Dispersion and sedimentation of titanium dioxide nanoparticles in freshwater algae and daphnia aquatic culture media in the presence of arsenate

Zhenhong Wang\textsuperscript{a,b}, Zhuanxi Luo\textsuperscript{a} and Yamen Yan\textsuperscript{a}

\textsuperscript{a}Key Laboratory of Urban Environment and Health, Institute of Urban Environment, Chinese Academy of Sciences, Xiamen, China; \textsuperscript{b}College of Chemistry and Environment, Fujian Province Key Laboratory of Modern Analytical Science and Separation Technology, and Pollution Monitoring and Control of Key Laboratory of Universities in Fujian Province, Minnan Normal University, Zhangzhou, China

ABSTRACT

Little information is available on Titanium dioxide nanoparticles (nTiO\textsubscript{2}) behavior in different culture media for aquatic organisms. This study aimed to accurately evaluate nTiO\textsubscript{2} dispersion and sedimentation in common freshwater algae (BG-11) and daphnia aquatic (SM7) culture media. We additionally investigated potential mechanisms of nTiO\textsubscript{2} stability under arsenate influence. Results showed that high ionic strength in culture media was probably a key reason for the acute nTiO\textsubscript{2} agglomeration found. Additionally, the hydrodynamic size of nTiO\textsubscript{2} suspension in the presence of arsenate was significantly larger, increasing with arsenate concentration in ultrapure water. Conversely, the hydrodynamic size in BG-11 and SM7 decreased with arsenate concentration. The nTiO\textsubscript{2} sedimentation rate increased significantly with arsenate concentration in ultrapure water but significantly decreased in BG-11 and SM7 culture media. Many nTiO\textsubscript{2} remained suspended after initial rapid sedimentation and the slight sedimentation that occurred in the subsequent 24 h, suggesting that algae and daphnia within the water column will be exposed to small nanoparticle aggregates for a long period of time. Such nTiO\textsubscript{2} behavior, especially in the presence of arsenate, requires more consideration than the different toxicological results reported in literature.

KEYWORDS

Culture medium; algae; daphnia; nanoparticle; stability

1. Introduction

Engineered nanoparticles (ENs) have received a lot of attention and recently caused great concern due to their rapidly increasing industrial applications and their subsequent release into the environment [1,2]. After entering the environment, ENs could potentially interact detrimentally with other elements within their neighborhood and cause adverse effects on organisms and on human health [3–5]. Comprehensive EN risk assessments remain a difficult task because the extent of such negative effects is dependent upon...
numerous variables, i.e. concentration, composition, stability, and bioavailability as well as the potential transformation of ENs as they interact with other elements in the environment [6–8]. Extensive insight into the environmental behavior and fate of ENs is therefore critical.

Among ENs, titanium dioxide nanoparticles (nTiO$_2$) are of great importance because of their wide applications in manufacturing products, such as pigments, coatings, sunscreen, and cosmetic additives. nTiO$_2$ has already been shown to be toxic to a wide range of aquatic and terrestrial organisms [9–11]. It is of particular importance to note that nTiO$_2$ is known to agglomerate immediately upon contact with numerous culture media, subsequently resulting in aiding sedimentation [12–14]. Given that different culture media possess different physicochemical dispersion and sedimentation properties, a great variety in nanoparticle stability can occur [15–17]. Therefore, it becomes difficult to obtain toxicity results under different culture media. This will likely result in inaccurate dose estimations and interpretation, which in turn obstructs sample comparisons [18–20]. For these reasons, this study aimed to understand nTiO$_2$ stability under different culture media in nanomaterial risk assessment studies.

Freshwater algae, being the primary producers in aquatic ecosystems, play an important role in speciation, distribution, and cycling of contaminations in freshwater environments. Daphnia, a genus of small planktonic crustaceans, is commonly used in certain environments to test the effects that nanoparticles have on an ecosystem. It is an indicator genus, and particularly useful because of its short lifespan and reproductive capacity. Numerous ways that nTiO$_2$ produce toxic effects on algal cells and daphnia (e.g. membrane damage, protein binding, loss of function, mutation, mitochondrial damage, lysis, adhesion, etc.) have been reported [21–25]. However, even though many studies have reported their findings from toxicological tests applying nTiO$_2$ to algal species and daphnia, nTiO$_2$ behavior cultivated within different culture media remains unclear.

