFe_3O_4, $nTiO_2$, and $nCeO_2$. The further comparisons on the bioaccessibility for nCuO and nZnO with non-nano-powder CuO and ZnO indicated that the aggregating size in suspension could play more important role in influencing the bioaccessibility than single particle size does. The present study reveals that aggregation of all studied nano-sized metal oxides occurred in body physiologic fluids and that nZnO and nCuO were easily dissolved in simulated physiologic fluids, suggesting more potential health risks from nZnO and nCuO's exposure.

Keywords In vitro test · Nano-sized metal oxides · Bioaccessibility · Simulated physiologic fluids · Health risk · Nano-medicine

Introduction

Nano-sized metal oxides such as nano-sized zinc oxide (ZnO), copper oxide (CuO), titanium dioxide (TiO_2), cerium dioxide (CeO_2), and Fe(II, III) oxide have been widely used in industrial catalysis, industrial additives, wastewater treatment, care products, paints, and other related areas (Kurlanda-Witek et al. 2014; Sun et al. 2015; Van Koetsem et al. 2015). The nano-sized metal oxides are potentially released to the environment during their application (Garaud et al. 2016). Their fate and eco-risks have received more and more attention (Dwivedi et al. 2015; Sharma et al. 2015). Generally, the dermal contact to these nano-sized metal oxides is

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considered of reasonable low risk. However, once they get into the body via the inhalation and oral ingestion, they may pose potential risks to human health (Chauhan et al. 2013; Lee et al. 2016; Pandurangan and Kim 2015; Sambale et al. 2015). On the one hand, their ultrafine size may make them easily entering into human's blood circulation, and then these ultrafine particulates deposit in tissues or cells which damage the normal physiological functions (Prow et al. 2008; Yang et al. 2004). On the other hand, the metals associated with these ultrafine particulates may be released during immersing in human body physiological fluids. These released metals can cause adverse effects even though the zinc, copper, and iron are essential elements for all living organisms unless present in excessive amounts (Evangelou et al. 2007; Twining et al. 2005).

In order to assess the potential health risks posed by these metals in the environmental medium, many in vitro and in vivo methods have been developed and validated (Juhasz et al. 2007; Li et al. 2016). The time- and cost-consuming and ethical constraints limit the wide application of in vivo methods (Denys et al. 2012). Therefore, in vitro methods to simulate the biological fluids (gastrointestinal phase or pulmonary alveoli area fluids) have been used in all kinds of environmental matrixes such as soil, sediments, dusts, and airborne particles. For example, Juhasz et al. used the solubility bioavailability research consortium (SBRC) in vitro assay to predict the lead/arsenic bioavailability in contaminated soils (Juhasz et al. 2014; Juhasz et al. 2009; Li et al. 2015). In vitro gastrointestinal (IVG) method is another widely used method for the assessment of these metals' bioaccessibility in those environment media (Szczeszak et al. 2015; Tao et al. 2009). The artificial lysosomal fluid (ALF) solution is analogous to the fluid with which inhaled particles would come into contact after phagocytosis by alveolar and interstitial macrophages in the lung (Cruz et al. 2015; Zereini et al. 2012). Gamble solution represents the interstitial fluid deep within the lung (Drysdale et al. 2012). ALF and Gamble solution methods are widely used to investigate the risk associated with the inhalation exposure to the airborne particulate matters (Julien et al. 2011).

Considering their potential health risks to human, more researches should be carried out on the bioaccessible fractions of metals in nano-sized metal oxides and the aggregating size in suspension. Thus, in this present work, the oral ingestion and inhalation bioaccessibility of nano-sized metal oxides (nZnO, nCuO, $nTiO_2$, $nCeO_2$, and nFe_3O_4) were investigated by the simulated gastrointestinal fluids (SBRC and IVG method) and simulated pulmonary fluids (ALF and Gamble solution method) mentioned above, respectively. Some non-nano-mineral powders (ZnO, $ZnSiO_3$, ZnS, and CuO) were investigated as the control. The aggregative effect in the simulated biological fluids of these nano-metal oxides was also studied by the dynamic light scattering (DLS) experiment and UV-Vis spectrometry (Schaudien et al. 2012). This study may provide a better estimate of the potential health risks from nano-sized metal oxides to human.

Material and methods

Reagents

Nano-ZnO, nCuO, $nTiO_2$, and $nCeO_2$ were bought from Sigma-Aldrich Corporation (for more information, see Table S1 in Supplementary Information). Nano-Fe_3O_4 was bought from Sinopharm Chemical Reagent Co., Ltd. Their average sizes were measured by X-ray diffraction (XRD) with Scherrer formula (The results are listed in Fig. S1 of the Supplementary Information) and ranged from 17 to 42 nm, matching the data provided by the manufacturers. The non-nano-mineral powders (ZnO, $ZnSiO_3$, ZnS, and CuO) were bought from Sinopharm Chemical Reagent Co., Ltd.

Physiologically based in vitro procedures

Oral ingestion and inhalation are the main exposure routes of nano-sized metal oxides to human (Twining et al. 2005). Therefore, the gastrointestinal bioaccessible metal fractions in nano-sized metal oxides were evaluated by using the SBRC and IVG method. The pulmonary bioaccessibility was evaluated by using the ALF and the Gamble solution. The components of these simulated biological fluids are listed in Tables S2–S4, and the operational parameters were cited from these literatures with minor modification (Denys et al. 2012; Drysdale et al. 2012; Juhasz et al. 2009; Julien et al. 2011; Schroder et al. 2004).

