Selective removal of ZnO nanoparticles from water using 3-aminopropyltriethoxysilane (APTES) and monitoring by meso-tetra(carboxyphenyl)porphyrin (TCPP)

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Research Article

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Abstract

Metal oxide nanoparticles (MONPs) such as TiO_2 and ZnO have been engineered for various industrial manufacturing processes with an annual global production exceeding millions of tonnes. Among MONPs, environmental exposure to ZnO nanoparticles may cause a significant toxicity due to bioaccumulation in the human body. Therefore, detection and removal of ZnO nanoparticles in the aqueous environment is highly desired. In this work, 3-aminopropyltriethoxysilane (APTES) was chosen to treat aqueous suspensions containing ZnO and other metal oxide nanoparticles. APTES first formed a thin surface layer on ZnO nanoparticles and continued in branching polymerization to reach out towards other APTES- ZnO nanoparticles. Eventually, a web-like sediment settled down onto the bottom. A high removal efficiency over 99% has attained for ZnO nanoparticles in aqueous suspension. The sedimentation was selective towards ZnO nanoparticles, due to the amino group of APTES preferentially interacting with ZnO to form a sturdy condensation coagulate. Other MONPs (such as Al_2O_3 , CaO , CeO_2 , CuO and TiO_2) did not form any web-like sediment with APTES but could co-precipitate with the ZnO nanoparticles. Meso-tetra(carboxyphenyl)porphyrin (TCPP) could be added as an indicator to co-precipitate with the sediment, with its color changing from red purple to bright green during the formation of APTES-TCPP- ZnO sediment.

Introduction

Numerous advances of nanoscience and nanotechnology have been reported in recent years on the use of metal oxide nanoparticles (MONPs) in enzymatic biosensors¹ and enzyme-linked immunosorbent assay¹. The massive production of MONPs for industrial manufacturing of consumer products leads to the inadvertent release of MONPs to the natural aquatic environment². This scenario raises great concerns because ZnO , TiO_2 and CeO_2 all exhibit toxicity towards microorganisms even though their bulk materials are generally considered to be non-toxic³. The toxicity is mainly due to induction of reactive oxygen species (ROS) that can lead to lipid peroxidation, DNA damage, and protein denaturation⁴. Ivask *et al.* identified the most toxic MONPs to be ZnO and CuO with average IC_{50} values of around $20 \mu\text{g/mL}$ against three mammalian cell lines (human alveolar epithelial cells A549, human epithelial colorectal cells Caco2, and murine fibroblast cell line Balb/c 3T3) at 24 h⁵. Jain *et al.* also reported in-vitro genotoxicity and cell death (on Chinese hamster lung fibroblast cells V-79) after exposure to $1\text{--}20 \mu\text{g/mL}$ ZnO nanoparticles for 6 hours⁶.

Most of the conventional wastewater treatment methods may not target the removal of MONPs and leaves a significant level of nanoparticles breakthrough ($2\text{--}96\%$ of ZnO and $0\text{--}4\%$ TiO_2) even with ultrafiltration simulated advanced treatment⁷. Thus, a simple detection and removal method towards MONPs is needed to test and treat wastewater. The recent common removal of MONPs is based on the enhanced coagulation and flocculation in the aqueous sample. While a single coagulant such as alum may not serve the best for MONPs removal, Sun *et al.* proposed the combination of enteromorpha polysaccharide and polyaluminum chloride can result in a 95% removal of ZnO nanoparticles along with

Zn²⁺ ⁸. You *et al.* also stated the combination of polyaluminum ferric chloride and cationic polyacrylamide is capable of removing both TiO₂ and ZnO nanoparticles ^{9,10}. Some organic ligands such as humic acid is proven to facilitate the coagulation of ZnO nanoparticles when using ferric chloride as a coagulant ¹¹. However, these methods are lack of selectivity towards one specific MONP.

Colorimetric detection has unexpectedly emerged as a research focus area in the field of analytical chemistry. Compared with instrumental methods of analysis, colorimetric methods offer many advantages such as adequate sensitivity, good selectivity, great convenience, low cost, as well as fast turnaround ¹². For the detection of metal oxide nanoparticles in aqueous suspension, surface reactivity of different metals can be the basis for selectivity in the development of novel colorimetric methods. Liu *et al.* carried out a comprehensive investigation of common MONPs for their ability to adsorb DNA, quench fluorescence, and release adsorbed DNA in the presence of target anions ¹³.