This study focused on accurately evaluating nTiO$_2$ dispersion and sedimentation in commonly used aquatic freshwater algae and daphnia culture media. A systematic examination of hydrodynamic size and sedimentation of nTiO$_2$ in three types of media was conducted. Additionally, arsenic, ubiquitous throughout global environments, is a noxious, nonessential metalloid that has been designated a priority pollutant [26]. Increasing concern has recently arose on how and to what extent changes in nTiO$_2$ cause co-occurring contaminants, such as arsenic, to react across phytoplankton–daphnia food chains [27]. Therefore, effort was also made to understand the potential mechanisms of nTiO$_2$ stability influenced by arsenate (As, predominant arsenic speciation in natural freshwater). Knowledge gained from results on nTiO$_2$ stability in different aquatic culture media will provide valuable basic information for studies on environmental implications of engineered nanoparticles.

2. Materials and methods

2.1. Sample particle stock solutions and culture media preparation

Titanium dioxide nanoparticles (nTiO$_2$, <25 nm, all 100% anatase) were purchased in powder form from Sigma-Aldrich Co. (St. Louis, MO, U.S.A.). Arsenate (Na$_3$AsO$_4$·12H$_2$O) was used for the preparation of As stock solutions for As(V). All additional chemical reagents used in this study were of analytical grade.
Na$_3$AsO$_4$·12H$_2$O and nTiO$_2$ in dry powder form were weighed on an analytical balance, suspended in ultrapure water (18.2 MΩ cm, Millipore Co.) at a concentration of 10 mM and 1000 ppm, respectively. The 10 mM As stock solution was diluted into 1 ~ 100 μM solutions of arsenate. The nanoparticle stock solution was then sonicated for 10 min at 35 ~ 40 W to aid in mixing and forming a homogeneous dispersion. The final concentrations of nanoparticle with 2 ~ 20 ppm were diluted from the nanoparticle stock solution separately with different aquatic culture media and different levels of arsenate solutions for further use. It is important to note that the selected culture media for nanoparticle dispersion have been widely used in current bioavailability, toxicity, and bioaccumulation studies of freshwater microalgae and daphnia. Freshwater microalgae were cultured in Blue-Green medium (BG-11) [13,28]. Daphnia magna was cultured in simplified Elendt M7 medium (SM7) [29]. The composition of the media employed in this study is provided in Table A.1. A systematic water quality analysis was conducted for each medium (Table 1). The ionic strength of BG-11 was higher than SM7. Compared to ultrapure water and surface water [30], both the algae and daphnia culture media exhibited high ionic strength (average of 23.44 and 20.56 mM, respectively) and conductivity (average of 1846 and 1619 ms/cm, respectively). The initial pH level of the two media was within a neutral range.

### 2.2. Scanning electron microscopy of nanoparticles

Visual characterization of nTiO$_2$ was conducted using Field Emission Scanning Electron Microscopy (FESEM, S-4800, Hitachi, Japan). Suspension of nTiO$_2$ was diluted with ultrapure water and placed on aluminum stubs to dry before SEM analysis was conducted at room temperature. Samples were then coated with a thin gold (Au) layer to increase the contrast and quality of images. Photographs were taken at a voltage of 5 kV.

### 2.3. DLS and laser Doppler velocimetry of nanoparticles in solutions

In order to characterize the size and zeta potential of selected nanoparticles in solutions, dynamic light scattering (DLS) and laser Doppler velocimetry (LDV) were conducted on a Zetasizer Nano ZS (Malvern Instruments Ltd., Malvern, U.K.). Samples were prepared as described in the sample particle stock solutions and media preparation section (2.1) and vortexed to provide a homogeneous solution. After which, 1.5 mL was transferred to a square cuvette for DLS measurements, and 1 mL was transferred to a Malvern Clear Zeta Potential cell for LDV measurements. Instrument temperature was set at 20 °C before sample measurements were conducted. Three independent measurements were taken with four prereading measures, each reading consisting of six runs of 10 s duration. Mean hydrodynamic size was calculated from the histogram of size distribution frequency for each nanoparticle. The polydispersity index (PDI) that provides the width of size distribution was also included. Solution pH levels for zeta potential measurements were measured using a Mettler Toledo FE20 pH meter.