Briefly, 0.050 g sample at the solid state was added to 50-mL polyethene tubes with the set volume (50 mL) of simulated biological fluids and kept in end-over-end shaker (250 rpm, 37 °C) for the set time (2, 4, or 24 h); then, 5 mL suspending solution was shifted and

centrifuged at 10000 rpm for 10 min. The supernatant (1 mL) was taken out carefully, diluted to 2 mL with 0.1 M HNO_3, and then stored at 4 °C. The conditions of centrifugation are discussed in Supplementary Information Formula S1 with Table S5. Sample (0.050 g) was extracted in gastric phase for 2 h, and the residue was then extracted by intestinal phase for 2 and 4 h (Table S4) (Denys et al. 2012). Considering the much longer resident period (generally for weeks or months) in the lung for particles (Twining et al. 2005), the exposure time in the simulated lung biological fluids was set as 4 and 24 h. And the kinetic model of dissolution was investigated with the typical particles: nZnO, ZnO, nCuO, and CuO within 5 days. The pH was measured and kept stable with 0.1 M HCl and 0.1 M NaOH in extraction processing. Triplicate samples were carried out. Duplicate blanks were also carried out to allow for background subtraction of elemental concentrations in the extraction solution.

Aggregation research

Five-milligram nano-sized metal oxide was added in 50 mL the simulated biological fluids (gastric SBRC, intestinal SBRC, gastric IVG, intestinal IVG, ALF, and Gamble solution, respectively, 100 mg L^{-1}). After 1 h ultrasonic processing (100 W), the real-time absorbance of the suspended liquid was monitored as the previous reports (Guo et al. 2007; Wang et al. 2010) with UV-Vis spectrometer (Nanjing Feile Instrument Co., Ltd., T-6) at 10, 20, 40, 60, 90 and 120 min, respectively. The Zeta potential was collected once at the end of ultrasonication, and the hydrodynamic radius was measured after diluted by 50-fold with DLS experiment at 0, 0.5, 1, and 2 h (additionally 4 h for ALF and Gamble solution) (Aerts et al. 2010; Follens et al. 2008) using particle sizer and Zeta potential analyzer (BIC, NanoBrook 90 Plus PALS). The suspended liquid was kept in a standing position without stirring until the measurement was ending.

Elemental determination and data analysis

Metal concentrations were measured using an inductively coupled plasma optical emission spectrometer (ICP-OES, PerkinElmer SCIEX, Optima 5300) (The instrumental conditions are listed in Table S6). The solutions of gastric SBRC, intestinal SBRC, gastric IVG, intestinal IVG, ALF, and Gamble solution were used as the calibration blanks, respectively. The calibration standard solutions were obtained by the dilution of the standard stock solutions (Custom Assurance Standard) purchased from SPEX CertiPreP (Zn, Fe, Cu 1000 mg L^{-1}, Lot number: 28-232CR; Ti 1000 mg L^{-1}, Lot number: 10-90CR; Ce 10 mg L^{-1}, Lot number: CL11-60YPY) with these simulated biological fluids. The relative percentage difference of parallel samples was within 20% (Relative percentage = |Conc. − Average|/Average × 100%, Average: the average concentration in parallel samples), or the experiments were repeated.

Statistical analysis

Statistical analysis was carried out using SPSS software. Statistically significant differences in nano-ZnO and CuO/non-nano-ZnO and CuO bioaccessibility in different simulated biofluids were analyzed using analysis of variance (Independent-sample T test, $*0.01 < p < 0.05$; $**p < 0.01$).

Results and discussion

Aggregative effect of nano-sized metal oxides in the simulated gastrointestinal and pulmonary physiological fluids

The aggregative effect of five nano-sized metal oxides suspending in the simulated biological fluids are summarized in Fig. S2 in the Supplementary Information. The overall hydrodynamic radii for these nano-sized metal oxides suspending in the physiological fluids at 2 mg L^{-1} ranged from 200 to 10,000 nm. The hydrodynamic radius of nZnO in gastric IVG was missing after 0.5 h because it dissolved greatly to lower concentrations, which was hard to be measured. The dissolution phenomenon also happened in intestinal SBRC of nZnO in that the hydrodynamic radius was decreased with time. The aggregative effect was obvious according to Fig. S2 except nZnO and $nCeO_2$ in intestinal SBRC and intestinal IVG. nFe_3O_4 aggregated most seriously especially in the simulated pulmonary biological fluids in which other nano-metal oxides were aggregating more gravely than those in the simulated gastrointestinal biological fluids (Fig. S2).

Interestingly, nZnO aggregating obviously in ALF, Gamble solution, and gastric IVG was less precipitated as indicated Fig. S3. This implied that aggregative

effects do not always bring in sediment. Similarly, it happened to $nCeO_2$ in Gamble solution. In fact, if the gravity of aggregates were not so strong that could overcome the applied forces from the fluid, the sediment would not form. The applied forces are related with the surface properties of nano-metal oxide and the components of the liquid (Gambinossi et al. 2015; Raza et al. 2016). On the contrary, the aggregating radius of nCuO in intestinal SBRC and intestinal IVG was relatively small but so much precipitation was generated as shown in Figs. S2 and S3, more likely owing to the weak applied forces to nCuO. Nevertheless, the other nano-metal oxides were aggregated obviously followed by lots of sedimentation. Accordingly, the suspending stability was in the order of nFe_3O_4 < nCuO < $nTiO_2$ < $nCeO_2$ < nZnO in all the simulated biological fluids as Fig. S3 reported.

The values of Zeta potential for suspended nano-sized metal oxides in Fig. S4 ranged from − 20 to 10 mV, implying the instability of nano-particles in the simulated biological fluids. The absolute values of Zeta potential for $nTiO_2$ in all of the simulated fluids were relatively large than others, resulting in its enhancement of suspending stability. However, nZnO was suspended stably with low Zeta potential in all these different simulated biological fluids similar as $nCeO_2$ in intestinal SBRC. This implied that the interactions between the fluid components with nano-particles in these suspensions made the difference.

