THE INTERACTION OF NANOPARTICLE TITANIUM DIOXIDE (n-TiO$_2$) WITH ORGANIC WASTEWATER CONTAMINANTS (OWCs): MECHANISMS, STABILITY, AND TRANSPORT

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Jaewoong Lee

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Jaewoong Lee, Ph.D.

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Advisor: Shannon L. Bartelt-Hunt

Wastewater treatment plants (WWPTs) play an important role in the environmental release of wastewater contaminants via physical and biological processes. However, the vast majority of WWTPs can not completely eliminate organic or inorganic contaminants. Newly emerging contaminants such as steroid hormones, pharmaceuticals, and nanoparticles (NPs) (i.e., TiO$_2$) are released from WWTP effluent into receiving waters.

In this study, we investigate the interaction between trace organic wastewater contaminants and nanoparticles after release from WWTPs in the aquatic environment. First, we investigated the stability of n-TiO$_2$ as a function of 17$\beta$-estradiol (E2) concentrations under various ionic strength conditions at fixed pH 7, which showed the effect of E2 on the stability and mobility of n-TiO$_2$. Second, we evaluated the stability of n-TiO$_2$ in the presence of humic acid (HA) and E2 concentration under high ionic strength conditions to simulate a landfill leachate. Third, quantitative structure-activity relationship (QSAR) models for prediction of n-TiO$_2$ aggregation by organic compound molecular descriptors were investigated using multiple linear regression (MLR) method.
Finally, we evaluated the deposition of n-TiO$_2$ under different surface roughness using a new technique, quartz crystal microbalance with dissipation (QCM-D). We observed that surface roughness resulted in higher deposition of n-TiO$_2$ under favorable condition compared to flat surfaces according to DLVO theory. These results represent the first effort to show the effects of surface roughness on the deposition of n-TiO$_2$. 
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Table of Contents

Acknowledgements .............................................................................................................. iv

Table of Contents ................................................................................................................. vi

List of Tables ........................................................................................................................ xi

List of Figures ......................................................................................................................... xiii

CHAPTER 1 PROJECT OVERVIEW AND OBJECTIVES ...................................................... 1

1.1 Project Overview .............................................................................................................. 1

1.2 Project Goals and Objectives ......................................................................................... 5

1.3 References ..................................................................................................................... 8

CHAPTER 2 EFFECT OF 17β-ESTRADIOL ON STABILITY AND MOBILITY OF
TiO₂ RUTILE NANOPARTICLES ............................................................................................ 11

2.1. Introduction .................................................................................................................. 11

2.2 Materials and Methods ................................................................................................. 14

2.2.1 Chemical reagents ..................................................................................................... 14

2.2.2 Preparation of n-TiO₂ suspensions and aggregation experiments ......................... 14

2.2.3 Measurements of zeta potential and particle size ..................................................... 15

2.2.4 Sorption isotherms .................................................................................................... 16

2.2.5 FTIR spectrometer analysis ....................................................................................... 16

2.3 Results and discussion ................................................................................................. 17

2.3.1 Sorption of 17β-estradiol to n-TiO₂ .................................................................... 17
2.3.2 Aggregation of n-TiO₂ in the presence of E2 .................................................. 20
2.3.3 Particle size distributions in the presence of E2 .............................................. 23
2.3.4 Zeta potentials in the presence of E2 ................................................................. 25
2.3.5 n-TiO₂ interactions ............................................................................................. 29
2.3.6 Implications on the transport in porous media .................................................. 32
2.4 References ............................................................................................................... 35

CHAPTER 3 AGGREGATION OF n-TiO₂ IN THE PRESENCE OF HUMIC ACID (HA) AND 17β-ESTRADIOL (E2) UNDER HIGH IONIC STRENGTH CONDITIONS ...... 38
3.1 Introduction ............................................................................................................... 38
3.2 Methods and materials ............................................................................................. 40
3.2.1 Chemical reagents .............................................................................................. 40
3.2.2 Preparation of n-TiO₂ suspensions and aggregation experiments .................... 41
3.2.3 Measurement of particle size and zeta potential ............................................... 42
3.3 Results and discussion ............................................................................................. 42
3.3.1 Aggregation of n-TiO₂ in the presence of humic acid ........................................ 42
3.3.2 Aggregation of n-TiO₂ in high ionic strength conditions .................................... 45
3.3.3 Aggregation of n-TiO₂ in the presence of HA at high ionic strength conditions ......................................................................................................................... 49
3.3.4 Aggregation of n-TiO₂ in the presence 17β-estradiol (E2) in high ionic strength .......................................................................................................................................................................................................................................................................................................................... 52
3.3.5 Aggregation of n-TiO$_2$ in the presence of HA and 17β-estradiol (E2) in high ionic strength conditions ........................................................................................................ 55

3.4 References ....................................................................................................................... 59

CHAPTER 4 QSAR MODELS TO PREDICT n-TiO$_2$ STABILITY AS A FUNCTION OF OWC MOLECULAR DESCRIPTORS .......................................................................................................................... 60

4.1 Introduction ....................................................................................................................... 60

4.2 Material and Methods ..................................................................................................... 62

4.2.1 Chemical Reagents .................................................................................................... 62

4.2.2 Preparation of n-TiO$_2$ Samples with Organic Wastewater Contaminants (OWCs) ........................................................................................................................................................................ 65

4.2.3 Determination of n-TiO$_2$ aggregation rate and aggregation kinetics ............... 66

4.2.4 QSAR Models for Predicting Aggregation of n-TiO$_2$ by molecular descriptors of OWCs .................................................................................................................................................................................. 67

4.3 Results and discussion .................................................................................................... 69

4.3.1 Aggregation of n-TiO$_2$ in the presence of OWCs..................................................... 69

4.3.2 MLR QSAR models for the 5 mg/L n-TiO$_2$ + 80% of C$_w$ of OWCs ................. 75

4.3.2.1 MLR QSAR models using fitlm function .............................................................. 75

4.3.2.2 MLR QSAR models using stepwiselm function ..................................................... 82

4.3.3 MLR QSAR models for the 1 mg/L n-TiO$_2$ + 0.2 mg/L of C$_w$ of OWCs ....... 93

4.3.3.1 MLR QSAR models using fitlm function .............................................................. 93

4.3.3.2 MLR QSAR models using stepwiselm function ..................................................... 99
CHAPTER 5 DEPOSITION OF n-TiO$_2$ ON ROUGH SURFACES USING QUARTZ CRYSTAL MICROBALANCE WITH DISSIPATION MONITORING (QCM-D) .... 107

5.1 Introduction ............................................................................................................. 107

5.2 Material and Methods ............................................................................................ 109
  5.2.1 Preparation of n-TiO$_2$ suspensions ................................................................. 109
  5.2.2 Combined quartz crystal microbalance with dissipation (QCM-D) and spectroscopic ellipsometry (SE) Experiments ............................................................. 110
  5.2.3 Preparation of surfaces ...................................................................................... 112

5.3 Results .................................................................................................................... 117
  5.3.1 Deposition and release of n-TiO$_2$ on flat surfaces under favorable condition 117
  5.3.2 Deposition and release of n-TiO$_2$ on flat surfaces under unfavorable condition ......................................................................................................................... 120
  5.3.3 n-TiO$_2$ deposition onto rough surfaces ............................................................. 124
    5.3.3.1 Deposition under favorable condition ......................................................... 124
    5.3.3.2 Release under favorable condition ............................................................. 126
    5.3.3.3 Deposition under unfavorable condition ................................................... 128
  5.3.4 Deposition of n-TiO$_2$ rutile particles with and without humic acid .......... 134

5.4 References .............................................................................................................. 139

CHAPTER 6 CONCLUSIONS AND FUTURE RESEARCH WORKS ......................... 142

6.1 Conclusions ............................................................................................................ 142
6.1.1 Effect of 17β-estradiol (E2) on stability and mobility of TiO₂ rutile nanoparticles ................................................................. 143

6.1.2 Aggregation of n-TiO₂ in the presence of humic acid (HA) and 17β-estradiol (E2) under high ionic strength conditions ......................................................... 144

6.1.3 QSAR models to predict n-TiO₂ stability as a function of OWC molecular descriptors ........................................................................................................ 145

6.1.4 Deposition of n-TiO₂ on rough surfaces using quartz crystal microbalance with dissipation monitoring (QCM-D) .................................................................. 146

6.2 Recommendations for Future Works ................................................................. 146

6.2.1 Future works on the stability/mobility and QSAR model of n-TiO₂ in the presence of various OWCs as well as natural organic matter .................................... 147

6.2.2 Future works on the deposition and transport of n-TiO₂ using a QCM-D technique .......................................................................................................... 147

SUPPLEMENTARY MATERIAL OF CHAPTER 2 ..................................................... 148
List of Tables

Table 1. 1 Ranges of concentration of E2, Ibuprofen, and n-TiO₂ in WWTP effluents..... 1
Table 4. 1 Molecular descriptors of our target chemical compounds................................. 63
Table 4. 2 Structure of our target chemical compounds ............................................... 64
Table 4. 3 Calculation of responses at t = 0 hr for the 5 mg/L n-TiO₂ + 80% of C_w of OWCs............................................................... 77
Table 4. 4 Calculation of responses at t = 12 hr for the 5 mg/L n-TiO₂ + 80% of C_w of OWCs.................................................................................. 78
Table 4. 5 Estimated coefficients and p-values created by MLR using fitlm function at 0 hr and 12 hr ........................................................................................................... 79
Table 4. 6 Estimated coefficients by stepwiselm regression model for dD/dt at t = 0 hr. 83
Table 4. 7 Estimated coefficients by stepwiselm regression model for increase rate of diameter at t = 0 hr ........................................................................................................... 85
Table 4. 8 Estimated coefficients by stepwiselm regression model for k₁ at t = 0 hr....... 87
Table 4. 9 Estimated coefficients by stepwiselm regression model for dD/dt at t = 12 hr.......................... 90
Table 4. 10 Estimated coefficients by stepwiselm regression model for increase rate of diameter at t = 12 hr ........................................................................................................... 91
Table 4. 11 Estimated coefficients by stepwiselm regression model for k₂ at t = 12 hr... 92
Table 4. 12 Calculation of responses at t = 0 hr for the 1 mg/L n-TiO₂ + 0.2 mg/L of C_w of OWCs ................................................................................................................................. 94
Table 4. 13 Calculation of responses t = 12 hr for the 1 mg/L n-TiO₂ + 0.2 mg/L of C_w of OWCs........................................................................................................................................... 95
Table 4. 14 Estimated coefficients and p-values created by MLR using fitlm function at 0 hr and 12 hr .......................................................... 96
Table 4. 15 Estimated coefficients by stepwiselm regression model for dD/dt at t = 0 hr .......................................................................................................................................................................................... 100
Table 4. 16 Estimated coefficients by stepwiselm regression model for increase rate of diameter at t = 0 hr .......................................................................................................................................................................................... 101
Table 4. 17 Estimated coefficients by stepwiselm regression model for dD/dt at t = 12 hr .......................................................................................................................................................................................... 102
Table 4. 18 Estimated coefficients by stepwiselm regression model for increase rate of diameter at t = 12 hr .......................................................................................................................................................................................... 103
Table 5. 1 Matrix of QCM-D/SE experiments .......................................................................................................................................................................................... 116
Table S. 1 Cumulative undersize distribution, C(d) and mobility distance of aggregated n-TiO₂ according to C(d) at 12hr. .......................................................................................................................................................................................... 159
List of Figures

Figure 1. 1 Titanium dioxide nanoparticles (n-TiO$_2$). The picture is obtained from the website from product of company (http://www.nanoamor-europe.com/). ...................... 7

Figure 2. 1 FTIR Spectra band of adsorbed E2 (3 mg/L) on n-TiO$_2$ (30 mg/L) in 10 mM NaCl, DI water, and n-TiO$_2$ powder. (A) overall bands and (B) characteristic stretching bands due to the sorption of E2................................................................. 19

Figure 2. 2 Aggregation of n-TiO$_2$ (5 mg/L) at pH 7 with different E2 concentrations and under different ionic strength: (A) DI water, (B) NaCl of 10 mM, (C) NaCl of 20 mM, and (D) CaCl$_2$ of 2 mM. Data plots were triplicate measurements. ......................... 22

Figure 2. 3 Intensity-normalized particle size distributions of n-TiO$_2$ as a function of applied E2 concentrations after 12 hr: (A) DI water, (B) NaCl of 10 mM, (C) NaCl of 20 mM, and (D) CaCl$_2$ of 2 mM. .................................................................................................................. 24

Figure 2. 4 Zeta potential of n-TiO$_2$ with different IS condition in the presence of E2 at 0 hr .......................................................... 26

Figure 2. 5 DLVO + steric interaction-energy and DLVO interaction-energy between n-TiO$_2$ in the presence of 3 mg/L E2 at various ionic strength conditions ................. 31

Figure 2. 6 Estimated mobility distance of n-TiO$_2$ in porous media based on the clean bed filtration model assuming a typical groundwater flow velocity of 1m/d in a coarse sandy media with mean grain diameter of 0.713 mm and volumetric water content of 0.31. .... 34

Figure 3. 1 (A) Average hydrodynamic diameter and (B) average zeta potential of n-TiO$_2$ in DI water at pH 7 in the presence of HA and in the absence of HA. ................. 44
Figure 3. 2 Average hydrodynamic diameter (A and B) and average zeta potential (C and D) of n-TiO$_2$ in 20 mM CaCl$_2$, 2 mM CaCl$_2$, 100 mM NaCl, 20 mM NaCl, 10 mM NaCl, and DI water.................................................................................................................................................. 48