| Medium | TDS (mg/L) | Conductivity (μs/cm) | Ionic strength (mM) | pH    |
|--------|------------|----------------------|---------------------|-------|
| BG-11  | 1239 ± 6   | 1846 ± 23            | 23.44 ± 0.30        | 7.13 ± 0.30 |
| SM7    | 419 ± 9    | 1619 ± 17            | 20.56 ± 0.20        | 7.76 ± 0.07 |

Table 1. Water chemistry analysis of algae and daphnia culture media.
2.4. UV-Vis spectra

UV-Vis spectrometry can be used to calculate sedimentation of nanoparticles based on their detected particle sizes [31,32]. Sedimentation of nTiO2 was characterized using a UV-Vis spectrometer (UV-2450, Shimadzu, Japan) with a wavelength of 262 nm for water and 216 nm for algae and daphnia magna media in accordance with the pre-experiment with a wavelength range from 200 to 800 nm and a resolution of 1 nm.

2.5. Statistics

All experiments were independently repeated three times and data were recorded as the mean with standard deviation (SD). The homogeneity of variance was evaluated and one-way analysis of variance (ANOVA) with the Tukey’s multiple comparison procedure was used to detect significant differences between the control and treated groups. For all data analyses, a < 0.05 P-value was considered statistically significant.

3. Results and discussion

3.1. TiO2 nanoparticle dispersion

SEM analysis revealed irregular morphology of nTiO2 in dispersed solutions. As shown in Figure 1 (a), nanoparticles dispersed in ultrapure water had a similar average diameter to the original dry nanopowder. In the BG-11 and SM7 media, nanoparticles aggregated more tightly with more smooth surfaces with average diameters of 400 and 600 nm, respectively (Figure 1 b-c).

DLS analysis revealed that nTiO2 suspended in deionized water at a 20 ppm concentration formed a relatively stable dispersion with a zeta potential of 22.9 mV (Table 2). Under optimum sonication conditions, particle size was ~200 nm. The much larger hydrodynamic size observed when compared to the primary particle size suggested that nTiO2 samples consisted of some hard aggregates that were not easily broken down by ultrasonication. At the same time, several dispersed nanoparticles can re-aggregate rapidly after ultrasonication. Similar observations were also made in some earlier studies [33–35]. When suspended in algae and daphnia culture media, TiO2 nanoparticles exhibited much poorer dispersion. DLS analysis of TiO2 suspensions at a 20 ppm concentration showed that agglomerate sizes were 544 and 605 nm for algae and daphnia media, respectively (Table 2). Consistent with this dramatic size increase, zeta potentials also dropped to

![Figure 1. SEM images of TiO2 nanoparticles dispersal in (a) ultrapure water, (b) BG-11, and (c) SM7.](image-url)
approximately −10 and 0 mV for algae and daphnia media, respectively (Table 2). Even though the pH level did not change significantly with an increase in nTiO2, it increased as arsenate concentrations increased in all media (Table 3).

In contrast to water, where hydrodynamic size remained similar in a wide range of nanoparticle concentrations (2 ~ 20 ppm), the nTiO2 agglomerate size increased with increasing nanoparticle concentrations in the presence of algae (200 ~ 500 nm) and daphnia (250 ~ 600 nm) culture media (Figure 2). High ionic strengths observed in algae and daphnia culture media were probably the key reasons for the significant increase in TiO2 nanoparticle agglomeration. According to the Derjaguin–Landau–Verwey–Overbeek (DLVO) theory, the stability of a colloidal suspension is based on the net balance of two forces: the electrostatic repulsion which prevents aggregation and a universal attractive van der Waals force which acts to bind particles together [36]. At lower electrolyte conditions, such as ultrapure water medium, a smaller nTiO2 suspension size was observed, which can be explained by the dominance of electrostatic repulsion and the relative weak van der Waals attraction. With increasing electrolyte concentrations, ionic strength increases. High ionic strength compresses the electrical double layer to improve van der Waals attraction, leading to nTiO2 agglomeration as observed in the aquatic culture media. It has been reported that additional salts caused a shift in nTiO2 suspension zeta potentials to a lower value [37]. Therefore, after the addition of aquatic culture media, zeta potential values were lower. The lower the absolute values of zeta potential were, the more instable nanoparticles became, and they became more obviously aggregated. This could explain the largest nTiO2 hydrodynamic size dispersion in SM7 medium because of its lowest zeta potential (Table 2). In addition, the point of zero charge of nTiO2 stabilization was approximately pH 4–6.5 [29,37,38]. Subsequently, at an alkaline pH level, repulsive interaction would likely increase in aquatic culture media due to surface ionization in nTiO2 suspension, improving overall nTiO2 dispersion [36,39]. It was therefore concluded