In a word, the aggregation and sedimentation of nano-metal oxides in a simulated biofluid are influenced by their surface properties and the ingredient of the liquid. The dissolution of a nano-metal oxide in fluids may decrease the aggregating radius in a way. In return, the aggregative effect can influence the solubility of metal elements and result in their weakened bioaccessibility. Actually, the aggregative effect of nano-sized metal oxides in the lung phase is beneficial to the clearance procedure such as discharge function due to movement of respiratory tract cilia and phagocytosis by macrophage cells (Kendall et al. 2004; Kendall et al. 2002). For example, larger particles were easily blocked at the inner surface of the respiratory tract; movement of respiratory tract cilia make these particles move backward and eventually expelled from the body with the sputum. However, the overloaded undissolved nano-metal oxides may cause a series of damages to pulmonary alveoli area (Kendall 2007; Wright 2005).

Gastrointestinal bioaccessibility of nano-sized metal oxides analyzed by using SBRC method and IVG method

Bioaccessibility (%) is defined as the soluble fraction of an element in nano-sized metal oxides that is dissolved in the simulated gastrointestinal/pulmonary physiological fluids in the present study and calculated Formula S2 (Huang et al. 2016). The gastrointestinal bioaccessibility and standard deviation (SD) of the metals for the five nano-sized metal oxides are given in Fig. 1. Nano-ZnO showed the highest bioaccessibility (79.9 ± 4.6%) in the gastric SBRC while its bioaccessibility in the gastric IVG was lower (9.1 ± 0.3%). The similar phenomena were observed in the intestinal phase in that the bioaccessibility for nZnO in intestinal SBRC solution (15.4 ± 0.9%) was higher than that in intestinal IVG solution (2.6 ± 0.1%).

The bioaccessibility for nCuO in the gastric phase (24.4 ± 0.6%) and intestinal phase (13.8 ± 0.2%) of SBRC was higher than that in the gastric phase (1.1 ± 0.1%) and intestinal phase (~ 0.2%) of IVG. Cerium ions were comparatively difficult to be released from $nCeO_2$, which is partially dissolved in the gastric SBRC solution with a value of 5.5 ± 0.1%, and $nCeO_2$ was less dissolved in gastric IVG and intestinal IVG (~ 0.2%). It is worth to be mentioned that the nFe_3O_4 and $nTiO_2$ were all rarely dissolved in these simulated physiological fluids (< 0.03%) except $nTiO_2$ in SBRC intestinal phase (~ 0.9%).

The comparisons in intestinal bioaccessibility of nano-metal oxides, as well as other non-nano-metal compounds as controls, between 2 and 4 h are shown in Fig. 2. According to the assumption that the dissolving dynamic of nano-sized metal oxides in intestinal phase is a first order reaction (Wragg and Klinck 2007), the Cu compounds and less dissolved $nCeO_2$, nFe_3O_4, and $nTiO_2$ should have almost reached the dynamic equilibrium before 4 h, based on their bioaccessibility at 2 h close to that at 4 h, while Zn compounds except ZnS may not reach the equilibrium because the bioaccessibility at 2 h was far less than that at 4 h. Considering the short food gastrointestinal emptying time, 4 h is enough to estimate the bioaccessibility in the present study.

Pulmonary bioaccessibility of nano-sized metal oxides analyzed by using ALF and Gamble solution

Pulmonary bioaccessibility for the five nano-sized metal oxides is also listed in Fig. 1. Compared with the

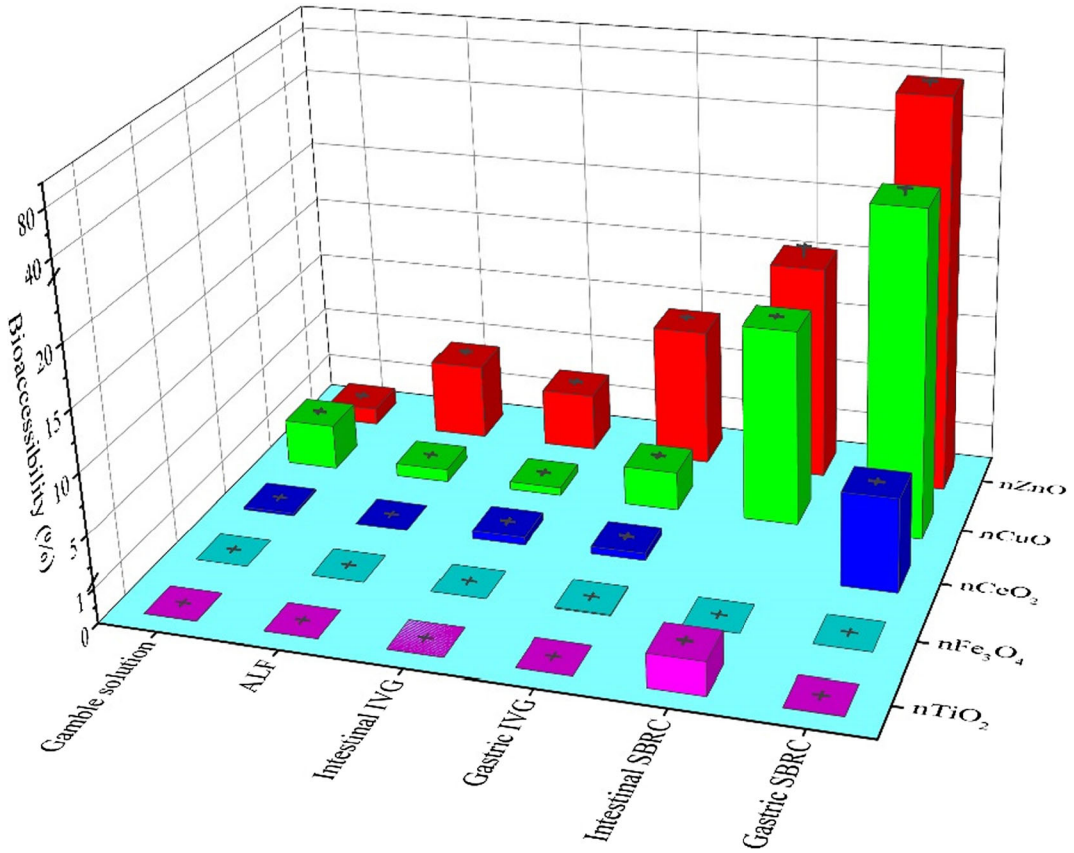

Fig. 1 The bioaccessibility (%) of metals for nano-sized metal oxides. Values are measured at 2 h for gastric phase, 4 h for intestinal phase, and 24 h for ALF and Gamble solution ($n = 3$). For detailed information, see Table S7

digestion tract, nano-sized metal oxides showed a different behavior with the low level of bioaccessibility in the lung phase. Generally, the values were below to 4.0% (the maximum value was 4.0 ± 0.3% of Zn in Gamble solution; Fig. 1). In the respiratory tract, Zn and Cu showed the obvious difference in bioaccessibility