Figure 3. 3 (A) Average hydrodynamic diameter and (B) average zeta potential of n-TiO$_2$ in 100 mM NaCl, 20 mM CaCl$_2$, and DI water in the presence and absence of HA (5.7 mg/L).................................................................................................................................................. 51

Figure 3. 4 Average hydrodynamic diameter (A and B) and average zeta potential (C and D) of n-TiO$_2$ in 100 mM NaCl, 20 mM CaCl$_2$, 20 mM NaCl, 10 mM NaCl, 2 mM CaCl$_2$, and DI water in the presence of E$_2$ (3 mg/L).................................................................................................................................................. 54

Figure 3. 5 Average hydrodynamic diameter (A and B) and average zeta potential (C and D) of n-TiO$_2$ at 20 mM CaCl$_2$ and 100 mM NaCl in the presence of HA (5.7 mg/L) and E$_2$ (3 mg/L).................................................................................................................................................. 58

Figure 4. 1 Aggregation of n-TiO$_2$ with 80% of C$_w$ of OWCs in 2 mM CaCl$_2$ at pH 7... 72

Figure 4. 2 Zeta potential of n-TiO$_2$ with 80% of C$_w$ of OWCs in 2 mM CaCl$_2$ at pH 7 73

Figure 4. 3 (A) aggregation and (B) zeta potential of 1 mg/L n-TiO$_2$ with 0.2 mg/L of C$_w$ of OWCs in 2 mM CaCl$_2$ at pH 7 .................................................................................................................................................. 74

Figure 4. 4 Relationship between modeled response and observed response using coefficients by fitlm function at 0 hr................................................................. 80

Figure 4. 5 Relationship between modeled response and observed response using coefficients by fitlm function at 12 hr............................................................................... 81

Figure 4. 6 Relationship between modeled response and observed response for dD/dt using coefficients by stepwise function at 0 hr .......................................................... 84

Figure 4. 7 Relationship between modeled response and observed response for increase rate of diameter using coefficients by stepwiselm function at 0 hr ......................... 86
Figure 4. 8 Relationship between modeled response and observed response for $k_1$ using coefficients by stepwiselm function at 0 hr ................................................................. 88

Figure 4. 9 Relationship between modeled response and observed response for $dD/dt$ using coefficients by stepwiselm function at 12 hr ................................................................. 90

Figure 4. 10 Relationship between modeled response and observed response for increase rate of diameter using coefficients by stepwiselm function at 12 hr ................................. 91

Figure 4. 11 Relationship between modeled response and observed response for $k_2$ using coefficients by stepwiselm function at 12 hr ................................................................. 92

Figure 4. 12 Relationship between modeled response and observed response for $dD/dt$ (A) and increase rate in diameter (B) using coefficients by fitlm function at 0 hr ...................... 97

Figure 4. 13 Relationship between modeled response and observed response for $dD/dt$ (A) and increase rate in diameter (B) using coefficients by fitlm function at 12 hr ...................... 98

Figure 4. 14 Relationship between modeled response and observed response for $dD/dt$ using coefficients by stepwiselm function at 0 hr ................................................................. 100

Figure 4. 15 Relationship between modeled response and observed response for increase rate of diameter using coefficients by stepwiselm function at 0 hr ................................. 101

Figure 4. 16 Relationship between modeled response and observed response for $dD/dt$ using coefficients by stepwiselm function at 12 hr ................................................................. 102

Figure 4. 17 Relationship between modeled response and observed response for increase rate of diameter using coefficients by stepwiselm function at 12 hr ................................. 103

Figure 5. 1 (A) Flat surface: Al$_2$O$_3$ or SiO$_2$ ALD and (B) Roughness surface: Si-SCTF with Al$_2$O$_3$ or SiO$_2$ ALD on QCM gold crystal sensor with a schematic and SEM of SCTF by NOVA NANOSEM 450 ................................................................. 114

Figure 5. 2 Picture of QCM-D/SE experimental setup ................................................................. 115
Figure 5.3 QCM-D/SE measurements on flat $\text{Al}_2\text{O}_3$ ALD in DI water (Table 5.1 (a)): (A) Frequency and dissipation, (B) surface density of n-$\text{TiO}_2$ both QCM and SE calculation, and (C) mass fraction as a ratio of surface density [SE/QCM]... 121

Figure 5.4 QCM-D/SE measurements on flat $\text{Al}_2\text{O}_3$ ALD in 10 mM NaCl (Table 5.1 (c)): (A) Frequency and dissipation, (B) surface density of n-$\text{TiO}_2$ both QCM and SE calculation, and (C) mass fraction as a ratio of surface density [SE/QCM]... 122

Figure 5.5 QCM-D/SE measurements on flat $\text{SiO}_2$ ALD in DI water (Table 5.1 (g)): Frequency and dissipation shifts... 123

Figure 5.6 QCM-D/SE measurements (Table 5.1 (d)): (A) Frequency and dissipation, (B) surface density of n-$\text{TiO}_2$ both QCM and SE calculation, and (C) mass fraction as a ratio of surface density [SE/QCM]... 129

Figure 5.7 QCM-D/SE measurements (Table 5.1 (e)): (A) Frequency and dissipation, (B) surface density of n-$\text{TiO}_2$ both QCM and SE calculation, and (C) mass fraction as a ratio of surface density [SE/QCM]... 130

Figure 5.8 QCM-D/SE measurements (Table 5.1 (f)): (A) Frequency and dissipation, (B) surface density of n-$\text{TiO}_2$ both QCM and SE calculation, and (C) mass fraction as a ratio of surface density [SE/QCM]... 131

Figure 5.9 QCM-D/SE measurements for deposition and release behaviors during at each 1 hr in DI water: (A) Frequency and dissipation, (B) surface density of n-$\text{TiO}_2$ both QCM and SE calculation, and (C) mass fraction as a ratio of surface density [SE/QCM]... 132

Figure 5.10 QCM-D/SE measurements (Table 5.1 (h) and (i)): (A) Frequency and dissipation (DI water) and (B) Frequency and dissipation (100 mM NaCl)... 133
Figure 5. 11 QCM-D/SE measurements (Table 5.1 (b-1)): (A) Frequency and dissipation, (B) surface density of n-TiO₂ both QCM and SE calculation, and (C) mass fraction as a ratio of surface density [SE/QCM]. ................................................................. 136

Figure 5. 12 QCM-D/SE measurements (Table 5.1 (b-2)): (A) Frequency and dissipation, (B) surface density of n-TiO₂ both QCM and SE calculation, and (C) mass fraction as a ratio of surface density [SE/QCM].................................................................. 137

Figure S. 1 Sorption isotherms of 17β-estradiol (E₂) on n-TiO₂ (5 mg/L) suspension in 10 mM NaCl at pH 7 and 25°C after 2 hr (n=3).................................................................. 154

Figure S. 2 The particle diameter changing of n-TiO₂ (5mg/L) by various E₂ concentrations at pH 7 during 12 hr: (A) 20 mM NaCl and (B) 2 mM CaCl₂. Data plots were triplicate measurements. * means p < 0.05 on unequal variances. ...................... 155

Figure S. 3 Zeta potential of n-TiO₂ at pH 7 under different E₂ concentrations and under different ionic strength during 12 hr: (A) DI-water, (B) 10 mM NaCl, (C) 20 mM NaCl, and (D) 2 mM CaCl₂. Data plots were triplicate measurements................................. 156

Figure S. 4 DLVO interaction-energy for (A) DI water, (B)10 mM NaCl, (C) 20 mM NaCl, and (D) 2 mM CaCl₂......................................................................................... 157

Figure S. 5 FTIR Spectra band of pure E₂......................................................................................... 158
CHAPTER 1 PROJECT OVERVIEW AND OBJECTIVES

1.1 Project Overview

Organic wastewater contaminants (OWCs), such as steroid hormones and pharmaceuticals are released into receiving waters in municipal wastewater effluent at concentrations ranging from ng/L to µg/L because wastewater treatment plants can not completely remove these compounds, which have diverse physical and/or chemical properties [1-6]. In addition, due to increasing applications of nanotechnology in various products, the production of TiO₂ nanoparticles (n-TiO₂) is estimated to increase to 201,500 tons in 2015 [7]. As a result of this growth in n-TiO₂ production, recent studies have reported the occurrence and detection of nanoparticles in effluents from wastewater treatment plants and in receiving surface waters [8-11]. Municipal wastewater can contain inorganic contaminants such as TiO₂ nanoparticles (n-TiO₂) at concentrations ranging from 181 to 1233 µg/L in influent and wastewater effluents can contain titanium oxide nanoparticle (TiO₅) at concentrations ranging from < 2 to 20 µg/L as TiOₓ [9]. Engineered n-TiO₂ was directly discharged from façade paints by runoff into surface waters with measured concentrations in surface water as high as 600 µg/L as well as the sunscreens originated from external sources into lakes were detected with less than 1.7 µg/L during a year [10, 11].

Table 1. 1 Ranges of concentration of E2, Ibuprofen, and n-TiO₂ in WWTP effluents

<table>
<thead>
<tr>
<th>E2 [ng/L]</th>
<th>Pharmaceutical (i.g. Ibuprofen) [ng/L]</th>
<th>n-TiO₂ [µg/L]</th>
</tr>
</thead>
</table>
Exposure to both emerging organic contaminants and nanoparticles have caused adverse consequences in aquatic environments. For instance, steroid hormones such as estrogens can cause feminization of male fish even at low concentrations in aquatic systems [13-15]. In addition, nanoparticles (NPs) have been reported to negatively affect the growth and reproduction of aquatic organisms as well as the biomass, composition, and community diversities of soil microbes [16-18]. There is limited information about the toxicity results from combined exposure to both nanoparticles and organic wastewater contaminants. Interestingly, Zhu et al. (2011) demonstrated increased toxicity of a marine antifouling compound, tributyltin (TBT) when it was associated with n-TiO$_2$ compared to TBT alone.

Some pharmaceutical compounds have been shown to undergo rapid sorption onto sediments (with 30 min) [19] and some steroid estrogens such as estrone (E1), 17β-estradiol (E2), and 17α-ethinylestradiol (EE2) were shown to undergo sorption onto sediments within 1 hr [20]. Therefore, it is likely that OWCs discharged in WWTP effluents may be associated with colloidal particles which may play a key role on the fate and transport of these compounds in aquatic systems.

Surface modification of NPs altered by surrounding water environment conditions such as pH, ionic strength, natural organic matter (NOM), and other organic contaminants can affect the stability and transport of NPs in aquatic systems [21-30]. The stability is influenced with pH because the surface charge of NPs is dramatically changed at the point of zero charge (pH$_{pzc}$). Below the pH$_{pzc}$, NPs have positive charges, while NPs have negative charges at above pH$_{pzc}$ due to the surface modification of NPs with hydrogen (H$^+$) or hydroxide (OH$^-$) molecules in water. As pH approaches the pH$_{pzc}$, the
stability dramatically decreases corresponding with lager aggregation and settlement due to decreasing electrostatic repulsion between NPs [26, 31, 32]. Electrolytes such as a monovalent cation (e.g. Na\(^+\)) and divalent cation (e.g. Ca\(^{2+}\)) commonly found in aquatic systems could affect the stability of NPs according to Derjaguin-Landau-Verwey-Overbeek (DLVO) theory [26, 30, 31, 33]. Based on DLVO theory, there are two interaction energies between electrical double-layer repulsion energy and van der Waals attraction energy. The electrical double-layer repulsion energy is the function of Debye length \((1/\kappa)\) that is proportional to the valence charge or concentrations of electrolytes [34].

\[
\kappa = \sqrt{\frac{2000 N_A I e^2}{\varepsilon_r \varepsilon_0 k_B T}}
\]

where \(N_A\) is the Avogadro number, \(e\) is the elementary charge, \(I\) is the ionic strength of the electrolyte, \(\varepsilon_r\) is the relative dielectric constant of water, \(\varepsilon_0\) is the permittivity of vacuum, and \(k_B\) is the Boltzmann constant, and \(T\) is the absolute temperature (K). Therefore, ionic strength and ion valence could eliminate repulsion layer between NPs and lead to aggregation of NPs.

Dissolved organic contaminants such as natural organic matter (NOM) could affect the stability of NPs. Both humic acid and fulvic acid of NOM could modify the surface of NPs by adsorption which could increase the stability of NPs due to electrostatic or steric repulsive interactions between NPs [25, 28, 35]. Other dissolved organic compounds such as oxalic acid [29], citric acid [27], and carboxymethyl cellulose (CMC) [36] were also attributed to alter the stability of NPs due to the adsorption of organic compounds.
The stability of NPs described above suggests that water chemistry significantly may alter physicochemical properties of NPs, and as a result, affect the fate and transport of NPs in aquatic systems. In addition, it is estimated that different stability in various water matrixes has resulted in aggregation behavior and settlement of NPs by adsorption of organic contaminants.