In this study, a novel screening test of MONPs was developed for the specific detection and removal of ZnO among several other transition metal oxides in aqueous suspension. 3-aminopropyl-triethoxysilane (APTES), a popular silanization grafting agent on MONPs for a broad range of applications, was used as a coagulation agent. Though the toxicity of APTES may be a concern, the resultant grafting on MONPs shows low cytotoxicity and high thermal stability ¹⁴. The APTES-modified MONPs are also commonly used for drug delivery ¹⁵. Meso-tetra(carboxyphenyl) porphyrin (TCPP) is a porphyrin derivative that has been proved to produce unique fluorescence emission with ZnO nanoparticles ¹⁶. The present test was based on specific colorimetric development from TCPP and obvious coagulate sedimentation of APTES-TCPP-ZnO. Analytical UV-visible spectroscopy and EDX were used to confirm the presence and estimate the quantity of ZnO nanoparticles. Both the colorimetric reagent TCPP and the coagulation agent APTES displayed excellent selectivity toward ZnO nanoparticles. The proposed method can be applied for convenient ZnO removal in the absence or presence of other TMONPs in wastewater.

Materials And Methods

Chemicals

3-aminopropyltriethoxysilane (APTES), meso-tetra(4-carboxyphenyl)porphyrin (TCPP), TiO₂ nanoparticles (21 nm, > 99.5%), CeO₂ nanopowders (< 25 nm), ZnO nanoparticles (< 50 nm particles, > 97%) were all obtained from Sigma-Aldrich (Oakville, Ontario, Canada). Mixed cellulose ester (MCE) filter membrane (0.45µm) was purchased from Millipore (Bedford, Massachusetts, US).

Removal of MONPs

The standard sample was prepared by dispersing individual MNOPs in distilled deionized water (DDW) using an ULTRASONICS ultrasonic processor 300W at a concentration of 1 mg/mL. A stock TCPP in ethanol was prepared at 1.0 mg/mL. For the removal of MONPs in aqueous suspension, a mixture of APTES (2%) and TCPP stock (5%) were added to treat the standard MONPs samples. The treated

samples were then set at room temperature for coagulation and sedimentation over 24 hours. The filter paper/membrane-assisted removal was done by placing a piece of filter paper or MCE membrane on the vial bottom to collect APTES-ZnO sediment.

Characterization

The resultant dispersion of APTES and TiO₂ nanoparticles mixture sample and the sediments scratched out from APTES-ZnO nanoparticles sample were studied using scanning electron microscopy (SEM) coupled with energy dispersive X-ray (EDX) analysis. The absorption spectra of ZnO nanoparticles, both the initial dispersion and the final supernatant after sedimentation, were measured on a Thermo Scientific GENESYS 10S UV-vis spectrophotometer with a slit width of 1.0 nm. The UV-vis spectrum of ZnO-APTES-TCPP sediment was measured directly from the vial wall.

APTES dosage and efficiency

To study the effect of APTES dosage on ZnO removal, six standard 1.0 mg/mL of ZnO nanoparticle dispersions were treated with 0.2% – 2% volume ratio of APTES. A series of ZnO nanoparticles in aqueous dispersion at various concentrations were prepared for treatment by spiking 2% by volume of APTES. The removal efficiency was then determined by UV-vis spectrophotometry.

Results And Discussion

Role of APTES

Addition of APTES by itself to the aqueous samples containing MONPs will experience a hydrolysis first on the epoxy group and result an increase of pH from 7 to 10 at the surface of ZnO nanoparticles¹⁷. This pH range exerts a maximum prohibition to the possible release of free metal ions for MONPs. For TiO₂ and ZnO nanoparticles that are more sensitive to the dissolution pH will experience an aggregation. Then, as of the multilayer APTES condensation that Bhushan et al. (2009) proposed, APTES will condensate on the surface of all MONPs and branch with other MONPs particles in high water content (Scheme 1)¹⁸. The higher degree of APTES branching condensation with ZnO nanoparticles is the leading cause forming a web-structured sediment. During the sedimentation, the accumulated APTES-ZnO on the vial bottom is immobilized by APTES condensed on the glass surface. Despite vigorous vortex, the sediment did not get dispersed and resuspended in the supernatant. However, there were no web-like sediments produced by APTES in other MONP dispersions.