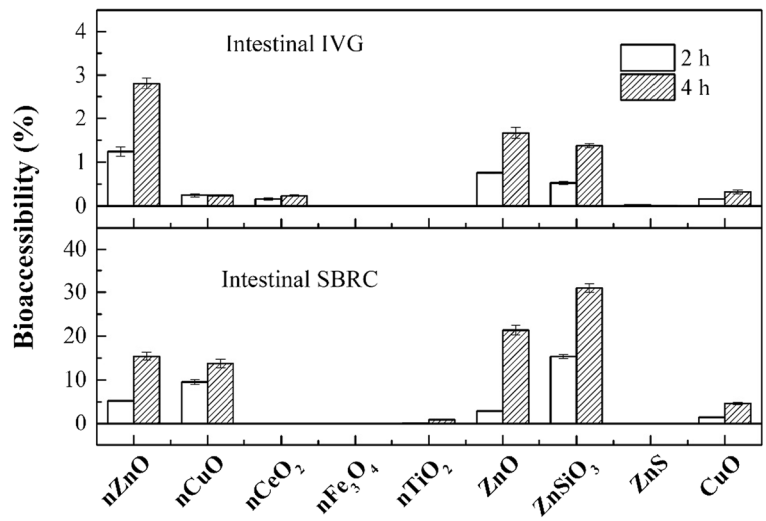

Fig. 2 The bioaccessibility in intestinal phase at different contact times

between the ALF method (Zn, 4.0 ± 0.3%; Cu, ~ 0.4%) and Gamble solution method (Zn, ~ 0.5%; Cu, 1.6 ± 0.1%). Nano-CeO_2 was less dissolved in both the ALF and Gamble solution (< 0.074%). Nano-Fe_3O_4 and nTiO_2 could be ignored with a little bioaccessibility.

Figure 3 shows the apparent difference on the bioaccessibility of Zn and Cu at different contact times. There were no significant differences on Cu bioaccessibility between 4 and 24 h exposure to ALF solution and Gamble solution, while Zn bioaccessibility increased significantly from 4 to 24 h in ALF solution (Fig. 3). According to the kinetic model of bioaccessibility shown in Fig. S5, these nano-particles reached the highest bioaccessibility within 8 h. Interestingly, nZnO in ALF solution dissolved very slowly within 4 h and then a rapid dissolution. This phenomenon was not observed for nCuO with its rapid dissolution within 1 h.

Gastrointestinal and pulmonary bioaccessibility of non-nano-sized Zn compounds and CuO

Considering the higher bioaccessibility of nZnO and nCuO, a further study was carried out on the non-nano-ZnO, non-nano-CuO, and the other species of Zn. The comparisons on the bioaccessibility of non-nano-ZnO and CuO with nZnO and nCuO are shown in Fig. 4. The nano-size particles showed a superior bioaccessibility in some extractions, like the bioaccessibility of nCuO that was significantly higher than non-nano-CuO in gastric SBRC and intestinal SBRC. Notably, the normal size particles might show a higher bioaccessibility than the nano-size ones, such as non-nano-ZnO in intestinal SBRC and ALF and non-nano-CuO in gastric IVG. The other cases that are shown in Fig. 4 said that the bioaccessibility of non-nano-sized ZnO/CuO was about the same as the nano-sized one. As well known, nano-size particles possess a lot of unique properties (Inerbaev et al. 2009). The great ratio of surface area to volume of nano-particles enhances the opportunity to contact with liquid which means more opportunity of the effect with contributing molecules or ions. Furthermore, the lower coordination number of nano-particle surface atoms leads to the rich defect where the metals escape easier (Weaver and Waddill 1991). Thus, the nano-sized metal oxides should show a higher bioaccessibility and a faster speed in dissolution than those with bigger size (Meunier et al. 2011). On the one hand, the aggregative effects of nano-particles also influence the surface effects, which result in the decrease of the bioaccessibility. On the other hand, considering the stability of nZnO in ALF, its induction period within 4 h may cause the reversal. Therefore, the simulated physiologic fluids may have greater influence on the bioaccessibility of metal oxides than the size of particles.

Due to the higher bioaccessibility of ZnO, three common Zn compounds (ZnO, $ZnSiO_3$, and ZnS powders) were further studied in in vitro tests. The different bioaccessibilities of these three compounds are shown in Fig. 5. Gastric bioaccessibility of these non-nano-Zn miners was significantly higher than those in intestinal phase and pulmonary phase (Fig. 5). Powder $ZnSiO_3$ and ZnO showed highest bioaccessibility in gastric