The deposition of NPs is a key process in evaluating the environmental fate of NPs in the subsurface. Two processes, transport and attachment, are considered in the deposition process [37]. Transport of colloidal particles is dominated by Brownian motion for nanoparticles or by fluid motion and settling for micron-sized particles [37, 38]. Attachment of colloidal particles to the vicinity collector surface is governed by interaction forces between particles and collector surfaces such as electrical double layer and van der Waals interactions, hydration forces, hydrophobic, and steric interactions [34, 37]. It is useful to estimate for the deposition of NPs in porous media due to the surface charge on NPs and collector surfaces according to the Derjaguin-Landau-Verwey-Overbeek (DLVO) theory [22, 34]. Recently, quartz crystal microbalance with dissipation (QCM-D) has been investigated for the deposition of NPs and well demonstrated the interaction processes between NPs and QCM-D surfaces based on DLVO theory [22, 35, 39]. However, particle size, surface roughness of particles and collectors, and heterogeneous surface charge have influenced on the discrepancy between theoretical and observed deposition of colloid particles by DLVO theory [37].

The stability of NPs will be influenced by aquatic chemistry and changes in conditions such as pH and ionic strength could result in different fate, transport, and toxicity behaviors of NPs in environment. Therefore, it is necessary to evaluate the
interaction between NPs and various organic contaminants under different IS and pH conditions. Moreover, surface modification of NPs and collectors may affect the different deposition behaviors of NPs in aquatic systems, thus we should investigate the deposition processes after surface modification of NPs and collectors.

1.2 Project Goals and Objectives

The objectives of this dissertation were to provide a better understanding of the interaction between steroid hormones and/or pharmaceuticals and an engineered NP, TiO$_2$ (Figure 1.1), under natural environmental conditions. These objectives were investigated by how OWC-NP interactions would influence the stability of n-TiO$_2$. The specific objectives of this proposal are as follows

1. Evaluate the stability of n-TiO$_2$ as a function of 17β-estradiol (E2) concentration [1] under various ionic strength conditions. This objective was achieved by batch and kinetic sorption experiments, particle size and zeta potential measurements, and Fourier Transform Infrared (FTIR) analysis. In addition, mobility analysis was investigated using the clean bed filtration theory in porous media.

2. Evaluate the aggregation of n-TiO$_2$ in the presence of humic acid (HA) and 17β-estradiol (E2) under high ionic strength conditions simulating a landfill environment.

3. Develop QSAR models to predict n-TiO$_2$ stability as a function of OWC molecular properties. This objective was achieved by conducting additional aggregation experiments for a range of OWCs and applying statistical
approaches of multiple linear regressions (MLR) to predict the stability of n-
TiO$_2$ by adsorption of OWCs.

4. Investigate the deposition of n-TiO$_2$ on various collector surface conditions
such as surface roughness about 100 nm Si slanted columnar thin films (Si-
SCTFs) coated with Al$_2$O$_3$ or SiO$_2$ or flat surface coated with either Al$_2$O$_3$ or
SiO$_2$ that are general soil compounds. This objective was achieved by using
quartz crystal microbalance with dissipation and spectroscopic ellipsometry
(QCM-D/SE).
Figure 1. 1 Titanium dioxide nanoparticles (n-TiO₂). The picture is obtained from the website from product of company (http://www.nanoamor-europe.com/).
1.3 References


CHAPTER 2 EFFECT OF 17β-ESTRADIOL ON STABILITY AND MOBILITY OF TiO₂ RUTILE NANOPARTICLES

2.1. Introduction

Titanium dioxide nanoparticles (n-TiO₂) are one of the most widely used metal oxide nanomaterials in a variety of commercial products and have a projected production of 201,500 tons in 2015 [1]. n-TiO₂ are released into surface water as traditional wastewater treatment plants (WWTPs) can only partially remove n-TiO₂ [2-4]. Municipal wastewater also contains various organic wastewater contaminants (OWCs) such as pesticides, steroid hormones, pharmaceuticals, and industrial chemicals. Steroids hormones have also been detected in WWTP effluent at concentrations ranging from ng/L to µg/L [5-7]. As a result, it is highly possible that n-TiO₂ and OWCs may co-exist in receiving waters, yet there is little research documenting the influence of OWCs on n-TiO₂ fate.

The interaction of OWCs and nanoparticles is of importance as several studies have demonstrated the individual toxicity of both OWCs and n-TiO₂ to organisms. 17β-estradiol (E2), one of estrogenic compounds detected in WWTP effluent, has been identified to cause reproductive impacts to aquatic organism even at low concentrations (ng/L) [8-10]. n-TiO₂ have been reported to cause oxidative stress in brain microglia cells[11], inflammation in rats and mice[12], cytotoxicity and genomic instability towards cultured WIL2-NS human lymphoblastoid cells [13], and DNA damage to goldfish skin cells (GFSk-S1) [14]. One study has demonstrated enhanced toxicity of n-TiO₂ complexed with an organic compound. Previous study revealed that n-TiO₂ can sorb tributyltin (TBT), a marine antifouling compound, and the combined TBT-TiO₂ complex
demonstrated increased toxicity to abalone compared with TBT alone, as n-TiO$_2$ attached on the surface of abalone could deliver more TBT to organisms [15]. This potential for enhanced toxicity further motivates a need to understand the behavior of OWC/n-TiO$_2$ complexes.

Multiple studies have demonstrated that n-TiO$_2$ will exist as n-TiO$_2$ aggregates in water rather than as individual nanoscale particles [16-19]. Surface properties of n-TiO$_2$ aggregates have been reported to be sensitive to factors such as pH, ionic strength, and natural organic matter in the aqueous phase [16, 20-23]. Larger and rapid aggregation of n-TiO$_2$ was observed at the point of zero charge (pH$_{zpc}$) [16, 22, 24] and was also observed in high ionic strength solution as Na$^+$ or Ca$^{2+}$ cations can reduce zeta potential as well as reduce repulsive forces on the surface of n-TiO$_2$ [16, 17, 25]. In addition, uptake of organic acid such as oxalic acid and citric acid have been reported to alter the surface charges of n-TiO$_2$ [20, 21]. n-TiO$_2$ suspensions were found to be more stable in the presence of natural organic matter (NOM), due to steric repulsion created by the NOM sorbed on the surface [25-27]. Presumably, coexistence of OWCs and n-TiO$_2$ may also modify the surface properties of n-TiO$_2$, however, little is known about how interaction with OWCs would influence n-TiO$_2$ aggregates.

The stability of OWC/n-TiO$_2$ suspensions will affect their mobility in porous media. The size of n-TiO$_2$ will impact the relative contributions of diffusion, interception and sedimentation processes when they approach the porous medium surface. Nanoparticles with less negative surface potential tend to deposit more in the porous media due to more attractive interactions between nanoparticles and the porous medium surface. In addition, adsorption of surfactants, carboxymethyl cellulose (CMC), and
humic acid on n-TiO$_2$ was found to enhance the mobility of n-TiO$_2$ in saturated porous media due to increased repulsive forces [28-30]. However, little is known about the mobility of OWC/n-TiO$_2$ complexes in porous media.

In this study, we investigated the interaction of TiO$_2$ nanoparticles and E2. E2 was chosen as a representative OWC because it is routinely detected in WWTP effluents [8, 31]. A reductionist approach to water chemistry (i.e. a limited range of ionic strength and no NOM) was deliberately chosen, so that the basic interactions between E2 and n-TiO$_2$ could be elucidated. In this study, the concentration of n-TiO$_2$ is kept as 5 mg/l, which is significantly higher than the modeled n-TiO$_2$ concentration in treated wastewater (2.5 to 10.8 µg/l) and surface water (12-57 ng/l) [32]. It is recognized that a higher particle concentration implies higher aggregation kinetics due to higher collision frequency between nanoparticles, which, therefore requires longer time to reach an equilibrium suspension. This concentration, however, is on the lower end of concentrations used in the other n-TiO$_2$ fate and transport studies [33], where higher concentrations were used to overcome the experimental detection restrictions. On the other hand, concentrations of steroids hormones detected in wastewater effluent ranged from ng/L to µg/L [5-7], which are in the same order of magnitude as modeled n-TiO$_2$ concentration. Therefore, a range of E2 concentrations (i.e. 0, 0.1, 0.5, 1, and 3 mg/L) were chosen to study, which kept the mass ratio between E2 and n-TiO$_2$ relevant to environmental condition, although E2 concentrations were largely more elevated than actual environmental concentrations.

Batch sorption experiments were first conducted to measure both the equilibrium and kinetics of E2 sorption onto n-TiO$_2$ in DI water. FTIR analysis was performed to
elucidate the interactions between E2 and n-TiO₂. Aggregation of n-TiO₂ was measured in a range of E2 concentrations (i.e. 0, 0.1, 0.5, 1, and 3 mg/L) in various environmentally-relevant ionic strength conditions (i.e. DI water, 10mM NaCl, 20 mM NaCl, and 2 mM CaCl₂). Interaction energies between n-TiO₂ aggregates in the suspension were analyzed based on an extended Derjaguin, Landau, Verwey, and Overbeek (DLVO) theory. The implication on the mobility of n-TiO₂ in porous media was evaluated based on the clean bed filtration theory.

2.2 Materials and Methods

2.2.1 Chemical reagents

n-TiO₂ (rutile) was purchased from Nanostructured & Amorphous Material Inc. (Los Alamos, NM, USA) with average particle size of 10×40 nm, 98% purity, specific surface area of 160 m²/g, and coated with less than 5% SiO₂. The 17β-estradiol (purity ≥ 97%) and methanol (Optima grade) were purchased from Aldrich Corporation and Fisher Scientific, respectively. Sodium chloride and calcium chloride dihydrate were purchased from Fisher Scientific and Acros Organics, respectively.

2.2.2 Preparation of n-TiO₂ suspensions and aggregation experiments

n-TiO₂ suspension were prepared as described in French et al. (2009) and Chen et al. (2011) with modifications. All n-TiO₂ suspension were prepared in DI water (18.0 MΩ-cm, Barnstead Nanopure system). 1.5 mg of n-TiO₂ was placed in 300 ml DI water, 10 mM NaCl, 20 mM NaCl, or 2 mM CaCl₂ in a 500 ml glass flask resulting in a final n-TiO₂ concentration of 5 mg/L. Suspensions were immediately stirred using a magnetic stirplate for 30 s followed by sonication in an ultrasonic water bath (FS 60, 100 W, 42 kHz, Fisher Scientific, Pittsburg, PA) for 1 hr to obtain a homogeneous stock suspension.
After sonication, the flask was cooled to 25 °C and then 0.01 M NaOH was added to adjust pH to 7 ± 0.1. After pH adjustment, the n-TiO₂ suspension was aliquoted into 15 mL borosilicate glass tubes and spiked with a 300 mg/L 17β-estradiol (E2) stock suspension in methanol. The methanol concentration in the final solution was kept less than 1% (v/v) to minimize the potential for cosolvent effects on n-TiO₂ aggregation.

Aggregation experiments were conducted over a 12 hr period in triplicate. All samples contained a n-TiO₂ concentration of 5 mg/L in DI water and initial aqueous 17β-estradiol (E2) concentrations ranging from 0.1 mg/L to 3 mg/L. An aggregation experiment was also performed without E2 as a negative control. During the aggregation experiments, tubes were covered with aluminum foil to preventing the photodegradation or photocatalytic degradation of E2 by light and incubated in covered chamber at room temperature over 12 hr. Aggregation experiments were subsequently taken at 0 hr, 1 hr, 3 hr, 6 hr, and 12 hr to analyze aggregation sizes and zeta potential. To make sure a representative sample, n-TiO₂ suspension was vigorously shaken for seconds before taking 3 mL aliquot for particle size and zeta potential analysis.

2.2.3 Measurements of zeta potential and particle size

The zeta potentials of n-TiO₂ in the suspension were determined under various solution chemistries using a ZetaPALS zeta potential analyzer (Brookhaven Instruments Corporation, Holtsville, NY). ZetaPALS utilizes phase analysis light scattering (PALS) technique, an extension of electrophoretic light scattering, to measure the electrophoretic mobility, which was then converted to zeta potential base on a Smoluchowski equation. The particle size and distribution were measured using a 90Plus Particle Size Analyser (Brookhaven Instruments Corporation, Holtsville, NY) based on a dynamic light
scattering (DLS) principle. Each measurement was repeated using at least three different samples.

2.2.4 Sorption isotherms

The sorption isotherms for E2 on n-TiO$_2$ were conducted with initial aqueous E2 concentrations ranging from 0.05 mg/L to 2 mg/L. n-TiO$_2$ suspensions were prepared in DI-water at pH 7 as described above. Preliminary kinetic experiments showed that E2 adsorption onto n-TiO$_2$ reached equilibrium in 24 hr, so sorption isotherm experiments were conducted with a 24 hr equilibration time. Each E2 concentration was evaluated in duplicate and tubes were incubated on a rotary shaker at 150 rpm and 25°C. All samples were covered with aluminum foil to minimize E2 degradation by photocatalysis. Samples were centrifuged at 11,000 rpm for 40 min to separate the solid phase and aqueous phase [34]. The supernatant was scanned using a UV-Vis spectrophotometer at a wavelength of 301 nm to ensure that all the n-TiO$_2$ in the suspension has been settled by centrifugation. E2 in the aqueous phase was extracted by solid phase extraction (SPE) and analyzed by GC-MS as described in the Supplementary Material. A Freundlich model was used to describe the isotherms.