Removal efficiency

The removal efficiency is measured by the UV-vis spectra of ZnO nanoparticles dispersion before and after the addition of APTES. Figure 1 shows the calibration curve of standard ZnO nanoparticles dispersion to be linear within the range between 0.1 and 0.5 mg/mL with detection limit down to 0.01mg/mL. Figure 2 shows the difference of the APTES and ZnO mixture before and after 24 hours

sedimentation. APTES shows nearly no absorbance, so that the removal efficiency was calculated to be over 99% by taking the detection limit as the final ZnO concentration (Fig. 2).

When APTES is coated on MONPs' surface, the density of APTES permits a different ratio between NH_2 groups and hydroxylated metal groups. Moreno et al. (2020) proposed a computational method to find the binding energy of the common aquatic pollutant species of heavy metals ($\text{As}(\text{OH})_3$ and $\text{Cd}(\text{OH})_2$) and concluded that low APTES coverage on SiO_2 nanoparticles is favourable for the adsorption As, while Cd has favourable binding with the high APTES coverage¹⁹. This approach allows selective adsorption of certain heavy metal ions by adjusting the APTES coverage rate as high NH_2 surface can facilitate the adsorption by electrostatic interaction. The coverage ratio can be implemented to maximize the adsorption of MONPs since the surface charge of each nanoparticle is different. In this approach, the polymerization level of the condensed APTES resulted a specific NH_2 ratio and pore size in aqueous sample, which can grant a preferable adsorption toward ZnO nanoparticles. The electrostatic interaction between ZnO surface and NH_2 in APTES complex allows a more stable and sturdy structure and thus a quicker sedimentation.

Role of TCPP

Upon the addition of APTES and TCPP into the aqueous samples containing MONPs, TCPP as an indicator appeared to be green only in the presence of ZnO (Fig. 3A). After a 24-hour period of sedimentation, the vial of ZnO nanoparticles revealed a clear supernatant sitting on top of a green sediment with a web-like microstructure adhering onto the vial bottom (Fig. 3B). The bright green color produced by TCPP can be related to the interaction with ZnO nanoparticles in the porphyrin center. This ZnO-TCPP complex can be co-precipitated by APTES through either the electrostatic interaction or hydrolysis between TCPP's carboxyl (COOH) group and NH_2 during the sedimentation process. The addition of TCPP show no influence to the sedimentation but can be used as a convenient visual indicator for the majority of ZnO removal. The supernatant of APTES-TCPP-ZnO sample was later verified by EDX to comprise no significant amount of Zn.

Characterization of APTES-TCPP-MONPs

SEM images of APTES-TCPP-MONPs

The SEM images (Fig. 4) show the overall morphology difference of the APTES-TCPP sediments produced by TiO_2 nanoparticles and ZnO nanoparticles. Although they both exhibited the branching polymerization of APTES, the APTES-TCPP-ZnO sediment possessed a more uniform web-like microstructure. According to EDX analysis results, the APTES sediment had a relatively consistent distribution of Zn content throughout the web-like microstructure. In contrast, Ti content was only detected in a few spots of the APTES-TCPP- TiO_2 sediment. This result suggests that the branching polymerization of APTES with TiO_2 in the presence of TCPP happened slower than the

aggregation/agglomeration of TiO_2 nanoparticles themselves. Consequently, most of TiO_2 nanoparticles formed agglomerates during the APTES branching polymerization.