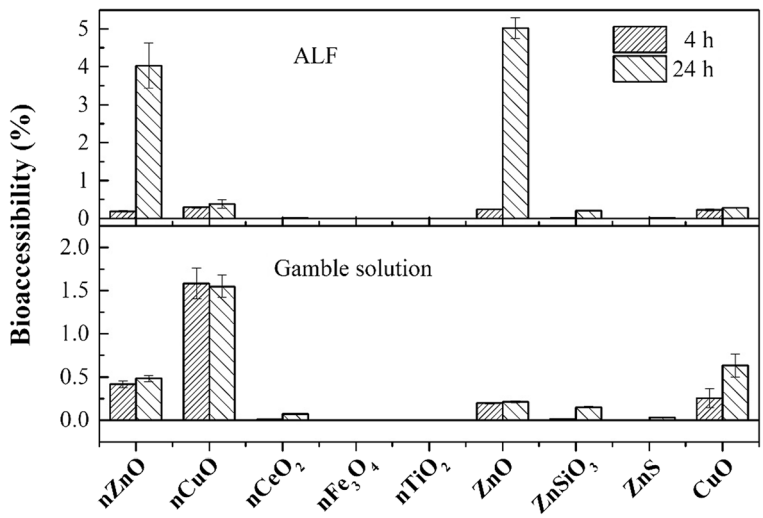

Fig. 3 The bioaccessibility in lung phase at different contact times

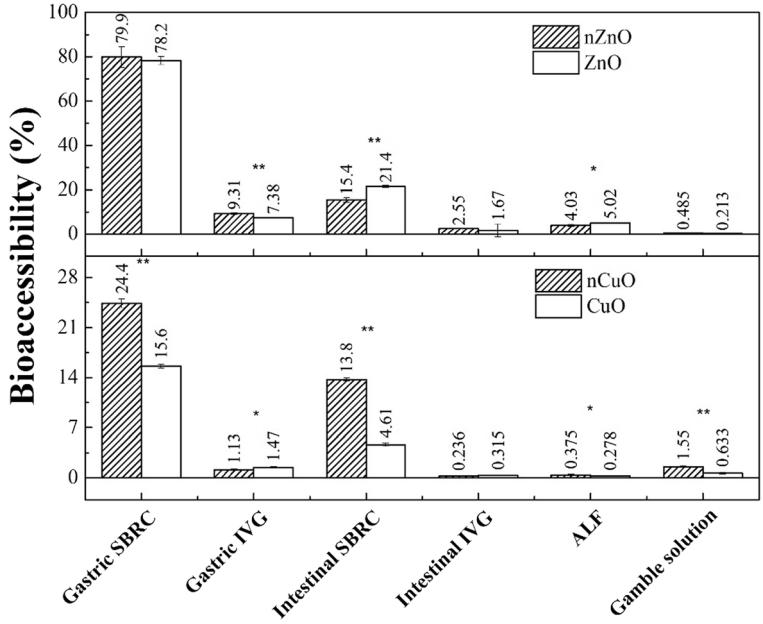

Fig. 4 The comparisons on in vitro bioaccessibility of nano- and non-nano-ZnO and CuO (*$0.01 < p < 0.05$; **$p < 0.01$)

phase for SBRC, about 2–3-fold higher than that in gastric phase for IVG (Fig. 5). This is consistent with the results of nZnO. The bioaccessibility of powder ZnS was very low in all gastrointestinal and pulmonary phases (< 0.03%) (Fig. 5), which might be due to its acid tolerance and water insolubility. Figure 5 also shows that the bioaccessibility for the powder ZnO was lower than that for the powder $ZnSiO_3$ in intestinal SBRC (21.4% for powder ZnO and 31.0% for powder $ZnSiO_3$). In general, $ZnSiO_3$ is easy to acidolyse in HCl solution to produce a water insoluble silicic acid. The silicic acid swelled in the simulated biological fluids with end-over-end shaking and incorporated with some Zn ions, leading to the 64.8% $ZnSiO_3$ versus 78.2% ZnO in gastric SBRC. While the silicic acid was treated with the intestinal SBRC, the incorporated Zn ions rereleased to the fluid and resulted in the higher bioaccessibility.

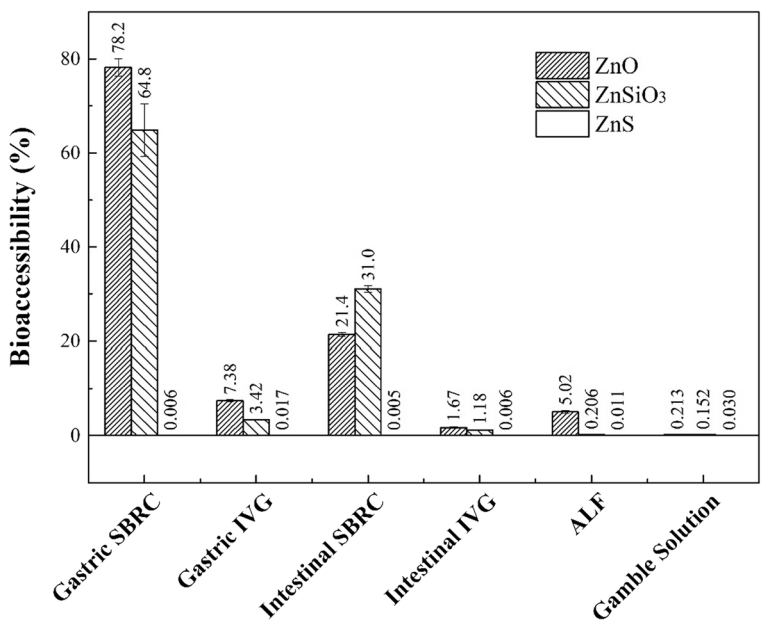

Fig. 5 The comparisons on in vitro bioaccessibility of non-nano-powders of Zn minerals

Environmental implication of the gastrointestinal and pulmonary bioaccessibility of nano-metal oxides

The components and pH of a simulated biological fluid are the important factors for the great differences on the gastrointestinal and pulmonary bioaccessibility of nano-metal oxides (Smith et al. 2014). For example, the main components of the gastric phase were 30.03 g L^{-1} glycine (adjusted to pH 1.5 with concentrated HCl) for SBRC and 0.15 M NaCl and 1% porcine pepsin (pH 1.8 adjusted with concentrated HCl) for IVG. The gastric phase of SBRC solution could provide sufficient HCl with the buffering effect of glycine for dissolving nZnO and nCuO, while the gastric phase of IVG solution without buffer was difficult to control solution pH. To maintain a stable solution, pH is the key to obtain an over-estimation of bioaccessibility and thus a precautionary methodology for risk assessment purpose (Oomen et al. 2002). Therefore, the SBRC method should be superior to the IVG method for the research of HCl consuming particles.