2.2.5 FTIR spectrometer analysis

Thermo Avatar 360 FTIR (Fourier Transform Infrared) Spectrometer, which is equipped with ISR ATR assembly (diamond crystal) and a routine-use with mid-IR scan ranges (650 - 4000 cm$^{-1}$), 2 cm$^{-1}$ resolution, and 32 scans, were used to measure the specific bands that could indicate the interaction between E2 and n-TiO$_2$ surfaces. 30 mg/L n-TiO$_2$ suspension in various IS conditions spiked with 3 mg/L E2 was used in FTIR analysis. Samples were centrifuged at a rate of 1500 rpm for 40 min to separate E2
– n-TiO₂ solid phases with aqueous phases. Then, only water was removed from glass bottles using glass pipette and E2 – n-TiO₂ solid phases were dried overnight at around 50 °C in dark oven chamber until residual water was completely removed. Finally, dried solids were carefully collected and measured by FTIR instrument at room temperature.

2.3 Results and discussion

2.3.1 Sorption of 17β-estradiol to n-TiO₂

A sorption isotherm of E₂ on n-TiO₂ in 10mM NaCl at pH 7 is presented in the Supplementary Material (Figure S.1). Using a Freundlich sorption model, we determined the sorption constant (1/n) and sorption coefficient (log Kᵢ) was 1.22 and 2.27 (R² = 0.985), respectively. Previous studies of E₂ sorption onto sediment or soil minerals have reported 1/n values in the range of 0.67 to 2.00 for and log Kᵢ values in the range of 0.004 to 6.67 for [35, 36]. The variability of previously reported soil log Kᵢ values could be due to different properties of soils used in these studies. Although the log Kᵢ value obtained in our study is within the literature reported range, it is difficult to make meaningful comparison. In our results, 1/n is slightly higher than 1, which indicates that E₂ has high affinity for n-TiO₂.

To further confirm the sorption of E₂ on n-TiO₂, FTIR analysis was performed for pure n-TiO₂ power, n-TiO₂ suspension in DI water, and n-TiO₂ in 10 mM NaCl solution with 3mg/l E₂. As shown in Figure 2.1 (A), all of the samples have an -OH stretching absorbance at 3390 cm⁻¹ from surface TiOH. In all the data, TiO and Ti-O-Ti maximum absorb from 1090-1060 cm⁻¹. For the 10 mM NaCl sample, there is a stretching mode at 1650 cm⁻¹. In this sample are stronger absorbances at 1020-1100 and 950-880 cm⁻¹. Absorption changes with ionic strength and cation and can be explained as a TiOM
stretching, where $M$ represents sodium ($\text{Na}^+$). The sorption of E2 on the surface of TiO$_2$ shows three characteristic stretching bands from CH$_2$, CH$_2$O and CH$_2$OH vibration at 1274, 1203, and 1153 cm$^{-1}$ (Figure 2.1 (B)). These are broader and shifted compared to the absorption of E2. A FTIR scan of pure E2 is provided in the supporting information (Figure S.5).
Figure 2. FTIR Spectra band of adsorbed E2 (3 mg/L) on n-TiO$_2$ (30 mg/L) in 10 mM NaCl, DI water, and n-TiO$_2$ powder. (A) overall bands and (B) characteristic stretching bands due to the sorption of E2.
2.3.2 Aggregation of n-TiO$_2$ in the presence of E2

Aggregation studies of n-TiO$_2$ were conducted in the presence and absence of E2 as a function of ionic strength (Figure 2.2). The average-hydrodynamic diameter of n-TiO$_2$ aggregates in DI water was approximately 190 nm when TiO$_2$ stock suspension was sonicated for 1 hr. This result was in a good agreement with previous studies, which reported n-TiO$_2$ aggregates mean hydrodynamic diameters of 150 nm or 194 nm in DI water [18, 27]. Figure 2.2 shows the influence of E2 on average n-TiO$_2$ hydrodynamic diameter as a function of ionic strength at pH 7 over a 12 hr period. In the absence of E2, the average hydrodynamic diameters of n-TiO$_2$ were approximately 200 nm, 280 nm, 400 nm, and 800 nm in DI-water, 10 mM NaCl, 20 mM NaCl, and 6 mM CaCl$_2$, respectively.

The impacts of E2 on n-TiO$_2$ aggregation under different electrolyte solutions were also observed. In DI water and 10 mM NaCl, E2 concentrations up to 3 mg/L resulted in no significant changes in the average hydrodynamic diameter of n-TiO$_2$ (Figure 2.2 (A) and (B)). In contrast, we observed an increase of n-TiO$_2$ average hydrodynamic diameters in 20 mM NaCl and 2 mM CaCl$_2$ (Figure 2.2 (C) and (D)). In 20 mM NaCl, the average hydrodynamic diameter increased from 330 nm in the absence of E2 to 750 nm at 3 mg/L E2 over 12 hr. In 2 mM CaCl$_2$, the average hydrodynamic diameters increased from 730 nm in the absence of E2 to 2160 nm at 3 mg/L E2 (Figure 2.2 (C) and (D)). Higher hydrodynamic diameter of n-TiO$_2$ in the presence of divalent cation (Ca$^{2+}$) is consistent with previous studies of n-TiO$_2$ aggregation [16, 17].

The statistical significance of E2 on n-TiO$_2$ aggregation was evaluated using one-tailed student’s t-test assuming unequal variance ($\alpha < 0.05$) (Details in Supplementary Material Figure S.2). For 20 mM NaCl and 2 mM CaCl$_2$, the presence of E2 generally
resulted in statistically significant changes in the average hydrodynamic diameter of n-TiO$_2$ when compared to controls with no E2. In contrast, the presence of E2 in the DI water and 10 mM NaCl did not lead to statistically significant differences in the average hydrodynamic diameters of n-TiO$_2$. 
Figure 2. Aggregation of n-TiO₂ (5 mg/L) at pH 7 with different E2 concentrations and under different ionic strength: (A) DI water, (B) NaCl of 10 mM, (C) NaCl of 20 mM, and (D) CaCl₂ of 2 mM. Data plots were triplicate measurements.
2.3.3 Particle size distributions in the presence of E2

To gain additional insight into the aggregation behavior of n-TiO$_2$ due to the presence of E2, we investigated the representative intensity-normalized particle size distribution obtained from DLS measurements after 12 hr as shown in Figure 2.3, which shows the relative amount of intensity at each size. In the absence of E2, the intensity of particle distributions are generally sharp and narrow, with wider peaks observed for high ionic strength conditions. The 5$^{\text{th}}$ through 95$^{\text{th}}$ percentile of particle size distribution (Supplementary Material Table S.1) ranged from 104 nm to 366 nm and from 365 nm to 1578 nm in DI water and 2 mM CaCl$_2$, respectively.

The presence of E2 in the suspension not only led to an increased average diameter of aggregates (Figure 2.3), but also to a wider distribution of n-TiO$_2$ aggregate size. This effect is obvious under higher ionic strength conditions and in the CaCl$_2$ solution. The particle size distribution in the DI water (Figure 2.3 (A)) and 10 mM NaCl (Figure 2.3 (B)) remained very sharp and symmetric in the range of E2 concentration investigated. In comparison, the particle distribution in 20 mM NaCl and 2 mM CaCl$_2$ skewed towards larger particle sizes. In 2 mM CaCl$_2$ solution, the 5$^{\text{th}}$ through 95$^{\text{th}}$ percentile of particle size distribution ranged from 881 nm to 4925 nm when E2 concentration increased to 3 mg/L (Supplementary Material Table S1). Recent studies have also reported increased aggregation distributions of n-TiO$_2$ after adsorption of organic acids [20, 21]. Although no settlement was visually observed, it is possible that certain concentrations of E2 may destabilize n-TiO$_2$ suspensions.
Figure 2. 3 Intensity-normalized particle size distributions of n-TiO$_2$ as a function of applied E2 concentrations after 12 hr: (A) DI water, (B) NaCl of 10 mM, (C) NaCl of 20 mM, and (D) CaCl$_2$ of 2 mM.
2.3.4 Zeta potentials in the presence of E2

As seen in Figure 2.4, zeta potential of n-TiO₂ becomes less negative with increasing ionic strength, due to the compressing of the electrostatic double layer. Initial zeta potential values are the closest to neutral in 2 mM CaCl₂, which can be attributed to the strong screening effect of Ca²⁺ ions and possible specific adsorption of Ca²⁺ ions [18, 25]. This finding is consistent with our observation of larger n-TiO₂ aggregate size in higher ionic strength and CaCl₂ solution. Zeta potential values remained relatively stable over a 12 hr period in DI water, 10 mM and 20mM NaCl solutions. But some increases of zeta potential (from -6.67 mV to 1.03 mV) were observed in 2 mM CaCl₂ at a concentration of 0.5 mg/L E2 (Supplementary Material Figure S.3).
Figure 2. 4 Zeta potential of n-TiO$_2$ with different IS condition in the presence of E2 at 0 hr.
Figure 2.4 also illustrates the impacts of E2 on the zeta potential of n-TiO₂ under different solution chemistry. In DI water, except at an E2 concentration of 0.1 mg/L, zeta potential of n-TiO₂ remained around -30 mV (±0.7) for E2 concentrations ranging from 0 to 3 mg/L. The minimal impact of E2 on the zeta potential of n-TiO₂ in DI water is consistent with the particle size distributions reported in Figure 2.3 (A), which are almost identical in the range of E2 concentration investigated. In other ionic strength conditions, E2 concentration increase will first result in more negative n-TiO₂ surface potentials. After reaching a minimum zeta potential, increasing E2 concentrations will lead to an increase of n-TiO₂ zeta potential. Such a trend appears true for all suspension investigated, including 10 mM NaCl, 20 mM NaCl, and 2 mM CaCl₂ solution.

Modification of n-TiO₂ zeta potential was previously reported for fulvic acid [26], citric acid [21], and humic acid [25, 28]. More negative electrophoretic mobilities of n-TiO₂ was observed in the presence of humic acid and fulvic acid [25, 26, 28]. At pH 6.0, the adsorption of citric acid on n-TiO₂ surfaces changed the zeta potential of n-TiO₂ from -33 mV to -45 mV [21]. The difference in the modification of n-TiO₂ zeta potential between these studies may be attributed to the different molecular structures and surface charges of these chemicals. E2 has two hydroxyl groups connected with four carbon rings. Experimental charge density study indicated that the phenolic hydroxyl group and the aromatic ring showed negative charges [37], while the middle nonpolar carbon rings are largely zero or weakly positively charged. Previous work has indicated that the nonpolar portion of the E2 molecules can bind to the uncharged surface hydroxyl groups on the mineral surfaces [38]. While zeta potential measurements of n-TiO₂ indicated an overall negatively charged surface, multiple researches have reported the surface charge
heterogeneity of nanomaterial aggregates in water [16, 39, 40]. Therefore, uncharged hydroxyle groups may exist on the surfaces of n-TiO$_2$ aggregate.

At a lower E2 concentration, it is possible that nonpolar portion of the E2 will preferentially bind onto the uncharged hydroxyl groups on the n-TiO$_2$ surface, but limit to a monolayer coverage. Orientation of the adsorbed E2 monolayer will allow the slightly negatively charged polar portion of the E2 directly contribute to the observed slightly more negative n-TiO$_2$ zeta potential. At a higher E2 concentration, multiple layers of E2 could be adsorbed onto the surface of n-TiO$_2$. This is partially supported by our estimation of the thickness of E2 layer sorbed on the surface of n-TiO$_2$ in 10 mM NaCl solution. As detailed in the supplemental material, our estimation based on the sorption data and molecular size of E2 indicated that multilayer of E2 molecules were sorbed on the surface of n-TiO2 when E2 concentration is higher than 1 mg/L. It is possible that the orientation of E2 adsorbed in multiplayer adsorption is different from monolayer adsorption on the surface of n-TiO$_2$, such that multilayer adsorption tends to hide or screen their weak negative charge. Such effect could be even more facilitated by the presence of the salt required in the system for multilayer adsorption, as counter ion adsorption tends to neutralize the surface charge. Therefore, additional sorption of E2 resulted in the shift of zeta potential from more negative to less negative. According to the DLVO theory, less negative value of zeta potential on the surface of n-TiO$_2$ will lead to a reduced energy barrier. Therefore, more aggregation will occur, and larger particle size will be observed.
2.3.5 n-TiO\textsubscript{2} interactions

Total interaction forces between n-TiO\textsubscript{2} based on the DLVO theory was calculated and details of the equations used for the DLVO interaction calculations are provided in the Supplementary Material (Figure S.4). According to these calculations, n-TiO\textsubscript{2} will be the most stable in DI water, where a wide repulsive energy barrier between n-TiO\textsubscript{2} exists. A sizable energy barrier (< 40 k\textsubscript{B}T) also exists in 10 mM NaCl, and only the particles with sufficient energy to overcome this barrier will form larger aggregates. In 20 mM NaCl and 2 mM CaCl\textsubscript{2}, except a small energy barrier (< 10 k\textsubscript{B}T) in 20 mM NaCl at an E2 concentration of 0.5 mg/L. DLVO calculation indicated a net attractive energy between n-TiO\textsubscript{2} particles (Figure S.4). However, no evidence of destabilization was visually observed in any these conditions.

Previous studies have reported that steric effects due to the adsorption of fulvic acid [26], citric acid [21], and humic acid [28] could enhance the stability of n-TiO\textsubscript{2} suspension. Therefore, we investigated the role of steric repulsion in n-TiO\textsubscript{2} – E2 system by incorporating a steric repulsion energy into the classical DLVO interaction energy curve. Based on the equilibrium sorption data and the molecular size of E2, the thickness of the E2 layers were estimated as 0.03, 0.19, 0.42, and 1.48 nm, for E2 concentrations of 0.1, 0.5, 1.0, and 3.0 mg/L, respectively. Based on the estimated thickness, the steric repulsion can be calculated using the Alexander-de Genne equations, as detailed in Supplementary Material.