UV-vis spectra of ZnO-APTES-TCPP

As presented by the UV-vis spectra in Fig. 5, TCPP exhibits a sharp absorption peak at 415 nm, and ZnO nanoparticles have a broad absorption peak from 250 to 360 nm. The freshly prepared mixture containing APTES, TCPP and ZnO nanoparticles shows a combination of TCPP and ZnO absorption peaks. However, after sedimentation over 24 hours, both the absorbance peaks of TCPP and ZnO diminish for the clear supernatant. The UV-vis spectrum of the precipitate showed a shift of TCPP peak from 415 nm to 440 nm; and an enhanced absorbance from 550 nm to 650 nm, which explained the presence of green color. This is a strong evidence that supports the co-precipitate of TCPP during the removal of ZnO in the aqueous sample.

Effect of other TMONPs

CuO nanoparticles is another one of the most toxic MONPs, and TiO_2 nanoparticles are commonly present in the wastewater along with ZnO nanoparticles due their similar photocatalytic applications. While there was no sedimentation of neither CuO or TiO_2 nanoparticles observed upon addition of APTES, potential interference of these TMONPs with ZnO sedimentation were determined individually. Identical parameters were applied in the sedimentation of ZnO in mixture with TiO_2 or CuO nanoparticles. The results showed that a less structured sediment formed on the vial bottom and some particles were suspending in the supernatant. Though the presence of other TMONPs might interfere the efficiency of ZnO nanoparticles sedimentation, their removal was not significantly compromised. Thus, this method could be applied for the removal of many other toxic TMONPs together with the sedimentation of ZnO nanoparticles.

Effect of APTES dosage

The APTES dosage was found to have optimal sedimentation effect at 2% v:v to a 1.0 mg/mL ZnO nanoparticles dispersion. With lower APTES ratio, the interaction of ZnO-APTES conjugate was rather limited to form sedimentations within 24 hours. For the 2% v:v APTES to water ratio, maximum treatment capacity could reach up to 2 mg/mL of Zn nanoparticles. Another interesting finding was that even completely sedimented ZnO-APTES conjugates were capable of removing an additional dosage of ZnO nanoparticles freshly dispersed in the supernatant water. After 24 hours were allowed for sedimentation, the removal efficiency reached over 99% and the supernatant appeared clear again (as evidenced in Fig. 6).

Membrane-assisted removal

To facilitate sustainable removal, a filter paper or mixed cellulose ester (MCE) membrane was placed on the glass vial bottom to collect the APTES-ZnO sediment. Figure 7 shows the appearance of APTES-ZnO

deposited on either filter paper or MCE membrane in a uniform layer. The filter paper or MCE membrane could be retrieved (after discarding the water on top) and then regenerated by ultrasonic cleaning for reuse.

Conclusion

In this work, APTES was found to afford a selective removal of ZnO nanoparticles among other MONPs (Al_2O_3 , CaO, CeO_2 , CuO and TiO_2) in aqueous suspensions through branching polymerization. A steady web-like sediment settled down onto the bottom with high removal efficiency over 99%. A colorimetric indicator of ZnO nanoparticles, TCPP, verified the completion of removal. With a filter paper or MCE membrane placed on the container bottom, convenient and sustainable disposal can be achieved simply by discarding the paper or regenerating the membrane.

Declarations

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Compliance with Ethical Standards

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Figures

Figure 1

(A) UV-vis spectra of ZnO nanoparticles and (B) calibration curve of absorbance between ZnO nanoparticles concentration.

Figure 2

UV-vis spectra of APTES and APTES-ZnO nanoparticles mixture before sedimentation and the supernatant after 24 hours sedimentation. (inset) expanded spectrum of APTES-ZnO supernatant.

Figure 3

Sediment formation of APTES-TCPP in aqueous samples containing different MONPs (A) and detailed appearance of APTES-TCPP-ZnO adhered onto the vial bottom.

Figure 4

Scanning electron microscopy (SEM) image of APTES-TCPP sediment with TiO₂ nanoparticles (A) and with ZnO nanoparticles (B).

Figure 5

UV-vis spectra of APTES, TCP, freshly prepared mixture of APTES/TCP/ZnO, and the supernatant after sedimentation over 24 hours.

Figure 6

UV-vis spectra of before and after the removal upon the additional dosage of ZnO nanoparticles

Figure 7

Morphology of APTES-ZnO depositing on filter paper (left) and MCE filter membrane (right)

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [Scheme1.jpeg](#)