The bioaccessibility of nZnO and nCuO extracted by using ALF and Gamble solution was obviously lower than that in the intestinal phase (SBRC and IVG), although pH of the simulated intestinal solution of SBRC and IVG was similar to that for ALF and Gamble solution. This might be due to the sequential extraction with the gastric phase and then the intestinal phase of SBRC and IVG. Therefore, the acid activation of nZnO and nCuO in gastric phase may influence the surface properties of nZnO and nCuO, and the soluble Zn and Cu in gastric phase solution were also combined into intestinal phase solution. Accordingly, the gastrointestinal phase would lead to the same bioaccessibility of Pb from soils at pH > 5 (Juhasz et al. 2009). It implied that a weak acid condition produces indistinctive bioaccessibility with a neutral condition about certain particles. Considerably, different bile concentrations may also induce the different bioaccessibilities of the different intestinal methods (Oomen et al. 2002).

pH of ALF (pH 4.5) has less of a predicted influence on measured bioaccessibility of Cu. The present study showed that Gamble solution (pH 7.3) produced a higher value of bioaccessibility than ALF. The similar result has been observed by Zereini et al. that the average values for Pt bioaccessibility in PM_{10} samples were 23% for ALF and 24% for Gamble solution after 24 h extraction (Zereini et al. 2012). There are two possible reasons. On the one hand, the influence of the weak acid condition should be ignored according to the analysis above. On the other hand, DTPA, one of the constituents of Gamble solution that make the difference, is a good chelating agent that can chelate Cu ions well (Pastor et al. 2007). It may help Cu to be released from nCuO. In a word, this suggested that the strong chelating effect contributes more release of elements from nano-sized metal oxides than the weak acid effect does.

The properties of nano-metal oxides are other important factors for the great differences on their gastrointestinal and pulmonary bioaccessibility. Compared with the inhalation tract, the oral tract should be considered to the main contributor of the bioaccessibility of nZnO and nCuO owning to their acid solubility. But there is no distinction in different phases with nFe_3O_4, $nTiO_2$, and $nCeO_2$ which were coincident at a low value of bioaccessibility for their acid tolerance. However, the low value of bioaccessibility should not be ignored and misread to be a safe level to health. As a matter of fact, the over-take and long period exposure may lead to a warning level of released concentration (refer to Table S8).

Conclusions

Solubility and aggregation of nano-sized metal oxides in physiological body fluids are the key factors influencing their toxic effects. Firstly, the aggregation of nano-sized metal oxides was confirmed in the in vitro simulated lung fluids (ALF and Gamble solution) and gastrointestinal fluids (SBRC and IVG) at 2 mg L^{-1} of nano-sized metal oxides. The aggregation in pulmonary phase was more serious than that in gastrointestinal phase. The aggregation may decrease the toxic effects caused by the size effects of nano-sized metal oxides. Although the aggregating level was rather complicated among the different suspensions, the suspending stability was generally in the order of Fe_3O_4 < CuO < TiO_2 < CeO_2 < ZnO. Secondly, nZnO showed the highest gastrointestinal bioaccessibility (79.9 ± 4.6% in gastric SBRC), and then nCuO (24.4 ± 0.6% in gastric SBRC), and nFe_3O_4, $nTiO_2$ and $nCeO_2$ have the lowest bioaccessibility, while nZnO showed the highest pulmonary bioaccessibility (4.0 ± 0.3% in Gamble solution), and then nCuO (1.6 ± 0.1% in ALF), and nFe_3O_4, $nTiO_2$, and $nCeO_2$ have the lowest bioaccessibility. It is found that low bioaccessibility of nFe_3O_4, $nTiO_2$, and $nCeO_2$ means low toxic effects from the released metal ions. Thirdly,

the comparisons on the bioaccessibility of nano- and non-nano-CuO and ZnO indicated that the aggregating size in suspension could play more important role in influencing the bioaccessibility than single particle size does. In summary, our investigations on solubility and aggregation of nano-sized metal oxides are helpful not only to explain the toxic components of the nano-sized metal oxides but also to in vitro evaluate the health risks of nano-sized metal oxides via oral ingestion and inhalation exposure.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

References

Aerts A, Haouas M, Caremans TP, Follens LR, Van Erp TS, Taulelle F, Vermant J, Martens JA, Kirschhock CE (2010) Investigation of the mechanism of colloidal silicalite-1 crystallization by using DLS, SAXS, and 29Si NMR spectroscopy. Chemistry 16:2764–2774. https://doi.org/10.1002/chem.200901688

Chauhan N, Hooda V, Pundir C S (2013) In vitro effects of metal oxide nanoparticles on barley oxalate oxidase. J Nanopart Res 15. https://doi.org/10.1007/S11051-013-1493-9

Cruz N, Rodrigues SM, Tavares D, Monteiro RJ, Carvalho L, Trindade T, Duarte AC, Pereira E, Romkens PF (2015) Testing single extraction methods and in vitro tests to assess the geochemical reactivity and human bioaccessibility of silver in urban soils amended with silver nanoparticles. Chemosphere 135:304–311. https://doi.org/10.1016/j.chemosphere.2015.04.071

Denys S, Caboche J, Tack K, Rychen G, Wragg J, Cave M, Jondreville C, Feidt C (2012) In vivo validation of the unified BARGE method to assess the bioaccessibility of arsenic, antimony, cadmium, and lead in soils. Environ Sci Technol 46:6252–6260. https://doi.org/10.1021/es3006942

Drysdale M, Ljung Bjorklund K, Jamieson HE, Weinstein P, Cook A, Watkins RT (2012) Evaluating the respiratory bioaccessibility of nickel in soil through the use of a simulated lung fluid. Environ Geochem Health 34:279–288. https://doi.org/10.1007/s10653-011-9435-x