As illustrated in Figure 2.5, incorporation of steric interaction revealed the existence of a significant energy barrier at a distance close to the surfaces for 10 mM NaCl, 20 mM NaCl, and 2 mM CaCl\textsubscript{2}, which is consistent with observed stable
suspension under these conditions. Further, the size of energy barrier is decreasing with
the increase of ionic strength and the presence of Ca\(^{2+}\), which again qualitatively agrees
with observed higher average particle size and broader particle size distributions,
indicating increased aggregation under such conditions.
Figure 2. 5 DLVO + steric interaction-energy and DLVO interaction-energy between n-TiO$_2$ in the presence of 3 mg/L E2 at various ionic strength conditions.
2.3.6 Implications on the transport in porous media

Under a range of environmentally relevant water chemistry conditions, our data indicated that E2 may quickly adsorb onto n-TiO\(_2\) surfaces, modify its surface charge and particle size distributions, and result in stable suspensions. We further explore the implication of these observations on the transport of n-TiO\(_2\) in porous media. Assuming a typical groundwater flow velocity of 1 m/d in a coarse sandy media with mean grain diameter of 0.713 mm and volumetric water content of 0.31, a mobility distance \((L)\), which describes the distance over which 99% of n-TiO\(_2\) will be removed within the porous media, can be estimated based on the clean-bed filtration (CFT) model:

\[
L = -\frac{2d_c}{3(1-\theta_w)\eta_o\alpha} \ln 0.01 \quad (1)
\]

Here, \(d_c\) (mm) is the average grain size of porous media; \(\theta_w\) (%) is the volumetric water content; \(\eta_o\) is the single-collector contact efficiency, defined as the frequency of particle collisions with grain surfaces, which was estimated based on a correlation developed by Tufenkji and Elimelech [41]. \(\alpha\) is the attachment efficiency factor, representing the fraction of the collision that lead to successful deposition, which was estimated based on a correlation developed by Elimelech [42].

Our calculations indicated that ionic strength conditions had a predominant effect on the estimated mobility distance of n-TiO\(_2\) (Supplementary Material Table S1). Estimated mobility distances for n-TiO\(_2\) with mean particle diameter (50\(^{th}\) percentile) ranged from 8.0 to 12.0 m, 0.06 to 0.08 m, 0.06 to 0.11 m, and 0.11 to 0.21 m in the DI water, 10 mM NaCl, 20 mM NaCl, and 2 mM CaCl\(_2\), respectively, for the range of E2 concentration investigated (Supplementary Material Table S1). The significantly higher
mobility distance in the DI water was due to much lower $\alpha$ values of about 0.004, comparing with $\alpha$ values in the range of 0.78 to 0.95 for 10 mM NaCl, 0.98 to 1.0 for 20 mM NaCl, and 1.0 for 2 mM CaCl$_2$ solutions, which are consistent with a recent study on the deposition of n-TiO$_2$ onto silica oxide surfaces [43]. A trend of increasing mobility distance in the order of 10 mM NaCl > 20 mM NaCl > 2 mM CaCl$_2$ can be attributed to an increase of in the average n-TiO$_2$ particle diameter. In the particle size range studied here (i.e. several hundred nanometers), a larger particle diameter will lead to a reduced diffusion process, therefore smaller mobility. Comparing with the influence of ionic strength, the impact of E2 on the mobility distance of n-TiO$_2$ is minimal. As illustrated in Figure 2.6, a slight decrease of the mobility distance with the increase of E2 concentration was estimated in DI water and 10 mM NaCl. In 20 mM NaCl and 2 mM CaCl$_2$, the presence of E2 will slightly increase the mobility of n-TiO$_2$ in porous media. It should be noted that the correlation to estimate $\alpha$ did not directly incorporate the possible influence of steric repulsion on transport, although it did incorporate the zeta potential change of n-TiO$_2$ to estimate the mobility of n-TiO$_2$ due to the presence of E2, thus, the actual mobility distances may be underestimated.
Figure 2. Estimated mobility distance of n-TiO$_2$ in porous media based on the clean bed filtration model assuming a typical groundwater flow velocity of 1m/d in a coarse sandy media with mean grain diameter of 0.713 mm and volumetric water content of 0.31.
2.4 References


CHAPTER 3 AGGREGATION OF n-TiO₂ IN THE PRESENCE OF HUMIC ACID (HA) AND 17β-ESTRADIOL (E2) UNDER HIGH IONIC STRENGTH CONDITIONS

3.1 Introduction

The stability of TiO₂ nanoparticles (n-TiO₂) is strongly influenced by surrounding water chemistry such as pH, ionic strength (IS), and the presence of organic compounds (e.g. natural organic matter) [1-5]. At fixed pH, the influence of ionic strength on the stability of n-TiO₂ can be explained based on the Debye length (1/κ). The inverse of Debye length is proportional to the ionic strength and the valence charge of the electrolyte [6]:

\[ \kappa = \frac{2000N_A I e^2}{\varepsilon_r \varepsilon_0 k_B T} \]

where \( N_A \) is the Avogadro number, \( e \) is the elementary charge, \( I \) is the ionic strength of the electrolyte, \( \varepsilon_r \) is the relative dielectric constant of water, \( \varepsilon_0 \) is the permittivity of vacuum, and \( k_B \) is the Boltzmann constant, and \( T \) is the absolute temperature (K).

Increasing IS has been shown to result in aggregation of n-TiO₂ due to compressing the diffuse double layer and decreasing zeta potential, which eliminate repulsive interactions [1, 2]. Divalent cations such as Ca\(^{2+}\) have been shown to more effectively decrease zeta potential than monovalent cations such as Na\(^{+}\) at a given pH, resulting in an increase in aggregation size of n-TiO₂ aggregates [1, 7].

Natural organic matter (NOM) commonly occurs in aquatic environments, so colloidal stability can be strongly influenced by adsorption of NOM onto nanoparticles.
(NPs), an effect that is well documented in several previous studies [4, 8, 9]. The adsorption of NOM on NPs can modify the surface characteristics of NPs such as surface charge, reactivity, and aggregation behavior [4, 9, 10]. Domingos et al. (2009) reported decreased n-TiO$_2$ aggregate diameters in the presence of fulvic acid ($\geq$ 1 mg/L) due to steric repulsion of fulvic acid on the surface of n-TiO$_2$. However, they observed the aggregation of n-TiO$_2$ at low fulvic acid concentration ($\leq$1 mg/L) at pH $>$ pH$_{zpc}$ because of bridging effect of fulvic acids. In addition, they reported increased stability was observed at high ionic strength conditions due to high adsorption of fulvic acid, which resulted in high steric repulsion. In low ionic strength conditions, fulvic acid led to increasing rigidity that resulted in bridging flocculation. Similarly, Thio et al. (2011) reported that the presence of salt would enhance the adsorption of humic acid on the surface of n-TiO$_2$ increasing the steric repulsion that resulted in increasing the stability of n-TiO$_2$ [8].

In addition, transport and deposition of nanoparticles were remarkably influenced when NOM or ions were present in aqueous solution. Transport of nanoparticles decreased due to enhanced deposition with increasing ionic strength as electrostatic repulsion energy barriers were reduced according to DLVO theory [5, 9, 11]. In contrast, in the presence of NOM or surfactants, transport of nanoparticles increased and deposition of nanoparticles decreased due to increased steric repulsion [5, 9].

Landfill leachate is an environment where high NOM and ionic strength are observed. Huo et al. (2008) reported NOM concentrations in leachate samples that ranged 80 - 632 mg/L (Humic acid) and 389 - 4420 mg/L (Fulvic acid), respectively [12]. Christensen et al. (2001) also reported high concentration of organic matter ranging from
30 to 29,000 mg as TOC/L as well as high concentrations of inorganic compounds such as sodium and calcium ranging from 70 to 7,700 mg/L and from 10 to 7,200 mg/L in landfill leachate, respectively [13]. The observed pH values of landfill leachate ranged from 4.5 to 9.4 [12-14]. It is necessary to evaluate nanoparticle aggregation in high organic matter and ionic strength concentrations as stability of nanoparticles can be strongly influenced by conditions such as pH, NOM, and ionic strength.

The objective of this study was to examine the aggregation of n-TiO$_2$ in the presence of humic acid under high ionic strength conditions simulating a landfill environment at fixed pH. Subsequently, we evaluated the aggregation of n-TiO$_2$ in the presence of 17β-estradiol as a representative organic contaminants under the same humic acid and ionic strength conditions. Studies have documented the presence of emerging contaminants, including 17β-estradiol in landfills.

3.2 Methods and materials

3.2.1 Chemical reagents

n-TiO$_2$ (rutile) was purchased from Nanostructured & Amorphous Material Inc. (Los Alamos, NM, USA) with average particle size of 10×40 nm, 98% purity, specific surface area of 160 m$^2$/g, and coated with less than 5% SiO$_2$. The 17β-estradiol (purity ≥ 97%), methanol (Optima grade), and humic acids were purchased from Aldrich Corporation and Fisher Scientific, respectively. Sodium chloride and calcium chloride dihydrate were purchased from Fisher Scientific and Acros Organics, respectively.
3.2.2 Preparation of n-TiO$_2$ suspensions and aggregation experiments

n-TiO$_2$ solutions were prepared as described in French et al. (2009) and Chen et al. (2011) with modifications. All n-TiO$_2$ solutions were prepared in DI water (18.0 MΩ-cm, Barnstead Nanopure system). 1.5 mg of n-TiO$_2$ was placed in 300 ml 100mM NaCl or 20mM CaCl$_2$ in a 500 ml glass flask resulting in a final n-TiO$_2$ concentration of 5 mg/L. Suspensions were immediately stirred using a magnetic stirplate for 30 s followed by sonication in an ultrasonic water bath (FS 60, 100 W, 42 kHz, Fisher Scientific, Pittsburg, PA) for 1 hr to obtain a homogeneous stock solution. After sonication, the flask was cooled to 25 °C and then 0.01 M NaOH was added to adjust pH to 7 ± 0.1. After pH adjustment, the stock solution of dissolved HA with 1000 mg/L in DI water was spiked into n-TiO$_2$ suspensions to make 5.7 mg/L HA in n-TiO$_2$ suspension at the final concentration, then the solution was vigorously mixed in magnetic stir for 30 sec. As well, 300 mg/L 17β-estradiol (E2) stock solution in methanol was spiked into n-TiO$_2$/HA solution that was kept less than 1% (v/v) to minimize the potential for cosolvent effects on n-TiO$_2$ aggregation. The final E2 concentration in n-TiO$_2$/HA suspension was 3 mg/L.

Aggregation experiments were conducted over a 12 hr period in triplicate. During the aggregation experiments, tubes were covered with aluminum foil to preventing the photodegradation or photocatalytic degradation of E2 by light and incubated at room temperature over 12 hr. Aggregation experiments were subsequently taken at 0 hr, 1 hr, 3 hr, 6 hr, and 12 hr to analyze aggregation sizes and zeta potential. To make sure a representative sample, n-TiO$_2$ solution was vigorously shaken for seconds before taking 3 mL aliquot from n-TiO$_2$/HA/E2 suspension to measure the particle size and zeta potential.
3.2.3 Measurement of particle size and zeta potential

The zeta potentials of n-TiO$_2$ in the suspension were determined under various solution chemistries using a ZetaPALS zeta potential analyzer (Brookhaven Instruments Corporation, Holtsville, NY). ZetaPALS utilizes phase analysis light scattering (PALS) technique, an extension of electrophoretic light scattering, to measure the electrophoretic mobility, which was then converted to zeta potential based on a Smoluchowski equation. The particle size and distribution were measured using a 90Plus Particle Size Analyzer (Brookhaven Instruments Corporation, Holtsville, NY) based on a dynamic light scattering (DLS) principle. Each measurement was repeated using at least three different samples.

3.3 Results and discussion

3.3.1 Aggregation of n-TiO$_2$ in the presence of humic acid

Figure 3.1 shows the change in hydrodynamic radius and zeta potential for n-TiO$_2$ in DI water in the presence and absence of humic acid (HA). At pH 7, the average hydrodynamic diameter and zeta potential of 5 mg/L n-TiO$_2$ was 195 nm and -30 mV, respectively, in the absence of HA. When HA was added at a concentration of either 0.57 or 5.7 mg/L, the initial radius and zeta potential was 175 nm and 194 nm and -31.3 mV and -27.57 mV, respectively. At 12 hrs, the average diameter of n-TiO$_2$/HA was 176.7 nm (0.57 mg/L) and 193.2 nm (5.7 mg/L), respectively. At both 0 and 12 hr, the hydrodynamic diameter of n-TiO$_2$ in the presence of HA (5.7 mg/L) was not significantly different when compared to the hydrodynamic diameter in the absence of HA (p-values 0.43 and 0.30, respectively), whereas significant differences were observed at lower HA concentration (0.57 mg/L). Similarly, Domingos et al. (2009) also reported a decreased
hydrodynamic diameter for n-TiO$_2$ (anatase, final concentration of 1 mg/L) at low fulvic acid (1 mg/L) compared with higher fulvic acid (> 1mg/L). Higher fulvic acid concentrations were attributed increased n-TiO$_2$ flocculation [4].