| Medium      | dH (nm) | PdI | \(\zeta\) (mV) | pH        |
|-------------|---------|-----|---------------|-----------|
| Ultrapure water | 206 ± 19 | 0.26 | 22.90 ± 0.15  | 6.96 ± 0.1 |
| BG-11       | 544 ± 135 | 0.39 | −10.90 ± 0.31 | 7.07 ± 0.3 |
| SM7         | 605 ± 157 | 0.40 | −0.71 ± 0.05  | 7.63 ± 0.3 |

| Culture media | As (µM) | \(\zeta\) (mV) | pH        |
|---------------|---------|---------------|-----------|
| Ultrapure water | 0    | 22.90 ± 0.15  | 6.41 ± 0.02 |
|               | 1    | 19.70 ± 0.21  | 6.84 ± 0.04 |
|               | 10   | 11.23 ± 0.19  | 6.38 ± 0.01 |
|               | 100  | 0.06 ± 0.00   | 7.27 ± 0.14 |
| BG-11         | 0    | −10.90 ± 0.30 | 7.16 ± 0.09 |
|               | 1    | −16.20 ± 0.17 | 7.31 ± 0.24 |
|               | 10   | −29.70 ± 0.09 | 6.82 ± 0.15 |
|               | 100  | −34.90 ± 0.37 | 7.39 ± 0.21 |
| SM7           | 0    | −0.71 ± 0.05  | 7.53 ± 0.06 |
|               | 1    | −4.90 ± 0.08  | 7.51 ± 0.07 |
|               | 10   | −15.20 ± 0.52 | 7.54 ± 0.1  |
|               | 100  | −29.70 ± 1.21 | 8.36 ± 0.07 |
that pH makes little difference in nTiO₂ dispersion because the pH level of the three culture media investigated was not significantly different [38].

The dispersed average nTiO₂ hydrodynamic size in ultrapure water, BG-11, and SM7 varied widely as previously reported. Tan et al. [29] demonstrated that the dispersed size of anatase nTiO₂ increased by approximately 189.0 and 266.1 nm with increasing concentrations from 0.5 to 2 mg/L in SM7 medium. At the same time, Seitz et al. [18] concluded a hydrodynamic size of >1000 nm for P25 nTiO₂ in ASTM medium (similar to SM7). In addition, Lin et al. [23] reported a hydrodynamic size of 940 nm for anatase nTiO₂ (100 ppm) in OECD medium (similar to BG-11). Even for the same dispersed culture medium, this indicates that the dispersion and sedimentation of nTiO₂ depend upon commercial sources, structure, concentration, as well as the dispersion operation method used. This wide discrepancy is more concerning than different toxic results reported.

3.2. TiO₂ nanoparticle sedimentation

After dispersion, aggregation of engineered nanoparticles in different culture media would inevitably occur to some extent. The ultimate consequence of aggregation is nanoparticle sedimentation. According to UV-Vis analysis, at a 20 ppm initial concentration, 55%, 85%, and 77% of TiO₂ nanoparticles remain in water, algae, and daphnia media supernatant, respectively, after a 24 h period (Figure 3). For all aquatic culture media, sedimentation rates of nanoparticles increased with increasing nTiO₂ concentrations (Figure 3). This trend was consistent with the dispersion status of the nanoparticles described above. Higher nTiO₂ dispersion was more difficult to aggregate and then settle in these culture media. In ultrapure water, nTiO₂ settlement was slowest followed in descending order by BG-11 and SM7. Brunellit et al. [20] reported that aggregation and sedimentation of nTiO₂ were primarily affected by initial concentrations. Our results indicated that culture
media were the primary factor that contributed to nTiO$_2$ aggregation and sedimentation, while their initial concentrations were the secondary factor.