Dwivedi AD, Dubey SP, Sillanpää M, Kwon Y-N, Lee C, Varma RS (2015) Fate of engineered nanoparticles: implications in the environment. Coordin Chem Rev 287:64–78. https://doi.org/10.1016/j.ccr.2014.12.014

Evangelou MW, Ebel M, Schaeffer A (2007) Chelate assisted phytoextraction of heavy metals from soil. Effect, mechanism, toxicity, and fate of chelating agents. Chemosphere 68:989–1003. https://doi.org/10.1016/j.chemosphere.2007.01.062

Follens LR, Aerts A, Haouas M, Caremans TP, Loppinet B, Goderis B, Vermant J, Taulelle F, Martens JA, Kirschhock CE (2008) Characterization of nanoparticles in diluted clear solutions for silicalite-1 zeolite synthesis using liquid 29Si NMR, SAXS and DLS. Phys Chem Chem Phys 10:5574–5583. https://doi.org/10.1039/b805520c

Gambinossi F, Mylon SE, Ferri JK (2015) Aggregation kinetics and colloidal stability of functionalized nanoparticles. Adv Colloid Interfac 222:332–349. https://doi.org/10.1016/j.cis.2014.07.015

Garaud M, Auffan M, Devin S, Felten V, Pagnout C, Pain-Devin S, Proux O, Rodius F, Sohm B, Giamberini L (2016) Integrated assessment of ceria nanoparticle impacts on the freshwater bivalve Dreissena polymorpha. Nanotoxicology 10:935–944. https://doi.org/10.3109/17435390.2016.1146363

Guo X, Jiang C, Shi TS (2007) Prepared chiral nanorods of a cobalt(II) porphyrin dimer and studied changes of UV-Vis and CD spectra with aggregate morphologies under different temperatures. Inorg Chem 46:4766–4768. https://doi.org/10.1021/ic0622149

Huang X, Betha R, Tan LY, Balasubramanian R (2016) Risk assessment of bioaccessible trace elements in smoke haze aerosols versus urban aerosols using simulated lung fluids. Atmos Environ 125:505–511. https://doi.org/10.1016/j.atmosenv.2015.06.034

Inerbaev TM, Masunov AE, Khondaker SI, Dobrinescu A, Plamada AV, Kawazoe Y (2009) Quantum chemistry of quantum dots: effects of ligands and oxidation. J Chem Phys 131:044106. https://doi.org/10.1063/1.3135193

Juhasz AL, Smith E, Weber J, Rees M, Rofe A, Kuchel T, Sansom L, Naidu R (2007) Comparison of in vivo and in vitro methodologies for the assessment of arsenic bioavailability in contaminated soils. Chemosphere 69:961–966. https://doi.org/10.1016/j.chemosphere.2007.05.018

Juhasz AL, Weber J, Smith E, Naidu R, Marschner B, Rees M, Rofe A, Kuchel T, Sansom L (2009) Evaluation of SBRC-gastric and SBRC-intestinal methods for the prediction of in vivo relative lead bioavailability in contaminated soils. Environ Sci Technol 43:4503–4509. https://doi.org/10.1021/es803238u

Juhasz AL, Herde P, Herde C, Boland J, Smith E (2014) Validation of the predictive capabilities of the SBRC-G in vitro assay for estimating arsenic relative bioavailability in contaminated soils. Environ Sci Technol 48:12962–12969. https://doi.org/10.1021/es503695g

Julien C, Esperanza P, Bruno M, Alleman LY (2011) Development of an in vitro method to estimate lung bioaccessibility of metals from atmospheric particles. J Environ Monit 13:621–630. https://doi.org/10.1039/c0em00439a

Kendall M (2007) Fine airborne urban particles ($PM_{2.5}$) sequester lung surfactant and amino acids from human lung lavage. Am J Physiol Lung Cell Mol Physiol 293:L1053–L1058. https://doi.org/10.1152/ajplung.00131.2007

Kendall M, Tetley TD, Wigzell E, Hutton B, Nieuwenhuijsen M, Luckham P (2002) Lung lining liquid modifies PM(2.5) in favor of particle aggregation: a protective mechanism. Am J Physiol Lung Cell Mol Physiol 282:L109–L114

Kendall M, Guntern J, Lockyer NP, Jones FH, Hutton BM, Lippmann M, Tetley TD (2004) Urban $PM_{2.5}$ surface

chemistry and interactions with bronchoalveolar lavage fluid. Inhal Toxicol 16(Supp l):115–129. https://doi.org/10.1080/08958370490443204

Kurlanda-Witek H, Ngwenya BT, Butler IB (2014) Transport of bare and capped zinc oxide nanoparticles is dependent on porous medium composition. J Contam Hydrol 162-163:17–26. https://doi.org/10.1016/j.jconhyd.2014.04.002

Lee SH, Wang TY, Hong JH, Cheng TJ, Lin CY (2016) NMR-based metabolomics to determine acute inhalation effects of nano- and fine-sized ZnO particles in the rat lung. Nanotoxicology 10:924–934. https://doi.org/10.3109/17435390.2016.1144825

Li HB, Li J, Zhu YG, Juhasz AL, Ma LQ (2015) Comparison of arsenic bioaccessibility in house dust and contaminated soils based on four in vitro assays. Sci Total Environ 532:803–811. https://doi.org/10.1016/j.scitotenv.2015.06.060

Li HB, Zhao D, Li J, Li SW, Wang N, Juhasz AL, Zhu YG, Ma LQ (2016) Using the SBRC assay to predict lead relative bioavailability in urban soils: contaminant source and correlation model. Environ Sci Technol 50:4989–4996. https://doi.org/10.1021/acs.est.6b00480

Meunier L, Koch I, Reimer KJ (2011) Effect of particle size on arsenic bioaccessibility in gold mine tailings of Nova Scotia. Sci Total Environ 409:2233–2243. https://doi.org/10.1016/j.scitotenv.2011.02.006