There was little change in zeta potential over 12 hours. At both HA concentrations, the zeta potential in Figure 3.1(B) indicated that the n-TiO$_2$ solution was stable due to high electrostatic repulsion. In addition, recent studies reported the zeta potential of n-TiO$_2$ was not changed with increasing HA or DOC concentrations but HA and DOC adsorbed on n-TiO$_2$ increased the stability of n-TiO$_2$ by inducing steric repulsion [7, 15]. Change of zeta potential by HA or DOC was observed at pH < pH$_{pzc}$ where the positive surface of n-TiO$_2$ could be modified with adsorption of HA or DOC, which have negative surface charges [7]. In our study, at pH 7 (> pH$_{pzc}$ = 4.2) both n-TiO$_2$ and HA are negatively charged which may limit the adsorption of HA onto n-TiO$_2$ due to high electrostatic repulsion between HA molecules and n-TiO$_2$ [9, 15]. Slightly more negative zeta potential was observed in the absence of HA than in the presence of HA at low ionic strength condition and pH 7. Zeta potential is not likely sensitive to adsorption of HA under unfavorable conditions [9].
Figure 3. 1 (A) Average hydrodynamic diameter and (B) average zeta potential of n-TiO$_2$ in DI water at pH 7 in the presence of HA and in the absence of HA.
3.3.2 Aggregation of n-TiO$_2$ in high ionic strength conditions

In Figure 3.2, differences in aggregation of n-TiO$_2$ was observed with various IS solutions under different dominant presence of cations. We investigated n-TiO$_2$ aggregation with Na$^+$ as the dominant cation at ionic strengths (IS) of 0.01, 0.02, and 0.1 M as well as with Ca$^{2+}$ as the dominant cation at IS of 0.006 and 0.06 M. Initially, with the Na$^+$ solutions, the hydrodynamic diameter was 193.2 nm (DI water), 303.2 nm (10 mM NaCl), and 766.5 nm (100 mM NaCl). With Ca$^{2+}$, hydrodynamic diameters were 874.2 nm (2 mM CaCl$_2$), and 925.7 nm (20 mM CaCl$_2$). The p-value was used to evaluate statistical significance of n-TiO$_2$ aggregation depending on the ionic strength. In all conditions, p-value showed significant changes in aggregation (p < 0.05). At times > 0 hr, minimal changes in hydrodynamic diameter were observed over 12 hr experimental period. The average zeta potential of n-TiO$_2$ was approximately -32 mV, -13 mV, and 5.7 mV at each IS, respectively. As IS increased, less negative zeta potentials was observed due to screening effects by cations.

Larger aggregate sizes and less negative zeta potentials were observed in 20 mM CaCl$_2$ compared to 100 mM NaCl (Figure 3.2). The divalent cation (Ca$^{2+}$) is more effective at decreasing Debye length and electrostatic repulsion barrier than the monovalent cation (Na$^+$), which resulted in larger n-TiO$_2$ aggregates [8]. Zeta potential of the 2 mM CaCl$_2$ and 20 mM CaCl$_2$ averaged -5.8 mV and +5.7 mV, respectively, whereas zeta potential was -14 mV in 100 mM NaCl. More aggregation occurred when the zeta potential was closer on the point of zero charge because more aggregation occurred at pH$_{pzc}$ due to little repulsion energy [1, 2]. Similarly, recent study reported that aggregated n-TiO$_2$ at pH 1 was stable during several months due to pH 1 is far from pH$_{pzc}$.
because decreasing repulsive energy could induce more aggregation of nanoparticles at various IS conditions [1, 3]. Here, our n-TiO₂ aggregate experiments showed that more aggregation occurred at increasing IS concentrations. Moreover, the divalent cation (Ca²⁺) is stronger at reducing repulsive energy on the surface of n-TiO₂ than the monovalent cation (Na⁺).
Figure 3. 2 Average hydrodynamic diameter (A and B) and average zeta potential (C and D) of n-TiO$_2$ in 20 mM CaCl$_2$, 2 mM CaCl$_2$, 100 mM NaCl, 20 mM NaCl, 10 mM NaCl, and DI water.
3.3.3 Aggregation of n-TiO$_2$ in the presence of HA at high ionic strength conditions

The stability of n-TiO$_2$ was also studied as a function of high ionic strength and the presence of HA (Figure 3.3). In the presence of 5.7 mg/L HA, different behavior was observed at 100 mM NaCl and 20 mM CaCl$_2$ over the 12 hour equilibration period. For the 100 mM NaCl solution, little difference in aggregation was observed in the presence and absence of HA. The average hydrodynamic diameter of n-TiO$_2$ was 786 nm (absence of HA) and 820 nm (presence of HA) at 12 hr. However, for the 20 mM CaCl$_2$ we observed significant increases in aggregation in the presence of HA compared to the absence of HA. In the presence of HA, the average hydrodynamic diameter of n-TiO$_2$ was approximately 1800 nm, which corresponds to a 71 % increased in particle diameter.

HA significantly changed the zeta potential of n-TiO$_2$ under high ionic strength conditions (Figure 3.3(B)). Zeta potential of n-TiO$_2$ was around -20 mV and -6 mV in the presence of HA at 100 mM NaCl and 20 mM CaCl$_2$, respectively. Zeta potentials of n-TiO$_2$ became more negative due to adsorption of HA on the n-TiO$_2$ surface, a result that is consistent with previous studies [4, 8, 9]. Particularly, the zeta potential of n-TiO$_2$ in 20 mM CaCl$_2$ changed from positive (ave. 5.7 mV) to negative (-6.5 mV) due to adsorption of HA [9]. Additionally, the zeta potential of n-TiO$_2$ in 100 mM NaCl became more negative from -12.7 mV (absence of HA) to -25.4 mV (presence of HA).

When we considered the effect of HA on aggregation and zeta potential of n-TiO$_2$ in high ionic strength conditions, the presence of HA play an important role on stability of n-TiO$_2$. HA changed the surface charges that induced more negative surface charges in both NaCl and CaCl$_2$. Moreover, n-TiO$_2$ aggregates in the presence of HA may be resulted from the bridging effects of HA on the surface of n-TiO$_2$ [4]. Particularly, larger
bridging effects of HA occurred in divalent (Ca$^{2+}$) than in monovalent (Na$^+$) even in lower divalent IS condition. French et al. (2009) reports the effect on n-TiO$_2$ aggregates between divalent and monovalent cation. They concluded that divalent cation (Ca$^{2+}$) showed stronger effects on n-TiO$_2$ aggregates compared to similar monovalent (Na$^+$) concentrations [1]. Therefore, our observation could explain the screening of Debye length that is proportional to the cation valences [1], which resulted in different n-TiO$_2$ aggregates between Na$^+$ and Ca$^{2+}$.
Figure 3. (A) Average hydrodynamic diameter and (B) average zeta potential of n-TiO$_2$ in 100 mM NaCl, 20 mM CaCl$_2$, and DI water in the presence and absence of HA (5.7 mg/L)
3.3.4 Aggregation of n-TiO$_2$ in the presence 17β-estradiol (E2) in high ionic strength

The stability of n-TiO$_2$ was evaluated in the presence of E2 at high ionic strength. In 100 mM NaCl, the increased aggregation rates of n-TiO$_2$ in the presence of E2 compared to the absence of E2 ranged from about 26% to 51% during 12 hr. (Figure 3.2 (B) and 3.4 (B)). In contrast, decreased hydrodynamic diameters of n-TiO$_2$ were observed in the presence of E2 at 10 mM NaCl compared to the absence of E2 (Figure 3.2 (B) and Figure 3.4 (B)). These results were explained in Chapter 2 due to steric repulsion of E2 adsorption on n-TiO$_2$ in 10 mM NaCl.

In 20 mM CaCl$_2$, we observed that E2 enhanced the aggregation of n-TiO$_2$ compared to n-TiO$_2$ aggregation in the absence of E2 over 12 hours. In the presence of E2 (Figure 3.4 (A)), the aggregation of n-TiO$_2$ was increased 44% from 994 nm to 1436 nm, whereas the aggregation of n-TiO$_2$ was increased 21% from 926 nm to 1119 nm in the absence of E2 (Figure 3.2 (A)). Particularly, larger n-TiO$_2$ aggregation was observed at 2 mM CaCl$_2$ compared to 20 mM CaCl$_2$ (Figure 3.4 (A)). These aggregation behaviors seemed to show a reverse trend that we expected at higher IS concentrations. However, these results may be related with heterogeneous surface with cations. Higher cation concentrations could make n-TiO$_2$ surfaces more homogeneously loaded with cations, which may result in increasing repulsion. Previous studies demonstrated those effects on aggregation of nanoparticles and attachment efficiency of colloidal particles due to surface heterogeneity or increasing repulsion at higher salt concentration [1, 16].

The zeta potential of n-TiO$_2$ in both 100 mM NaCl and 20 mM CaCl$_2$ was not influenced in the presence of E2 although a little decrease in zeta potential was observed in the presence of E2 in DI water.
Figure 3.4 Average hydrodynamic diameter (A and B) and average zeta potential (C and D) of n-TiO$_2$ in 100 mM NaCl, 20 mM CaCl$_2$, 20 mM NaCl, 10 mM NaCl, 2 mM CaCl$_2$, and DI water in the presence of E2 (3 mg/L).
### 3.3.5 Aggregation of n-TiO₂ in the presence of HA and 17β-estradiol (E₂) in high ionic strength conditions

The stability of n-TiO₂ was evaluated with the presence of HA or HA/E₂ under high ionic strength. In 100 mM NaCl, little aggregation was observed in presence of HA and E₂ over 12 hr (Figure 3.5 (B)). The average diameter of n-TiO₂ was 864 nm, and there was no difference with the aggregation in the presence of HA alone (Figure 3.3 (A)). However, more aggregation of n-TiO₂ was observed in the presence of E₂ alone compared to the presence of HA or HA/E₂ (Figure 3.5 (B)). Our results indicate that aggregation of n-TiO₂ was likely more influenced by the presence of HA rather than the presence of E₂. Humic acid consists of carboxylic groups and phenolic groups [9] that could be major functional groups for interaction with other organic contaminants. Therefore, n-TiO₂ coated with HA may react with E₂ but we couldn't observe the effect of E₂ on n-TiO₂ aggregation due to the presence of HA.

In 20 mM CaCl₂, increased aggregation of n-TiO₂ was observed in the presence of HA or HA/E₂ compared to the presence of E₂ alone (Figure 3.5(A)). In the presence of HA, we observed a slight increase in hydrodynamic diameter from 1644 nm to 1870 nm over 12 hr. In the presence of HA/E₂, n-TiO₂ aggregate was larger than in the presence of HA and ranged from 1857 nm to 2214 nm over 12 hr. Clearly, we observed more effect of HA or HA/E₂ on aggregation of n-TiO₂ at 20 mM CaCl₂ than 100 mM NaCl. These results may be related with higher bridging effect of HA with divalent cation than monovalent cations. Additionally, adsorption of E₂ on n-TiO₂ coated with HA enhanced n-TiO₂ aggregation.
Figure 3.5 (C and D) showed the zeta potential in the presence of HA or HA/E2 in 20 mM CaCl$_2$ and 100 mM NaCl. In both IS conditions, zeta potential was stable in the presence of HA over 12 hr. However, zeta potential increased in the presence of HA/E2 compared to the presence of HA alone that E2 may increase the surface charge of n-TiO$_2$. Further experiments are needed to understand the interaction mechanisms of E2 on the complex surface of n-TiO$_2$ in high ionic strength conditions and the presence of HA.

In conclusions, the aggregation of n-TiO$_2$ was influenced by the presence of HA and E2 in higher ionic strength solutions. Particularly, we observed more aggregation in the divalent cation solution than in the monovalent cation solution in the presence of HA and E2.
Ave. Hydrodynamic diameter [nm]

Time [hr]

20 mM CaCl₂ (HA 5.7 mg/L + E2 3 mg/L)
X 20 mM CaCl₂ (HA, 5.7 mg/L)
O 20 mM CaCl₂ (E2, 3 mg/L)

100 mM NaCl (HA 5.7 mg/L + E2 3 mg/L)
□ 100 mM NaCl (HA, 5.7 mg/L)
△ 100 mM NaCl (E2, 3 mg/L)
Figure 3. 5 Average hydrodynamic diameter (A and B) and average zeta potential (C and D) of n-TiO$_2$ at 20 mM CaCl$_2$ and 100 mM NaCl in the presence of HA (5.7 mg/L) and E2 (3 mg/L)
3.4 References


CHAPTER 4 QSAR MODELS TO PREDICT n-TiO₂ STABILITY AS A FUNCTION OF OWC MOLECULAR DESCRIPTORS

4.1 Introduction

Due to the application of nanoparticles (NPs) in consumer products, it is estimated that projected production of n-TiO₂ will be up to 201,500 tons in 2015 [1]. Therefore, it is assumed that NPs may be discharged into environment. Recent studies have reported the occurrence and detection of n-TiO₂ in effluent from wastewater treatment plants (WWTPs) as well as surface water from exterior facade paints and sunscreen products [2-5]. In addition, municipal wastewater contains various emerging organic wastewater contaminants (OWCs) such as pharmaceuticals, steroids, pesticides, and industrial compounds. OWCs are released from WWTPs into the aquatic environment because they are not completely removed during wastewater treatment processes [6-12]. Recent studies demonstrated the toxicity of n-TiO₂ as well as OWCs in ecosystems [13-18]. Therefore, it is estimated that n-TiO₂ and OWCs may co-exist in WWTP effluent and receiving water interactions such as sorption of OWCs on n-TiO₂, may occur. These interactions may influence the environmental fate of n-TiO₂ and OWCs.