Most importantly, after the initial rapid sedimentation and the slight sedimentation that occurred in the subsequent 24 h period, nTiO$_2$ was observed to stabilize in all of the three aquatic culture media investigated, indicating that many nTiO$_2$ particles remained suspended, especially in ultrapure water. Similar results were reported by Kller et al. [15]. In contrast, the lack of organic molecules in our culture media resulted in relatively higher sedimentation. Nevertheless, under such media conditions, aquatic organisms within the water column, such as algae and daphnia, will be exposed to small nanoparticle aggregates for a long period of time. However, at the initial stage, a rapid sedimentation of nTiO$_2$ may reduce the bioavailability of free ions due to their higher absorption capacity [40].

### 3.3. Effects of arsenate concentration on TiO$_2$ nanoparticle stability

Arsenate showed significant influences on nTiO$_2$ aggregation and sedimentation in the three culture media investigated (Figures 4 and 5). Compared to free arsenate counterparts, the hydrodynamic size of nTiO$_2$ suspension in the presence of arsenate was significantly larger, increasing with arsenate concentration in ultrapure water. It is interesting to note that the hydrodynamic sizes of nTiO$_2$ suspension in the presence of arsenate decreased with arsenate concentration in BG-11 and SM7 media. The sedimentation rate of nTiO$_2$ increased significantly in the presence of arsenic in ultrapure water but significantly decreased in BG-11 and SM7 media. This could be explained by negative charges related to arsenate, leading to significant changes in nTiO$_2$ surface charges and the subsequent variation in electrostatic repulsion. At the same time, increased pH levels in the presence of arsenate could increase negative surface charges of nTiO$_2$ suspension due to the relatively lower point of zero charge (ZPC). We concluded that after the addition of arsenate, positive charges of nTiO$_2$ suspension were significantly neutralized in ultrapure
water, while negative charges in BG-11 and SM7 were largely strengthened, supported by their zeta potential changes under nTiO2 suspension (Table 3).

With regards to nTiO2 risk assessments, the wide variation in stability of different culture media must be taken into account. In particular, after the addition of certain anion contaminants, such as arsenate and phosphate, the stability of nTiO2 suspension significantly increased, resulting in potential ecological accumulation of relative contaminants in aquatic organisms within the water column [16,40,41]. It is important to note that other aquatic culture media exist, such as those developed by ASTM (American Society for Testing and Materials), OECD, and EPA. Although they use similar ingredients to

Figure 4. Effects of arsenate concentration on TiO2 nanoparticles dispersion. nTiO2 concentration was 20 ppm for all media. Error bar: mean ± SD (n = 3). *denotes statistical differences from the control with P-values of <0.001.

Figure 5. Sedimentation of TiO2 nanoparticles under different arsenate concentrations. nTiO2 concentration was 20 ppm for all media.
support aquatic growth, further research on nTiO₂ stability is required in these media standards due to risks related to result presumptions.

4. Conclusions

It is of great value for nanomaterial risk assessment studies to understand the stability of nTiO₂ under different culture media due to their large production in manufacturing and wide application as well as their adverse impacts on the environment. In this study, culture media were the primary factor that contributed to nTiO₂ aggregation and sedimentation, while their initial concentrations were the secondary factor. The hydrodynamic size of nTiO₂ (~200 nm) remained similar in a wide range of nanoparticle concentrations (2~20 ppm) in ultrapure water, while it increased with increasing nanoparticle concentration in algae (200~500 nm) and daphnia (250~600 nm) culture media. It is important to note that high ionic strengths in algae and daphnia culture media are probably a key reason for the significant agglomeration of TiO₂ nanoparticle observed. Additionally, many nanoparticles remain suspended after initial rapid sedimentation and the slight sedimentation that occurred in the subsequent 24 h period, suggesting that algae and daphnia in the water column will be exposed to small nanoparticle aggregates for a long period of time. Wide dispersal variation in nTiO₂ hydrodynamic size was observed in ultrapure, BG-11, and SM7 media. Even for the same dispersed culture medium, nTiO₂ dispersion and sedimentation depended upon commercial sources, structure, concentration, as well as the dispersion operation method used. This wide discrepancy requires greater attention than reporting on different toxicological results. Understanding nTiO₂ stability in different aquatic culture media will provide greater insight into the study of environmental implications of engineered nanoparticles.

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ORCID

Zhuanxi Luo http://orcid.org/0000-0003-0164-4492
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