Oomen AG, Hack A, Minekus M, Zeijdner E, Cornelis C, Schoeters G, Verstraete W, Van de Wiele T, Wragg J, Rompelberg CJ, Sips AJ, Van Wijnen JH (2002) Comparison of five in vitro digestion models to study the bioaccessibility of soil contaminants. Environ Sci Technol 36:3326–3334. https://doi.org/10.1021/es010204v

Pandurangan M, Kim DH (2015) In vitro toxicity of zinc oxide nanoparticles: a review. J Nanopart Res 17:Artn 158. https://doi.org/10.1007/S11051-015-2958-9

Pastor J, Aparicio AM, Gutierrez-Maroto A, Hernandez AJ (2007) Effects of two chelating agents (EDTA and DTPA) on the autochthonous vegetation of a soil polluted with Cu, Zn and Cd. Sci Total Environ 378:114–118. https://doi.org/10.1016/j.scitotenv.2007.01.022

Prow TW, Bhutto I, Kim SY, Grebe R, Merges C, McLeod DS, Uno K, Mennon M, Rodriguez L, Leong K, Lutty GA (2008) Ocular nanoparticle toxicity and transfection of the retina and retinal pigment epithelium. Nanomedicine 4:340–349. https://doi.org/10.1016/j.nano.2008.06.003

Raza G, Amjad M, Kaur I, Wen DS (2016) Stability and aggregation kinetics of titania nanomaterials under environmentally realistic conditions. Environ Sci Technol 50:8462–8472. https://doi.org/10.1021/acs.est.5b05746

Sambale F, Stahl F, Bahnemann D, Scheper T (2015) In vitro toxicological nanoparticle studies under flow exposure. J Nanopart Res 17:Artn 298. https://doi.org/10.1007/S11051-015-3106-2

Schaudien D, Knebel J, Creutzenberg O (2012) In vitro study revealed different size behavior of different nanoparticles. J Nanopart Res:14, Artn 1039. https://doi.org/10.1007/S11051-012-1039-6

Schroder L, Basta NT, Casteel SW, Evans TJ, Payton ME, Si J (2004) Validation of the in vitro gastrointestinal (IVG) method to estimate relative bioavailable lead in contaminated soils. J Environ Qual 33:513–521. https://doi.org/10.2134/jeq2004.5130

Sharma VK, Filip J, Zboril R, Varma RS (2015) Natural inorganic nanoparticles—formation, fate, and toxicity in the environment. Chem Soc Rev 44:8410–8423. https://doi.org/10.1039/c5cs00236b

Smith E, Scheckel K, Miller BW, Weber J, Juhasz AL (2014) Influence of in vitro assay pH and extractant composition on As bioaccessibility in contaminated soils. Sci Total Environ 473:171–177. https://doi.org/10.1016/j.scitotenv.2013.12.030

Sun P, Shijirbaatar A, Fang J, Owens G, Lin D, Zhang K (2015) Distinguishable transport behavior of zinc oxide nanoparticles in silica sand and soil columns. Sci Total Environ 505:189–198. https://doi.org/10.1016/j.scitotenv.2014.09.095

Szczeszak A, Ekner-Grzyb A, Runowski M, Mrowczynska L, Grzyb T, Lis S (2015) Synthesis, photophysical analysis, and in vitro cytotoxicity assessment of the multifunctional (magnetic and luminescent) core@shell nanomaterial based on lanthanide-doped orthovanadates. J Nanopart Res 17:Artn 143. https://doi.org/10.1007/S11051-015-2950-4

Tao S, Lu Y, Zhang D, Yang Y, Yang Y, Lu X, Sai D (2009) Assessment of oral bioaccessibility of organochlorine pesticides in soil using an in vitro gastrointestinal model. Environ Sci Technol 43:4524–4529. https://doi.org/10.1021/es900188c

Twining J, McGlinn P, Loi E, Smith K, Giere R (2005) Risk ranking of bioaccessible metals from fly ash dissolved in simulated lung and gut fluids. Environ Sci Technol 39:7749–7756. https://doi.org/10.1021/es0502369

Van Koetsem F, Verstraete S, Van der Meeren P, Du Laing G (2015) Stability of engineered nanomaterials in complex aqueous matrices: settling behaviour of CeO_2 nanoparticles in natural surface waters. Environ Res 142:207–214. https://doi.org/10.1016/j.envres.2015.06.028

Wang C, Shang C, Westerhoff P (2010) Quantification of fullerene aggregate nC60 in wastewater by high-performance liquid chromatography with UV-vis spectroscopic and mass spectrometric detection. Chemosphere 80:334–339. https://doi.org/10.1016/j.chemosphere.2010.03.052

Weaver JH, Waddill GD (1991) Cluster assembly of interfaces: nanoscale engineering. Science 251:1444–1451. https://doi.org/10.1126/science.251.5000.1444

Wragg J, Klinck B (2007) The bioaccessibility of lead from Welsh mine waste using a respiratory uptake test. J Environ Sci Health A Tox Hazard Subst Environ Eng 42:1223–1231. https://doi.org/10.1080/10934520701436054

Wright JR (2005) Immunoregulatory functions of surfactant proteins. Nat Rev Immunol 5:58–68. https://doi.org/10.1038/nri1528

Yang CS, Chang CH, Tsai PJ, Chen WY, Tseng FG, Lo LW (2004) Nanoparticle-based in vivo investigation on blood-brain barrier permeability following ischemia and reperfusion. Anal Chem 76:4465–4471. https://doi.org/10.1021/ac035491v

Zereini F, Wiseman CL, Puttmann W (2012) In vitro investigations of platinum, palladium, and rhodium mobility in urban airborne particulate matter (PM_{10}, $PM_{2.5}$, and PM_1) using simulated lung fluids. Environ Sci Technol 46:10326–10333. https://doi.org/10.1021/es3020887