The stability of n-TiO₂ is influenced by surrounding water chemistry such as pH, ionic strength (IS), natural organic matter, and contaminants, which can result in significant surface modification of n-TiO₂ and influence n-TiO₂ aggregation behavior [19-22]. In addition, a study showed the enhanced toxicity of aggregated n-TiO₂ on a marine organism by complexation with tributyltin (TBT), a marine antifouling organic chemical compared with the toxicity of TBT alone [23]. Thus, interaction between n-
TiO₂ and OWCs should be considered for increasing knowledge about the toxicity and environmental fate of NPs and OWCs.

Sorption process of OWCs on n-TiO₂ may be important to understand the stability of n-TiO₂ and fate of OWCs based on the sorption of OWCs on solids. Previous studies estimated the sorption of OWCs to solids and sediments by quantitative structure-activity relationship (QSAR) model between K_{oc}, soil sorption coefficient and chemical descriptors of OWCs [24-26]. Recent studies have investigated relationships between biological toxicity of NPs and properties of NPs using QSAR model [27-30]. Deonarine et al. (2011) developed a linear regression between aggregation rate of ZnS nanoparticles and descriptors of natural organic matter (NOM). However, no QSAR model has been developed to predict stability of NPs based on descriptors of OWCs.

The purpose of this work is to propose a new statistical QSAR model for prediction of n-TiO₂ aggregation behavior by descriptors of OWCs. One of the different statistical approaches is multiple linear regression (MLR), which is commonly used among statistical modeling methods [27] for developing a QSAR model. Recently, Sathyamoorthy and Ramsburg derived a QSAR model for prediction of pharmaceutical sorption on sludge from multi-parameter models because increasing number of predictors demonstrated increasing R² values [26]. Therefore, in this study we investigated whether a QSAR model could be developed to describe how descriptors of OWCs could predict the aggregation of n-TiO₂-OWC complexes. Such a model would be useful to predict the fate and transport of nanoparticles coated with various organic contaminants in environment.
4.2 Material and Methods

4.2.1 Chemical Reagents

TiO$_2$ (Rutile) nanoparticles (n-TiO$_2$) were purchased from Nanostructured & Amorphous Material Inc. (Los Alamos, NM, USA) with average particle size of 10×40 nm, 98% purity, specific surface area of 160 m$^2$/g, and coated with less than 5% SiO$_2$. Organic wastewater contaminants (OWCs) such as pharmaceuticals (Ibuprofen, Azithromycin, Lincomycin), pesticides (Atrazine, Alachlor, Chlorthalonil, Cyanazine, Butylate, and Pendimethalin) and steroids (17β-Estradiol, Estrone, and 17α-Ethinylestradiol) were chosen to estimate the nanoparticle stability according to their molecular descriptors. Molecular descriptors and structure would be considered in predicting the stability of n-TiO$_2$ and details are provided in Table 4.1 and Table 4.2.
Table 4. Molecular descriptors of our target chemical compounds

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<th>log K_{ow}</th>
<th>M.W. [g/mol]</th>
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<th>Molar volume [cm³]</th>
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Table 4. Structure of our target chemical compounds

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4.2.2 Preparation of n-TiO₂ Samples with Organic Wastewater Contaminants (OWCs)

n-TiO₂ solutions of 5 mg/L were prepared with 2 mM CaCl₂ in 500 mL flask according to procedures described in chapter 2. After pH 7 adjustment, each chemical compounds were spikèd into n-TiO₂ solution and the final concentration of each compound in n-TiO₂ suspension was 80 % of water solubility of each compound. If methanol was used for solvent, the methanol concentration in final solution was kept less than 1 % (v/v) to minimize the cosolvent effect on n-TiO₂ aggregation.

Based on the results from chapter 2, n-TiO₂ aggregation strongly depends on ionic strength and compound’s concentration. Therefore, this study was conducted in the favorable conditions in 2 mM CaCl₂ that could show the aggregation of n-TiO₂ by OWCs. n-TiO₂ aggregation experiments in the presence of OWCs were measured for 12 hr and particle sizes and zeta potential were monitored. During the aggregation experiments, flasks were covered with aluminum foil to prevent the photodegradation or photocatalytic degradation of OWCs by light and stored in dark chamber at room temperature. To make a representative sample at each time, n-TiO₂/OWC was shaken for 30 seconds then 3 mL aliquot was taken from three different positions for triplicate measurements.

In addition, this study conducted a new aggregation experiment by changing concentrations of n-TiO₂ and OWCs that were set with 1 mg/L n-TiO₂ and 0.2 mg/L C_w of OWCs in 2 mM CaCl₂ at pH 7, respectively. Preparation methods of n-TiO₂ solution, OWCs stock solution, and spiking OWCs in n-TiO₂ suspension were same with previous aggregation experiments. Particle size and zeta potential of n-TiO₂ were measured at 0 hr and 12 hr. All measurements were triplicate.
4.2.3 Determination of n-TiO$_2$ aggregation rate and aggregation kinetics

n-TiO$_2$ aggregation was monitored using a 90Plus particle size analyzer (Brookhaven Instruments Corporation, Holtsville, NY) based on a dynamic light scattering (DLS) during 12 hr. Measured particle size represents the effective diameter of nanoparticles calculated for sphere particles from the Stokes-Einstein equation based on the translational diffusion coefficient, which is intensity-weighted. Therefore, each effective diameter of nanoparticles was intensity-weighted hydrodynamic diameter from accumulated autocorrelation function. Finally, the mean diameter was calculated by the sum of these effective diameters divided by N runs (3 runs in our experiments and 1 minute per 1 run). Zeta potential of n-TiO$_2$ was measured by ZetaPALS (Brookhaven Instruments Corporation, Holtsville, NY) using phase analysis light scattering (PALS) technique to measure the electrophoretic mobility according to a Smoluchowski equation that converted mobility to the zeta potential of particle.

To determine the increase rate of diameter and dD/dt between at 0 hr and 12 hr, the increase rate of diameter was measured by ratio between hydrodynamic diameters with OWCs and hydrodynamic diameter without OWCs and dD/dt was calculated with the differences of diameter between with OWCs and without OWCs by considering each time (Table 4.3 and 4.4). Additionally, n-TiO$_2$ aggregation kinetics ($k$) were calculated by applying the modified early-stage aggregation kinetic equation [31, 32] at between $t =$ 0 hr and $t =$ 12 hr and each aggregation kinetic was defined as $k_1$ or $k_2$, respectively,

\[
\left( \frac{\Delta D_H(t)}{dt} \right) \propto k_t N_0
\]
where \( D_H(t) \) is the hydrodynamic diameter of n-TiO\(_2\) at each time, \( N_0 \) is the concentration of n-TiO\(_2\), and \( k_t \) is the n-TiO\(_2\) aggregation kinetic at \( t=0 \) hr or \( t=12 \) hr. From the equation 1, \( k_1 \) and \( k_2 \) were summarized in Table 4.3 and Table 4.4, respectively.

### 4.2.4 QSAR Models for Predicting Aggregation of n-TiO\(_2\) by molecular descriptors of OWCs

The aggregation characteristic of n-TiO\(_2\) based on the molecular descriptors of OWCs was analyzed by quantitative structure-activity relationship (QSAR) model based on linear regression techniques that is a statistical modeling technique. MATLAB (2013b) program was used to develop a multiple linear regression model (MLR) between a response (\( Y \)) and one or more predictors (\( x_1, x_2, x_3, \ldots, x_n \)) [28]. The general MLR equation model is below:

\[
Y = a_0 + a_1.x_1 + a_2.x_2 + a_3.x_3 + \ldots + a_n.x_n
\]  

(2)

Recent study has reported about QSAR model development for predicting the sorption of pharmaceuticals (PhACs) on biosolids and showed the best model with increased \( R^2 \) values included multiple predictors [26]. In our study, 3 responses and 7 predictors (Table 4.3 and Table 4.4) were used to establish QSAR models between descriptors of OWCs and n-TiO\(_2\) aggregation behavior. To evaluate goodness of fit between observed data and modeled data, we evaluated R-square, adjusted R-square, and p-values.

R-square is called the square of the multiple correlation coefficient and the coefficient of multiple determination between the response values and predicted response
values that is ranged between 0 and 1, which R-square value close to 1 indicates a better model fit [33].

\[
R - square = \frac{SSR}{SST} = 1 - \frac{SSE}{SST}
\] (3)

where SSR is the sum of squares of the regression, SST is the total sum of squares, and SSE is the sum of squares of error.

Although R-square is useful to explain the model fit, R-square is not perfect for evaluating model fit when adding variables can increase the R-square values even though the model doesn't fit the data well. Therefore, adjusted R-square attempts to take care of this issue based on the residual degrees of freedom [33].

\[
\text{adjusted } R - square = 1 - \frac{SSE(n-1)}{SST(v)}
\] (4)

where v is the residual degrees of freedom that is defined as the number of n response variables minus the number of fitted coefficients, m. Generally, the residual degrees of freedom are proportional to the number of parameters such as n and m. Thus, adjusted R-square is always lower than R-square. The adjusted R-square ranges between 0 and 1 and a value closer to 1 means a better model fit. In addition, the p-value is obtained from a statistical test result based on a statistical significance level (p < 0.05) that indicate whether the coefficients of variables are statistically significant.

To predict a QSAR model of n-TiO\textsubscript{2} aggregation, two multiple linear regression (MLR) functions both fitlm and stepwiselm in MATLAB were used to estimate a QSAR model. The former function is to generate a linear model of the responses, \(Y\) to fit the predictors, \(X_n\). The latter function generates a linear model of responses to fit the
predictors using forward and backward stepwise regression to estimate a best model based on the criterion argument by adding or removing predictors corresponding to p-values less than 0.05.

4.3 Results and discussion

4.3.1 Aggregation of n-TiO₂ in the presence of OWCs

The aggregation behavior of n-TiO₂ in the presence of OWCs was investigated by the average hydrodynamic diameter of nanoparticles obtained from DLS measurements during 12 hr. We investigated the aggregation behavior of n-TiO₂ under favorable condition in 2 mM CaCl₂ and OWCs at a concentration equal to 80 % of the solubility of each OWC in 5 mg/L n-TiO₂ suspensions. The characteristics and structures of OWCs were shown in Table 4.1 and 4.2. The average hydrodynamic diameters of n-TiO₂ were measured for the aggregation behaviors of n-TiO₂ as a function of OWCs (Figure 4.1).

Different aggregation behavior of n-TiO₂ was observed in different OWCs. The stability of n-TiO₂ was strongly dependent on the presence of organic compounds. Some OWCs resulted in fast aggregation of n-TiO₂ initially (Figure 4.1 (A)), while other OWCs showed lesser and slower aggregation of n-TiO₂ during 12 hr (Figure 4.1 (B)). In previous study, a 25 % increase in diameter called the early-stage aggregation rate [32]. Thus, we applied this concept in analysis of our aggregation data. At 0 hr, some compounds such as estrone (E1), 17β-estradiol (E2), azithromycin, cyanazine, and butylate resulted in aggregation size up to over 25 % compared to the no OWCs TiO₂ control suspensions, while the other compounds showed less than 25 % increase of aggregation size compared to control n-TiO₂ suspension. During 12 hr, aggregation of n-TiO₂ increased to over 25 % of n-TiO₂ control suspensions for most of compounds except
for lincomycin which showed no aggregation over 12 hr. Some compounds such as estrone (E1), azithromycin, 17β-estradiol (E2), and butylate increased n-TiO₂ diameters by 3.1, 2.87, 2.85, and 2.69 times related to control n-TiO₂ suspensions. Ibupropen, cyanazine, chlorothalonil, pendimethalin, atrazine, 17α-ethinylestradiol, alachlor resulted in aggregation that was between 0.2 times and 1 time compared to control n-TiO₂ suspensions.

Simultaneously, we measured zeta potential of n-TiO₂ in the presence of OWCs (Figure 4.2 (A) and 4.2 (B)). In the control n-TiO₂ suspension, average zeta potential was -5.76 mV after 12 hr and average zeta potential in the presence of OWCs was more negative than the control except three compounds of 17β-estradiol (E2), cyanazine, and ibupropen. The most negative zeta potential (-10.29 mV) was measured in the presence of butylate, while the least negative zeta potential (-0.78 mV) was measured in the presence of 17β-estradiol (E2) after 12 hr. Larger aggregation diameters were observed in the presence of some compounds such as azithomycin, estrone (E1), and butylate despite the more negative zeta potentials for these compounds compared to the control, while 17β-estradiol (E2) and ibupropen also showed large aggregation with less negative charges of -0.78 mV and -2.77 mV, respectively. In the case of lincomycin, no aggregation was observed and the average zeta potential was -6.11 mV after 12 hr. These results suggest that trends in zeta potential are not consistent with aggregation behavior. Adsorption of OWCs on n-TiO₂ could be influencing n-TiO₂ aggregation as well as altering surface charge.

To evaluate the aggregation behavior of n-TiO₂ with a constant concentration ratios between n-TiO₂ and OWCs, we investigated aggregation of 1 mg/L n-TiO₂ in the
presence of 0.2 mg/L OWCs under the same conditions used in previous aggregation experiments. Figure 4.3 showed aggregation behavior (A) and zeta potential (B). All compounds showed increase aggregation. OWCs sorption resulted in more negative zeta potential except of estrone (E1) and 17β-estradiol (E2). Butylate and pendimethalin resulted in the most negative zeta potentials of -16.4 mV and -16.0 mV, respectively. Overall, our results reflecte that aggregation rates and zeta potential changes are related to the ratio of [n-TiO$_2$/[OWCs] in suspension. A recent study reported that growth rate of ZnS with NOM was sensitive to particle concentration as well as molecular size and structure of the NOM [34]. Therefore, the concentrations of nanoparticles as well as the OWC concentration could play a critical role in the aggregation and setting of nanoparticles in the aquatic environment. In addition, specific properties of OWCs may influence the aggregation behavior of n-TiO$_2$. 
Figure 4. 1 Aggregation of n-TiO$_2$ with 80% of C$_w$ of OWCs in 2 mM CaCl$_2$ at pH 7
Figure 4. 2 Zeta potential of n-TiO$_2$ with 80% of C$_w$ of OWCs in 2 mM CaCl$_2$ at pH 7.
Figure 4. 3 (A) aggregation and (B) zeta potential of 1 mg/L n-TiO$_2$ with 0.2 mg/L of C$_w$ of OWCs in 2 mM CaCl$_2$ at pH 7
4.3.2 MLR QSAR models for the 5 mg/L n-TiO$_2$ + 80% of C$_w$ of OWCs

Multiple linear regression was used to develop a QSAR model [26, 27, 35]. Here, we used three responses that were $\frac{dD}{dt}$, $k_1$ or $k_2$, and increase rate of diameter at 0 hr and 12 hr to evaluate a QSAR model according to responses (Table 4.3 and 4.4). Here, $\frac{dD}{dt}$ was obtained by the n-TiO$_2$ diameter changes after and before OWCs divided by time, $k_1$ or $k_2$ was calculated by $\frac{dD}{dt}$ was divided by the n-TiO$_2$ concentration, and increase rate in diameter was calculated by the diameter ratio between in the presence of OWCs and in the absence of OWCs. In addition, seven predictors listed in Table 4.1 were chosen that represented unique properties of each OWC as it is important to choose unbiased descriptors from many descriptors for nano-QSAR models [27].

Multiple linear regression models were performed using MATLAB functions of fitlm and stepwiselm. The best QSAR model was evaluated using the correlation coefficients ($R^2$ and Adj. $R^2$) associated with p-value ($< 0.05$). Detailed results for QSAR models were provided in below.

4.3.2.1 MLR QSAR models using fitlm function

Table 4.5 showed results of QSAR models for three responses that were evaluated by fitlm function, which provided correlation coefficients ($R^2$ and Adj. $R^2$) and p-value of each coefficient. Here, we assumed that all predictors strongly were related in a QSAR model. The correlation coefficients for three responses showed higher correlation values at 0 hr and 12 hr. For the best linear regressions, all responses presented a $R^2$ value of 0.92 at 0 hr. At 12 hr, the response of increase rate in diameter showed the highest $R^2$ value (0.9) but others also had high $R^2$ values of 0.87. In addition,
coefficients and p-value of each coefficient were calculated for the best QSAR models (Table 4.5). However, we observed that any p-value of coefficient didn’t show the significant relationship between predictors of OWCs and aggregation in spite of high correlation coefficients.

In this work, we evaluated relationship between modeled responses and observed responses using coefficients obtained from linear regression in Table 4.5. Figure 4.4 and 4.5 showed the relationship between model data and observed data between 0 hr and 12 hr, respectively. The $R^2$ was 0.66 for three responses at 0 hr and 0.43, 0.34, and 0.43 at 12 hr for three responses, respectively. The best correlation showed with 0.66 between modeled response and observe response at 0 hr, while correlation coefficients were significantly decreased at 12 hr that were 0.43 and 0.34 (Figure 4.4 and 4.5). From the correlation coefficient results, initial modeled response agreed with observed response using seven predictors with high correlation coefficient values than 12 hr.
Table 4. Calculation of responses at t = 0 hr for the 5 mg/L n-TiO₂ + 80% of C_w of OWCs

<table>
<thead>
<tr>
<th>Predictors</th>
<th>X</th>
<th>x2</th>
<th>x3</th>
<th>x4</th>
<th>x5</th>
<th>x6</th>
<th>x7</th>
<th>Diameter [nm], t = 0h</th>
<th>Diameter [nm] w/o chemicals</th>
<th>dD/dt</th>
<th>k₁, (nm·L·min⁻¹mg⁻¹), t = 0 h</th>
<th>Increase rate of diameter, t = 0 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Estrone</td>
<td>10.77</td>
<td>3</td>
<td>3.13</td>
<td>270.37</td>
<td>37.3</td>
<td>232.1</td>
<td>1</td>
<td>1391.2</td>
<td>874.2</td>
<td>57.45</td>
<td>11.49</td>
<td>1.591</td>
</tr>
<tr>
<td>17b-estradiol</td>
<td>10.71</td>
<td>3.6</td>
<td>4.01</td>
<td>270.39</td>
<td>40.46</td>
<td>232.6</td>
<td>2</td>
<td>1192.1</td>
<td>874.2</td>
<td>35.32</td>
<td>7.064</td>
<td>1.364</td>
</tr>
<tr>
<td>Azithromycin</td>
<td>8.74</td>
<td>7.09</td>
<td>4.02</td>
<td>748.99</td>
<td>180.08</td>
<td>169.9</td>
<td>5</td>
<td>1170.9</td>
<td>874.2</td>
<td>32.97</td>
<td>6.594</td>
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</tr>
<tr>
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<td>240.7</td>
<td>86.52</td>
<td>179.4</td>
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<td>1113.2</td>
<td>874.2</td>
<td>26.55</td>
<td>5.31</td>
<td>1.273</td>
</tr>
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<td>3.05</td>
<td>265.9</td>
<td>47.58</td>
<td>154.6</td>
<td>0</td>
<td>1053.2</td>
<td>874.2</td>
<td>19.89</td>
<td>3.977</td>
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<td>10.33</td>
<td>11.3</td>
<td>3.67</td>
<td>296.41</td>
<td>40.46</td>
<td>244.47</td>
<td>2</td>
<td>996.4</td>
<td>874.2</td>
<td>13.57</td>
<td>2.715</td>
<td>1.14</td>
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<tr>
<td>Ibuprofen</td>
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<td>3.97</td>
<td>206.28</td>
<td>37.3</td>
<td>200.3</td>
<td>1</td>
<td>966.4</td>
<td>874.2</td>
<td>10.24</td>
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<td>1.105</td>
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<td>62.73</td>
<td>169.9</td>
<td>2</td>
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<td>874.2</td>
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<td>1.81</td>
<td>1.093</td>
</tr>
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<td>269.77</td>
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<td>240.9</td>
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<td>874.2</td>
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<td>147.79</td>
<td>313.3</td>
<td>5</td>
<td>760.5</td>
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<td>-2.527</td>
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</tr>
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<td>231.5</td>
<td>1</td>
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<tr>
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<td>45</td>
<td>4.15</td>
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<td>45.61</td>
<td>211.945</td>
<td>0</td>
<td>1097.9</td>
<td>874.2</td>
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Table 4. Calculation of responses at \( t = 12 \) hr for the 5 mg/L \( n\text{-TiO}_2 \) + 80% of \( C_w \) of OWCs

<table>
<thead>
<tr>
<th>Predictors</th>
<th>x1</th>
<th>x2</th>
<th>x3</th>
<th>x4</th>
<th>x5</th>
<th>x6</th>
<th>x7</th>
<th>Diameter [nm], ( t = 12 ) h</th>
<th>Diameter [nm] w/o chemicals</th>
<th>( \frac{dD}{dt} )</th>
<th>( k_2 ) (nm·L)/(min·mg), ( t = 12 ) hr</th>
<th>Increase rate of diameter, ( t = 12 ) h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Compounds</td>
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<td></td>
<td></td>
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<td></td>
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<td>--------------------------------</td>
<td>------------------------------</td>
<td>----------------</td>
<td>-------------------------------</td>
<td>---------------------------</td>
</tr>
<tr>
<td>Azithromycin</td>
<td>8.74</td>
<td>7.09</td>
<td>4.02</td>
<td>748.99</td>
<td>180.08</td>
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<td>40.46</td>
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<td>244.467</td>
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<td>406.54</td>
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<td>231.5</td>
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<td>756.7</td>
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<td>2032.97</td>
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Table 4. Estimated coefficients and p-values created by MLR using fitlm function at 0 hr and 12 hr

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<th>Coefficient</th>
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<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th>R²</th>
<th>Adj. R²</th>
</tr>
</thead>
<tbody>
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<td></td>
<td>a0</td>
<td>a1</td>
<td>a2</td>
<td>a3</td>
<td>a4</td>
<td>a5</td>
<td>a6</td>
<td>a7</td>
<td></td>
</tr>
<tr>
<td>( \frac{dB}{dt} )</td>
<td>148.075</td>
<td>7.421</td>
<td>0.022</td>
<td>-13.291</td>
<td>0.039</td>
<td>0.338</td>
<td>-0.542</td>
<td>-24.881</td>
<td>0.916</td>
</tr>
<tr>
<td>p-value</td>
<td>0.162</td>
<td>0.167</td>
<td>0.854</td>
<td>0.375</td>
<td>0.623</td>
<td>0.359</td>
<td>0.371</td>
<td>0.167</td>
<td></td>
</tr>
<tr>
<td>Increase rate of diameter</td>
<td>2.513</td>
<td>0.076</td>
<td>0.000</td>
<td>-0.137</td>
<td>0.000</td>
<td>0.004</td>
<td>-0.006</td>
<td>-0.256</td>
<td></td>
</tr>
<tr>
<td>p-value</td>
<td>0.070</td>
<td>0.169</td>
<td>0.861</td>
<td>0.376</td>
<td>0.631</td>
<td>0.355</td>
<td>0.375</td>
<td>0.168</td>
<td></td>
</tr>
<tr>
<td>( k_1 )</td>
<td>29.665</td>
<td>1.491</td>
<td>0.004</td>
<td>-2.657</td>
<td>0.007</td>
<td>0.068</td>
<td>-0.109</td>
<td>-4.970</td>
<td></td>
</tr>
<tr>
<td>p-value</td>
<td>0.160</td>
<td>0.165</td>
<td>0.854</td>
<td>0.374</td>
<td>0.635</td>
<td>0.353</td>
<td>0.368</td>
<td>0.167</td>
<td></td>
</tr>
<tr>
<td></td>
<td>12 hr</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>a0</td>
<td>a1</td>
<td>a2</td>
<td>a3</td>
<td>a4</td>
<td>a5</td>
<td>a6</td>
<td>a7</td>
<td></td>
</tr>
<tr>
<td>( \frac{dB}{dt} )</td>
<td>6.4135</td>
<td>0.3789</td>
<td>0.0050</td>
<td>0.1782</td>
<td>-0.0004</td>
<td>0.0148</td>
<td>-0.0362</td>
<td>-0.9473</td>
<td>0.872</td>
</tr>
<tr>
<td>p-value</td>
<td>0.186</td>
<td>0.150</td>
<td>0.415</td>
<td>0.780</td>
<td>0.918</td>
<td>0.392</td>
<td>0.249</td>
<td>0.231</td>
<td></td>
</tr>
<tr>
<td>Increase rate of diameter</td>
<td>8.397</td>
<td>0.382</td>
<td>0.006</td>
<td>0.132</td>
<td>0.001</td>
<td>0.006</td>
<td>-0.041</td>
<td>-0.849</td>
<td></td>
</tr>
<tr>
<td>p-value</td>
<td>0.106</td>
<td>0.130</td>
<td>0.324</td>
<td>0.822</td>
<td>0.770</td>
<td>0.696</td>
<td>0.187</td>
<td>0.240</td>
<td></td>
</tr>
<tr>
<td>( k_2 )</td>
<td>1.2794</td>
<td>0.0756</td>
<td>0.0010</td>
<td>0.0351</td>
<td>-0.0001</td>
<td>0.0030</td>
<td>-0.0072</td>
<td>-0.1893</td>
<td>0.872</td>
</tr>
<tr>
<td>p-value</td>
<td>0.187</td>
<td>0.150</td>
<td>0.417</td>
<td>0.783</td>
<td>0.920</td>
<td>0.392</td>
<td>0.250</td>
<td>0.231</td>
<td></td>
</tr>
</tbody>
</table>
Figure 4. 4 Relationship between modeled response and observed response using coefficients by fitlm function at 0 hr
Figure 4. 5 Relationship between modeled response and observed response using coefficients by fitlm function at 12 hr
4.3.2.2 MLR QSAR models using stepwiselm function

To figure out the best QSAR model using MLR methods, we applied stepwiselm algorithms in MATLAB to create multiple linear regression models. Stepwiselm algorithms can determine the best multiple linear regression model by adding or removing predictors until p-value is at a minimum. For 0 hr, Table 4.6, 4.7, and 4.8 showed models estimated by stepwiselm algorithms according to different responses. Figure 4.6, 4.7, and 4.8 presented relationship between modeled response and observed response based on coefficients in Table 4.6, 4.7, and 4.8, respectively. As a result of stepwiselm model, p value of each predictor in Tables (4.6 - 4.8) were less than 0.05 indicating that all predictors were statistically significant. One additional predictor, x1:x7 was added to the model. p-values of these models were 0.0278, 0.029, and 0.0272 for Table 4.6, 4.7, and 4.8, respectively. In addition, R^2 and adj. R^2 in these model showed perfect linear correlation coefficient with > 0.99. From the coefficients in Table 4.6, 4.7, and 4.8, we obtained relationship between modeled response and observed response that was shown in Figure 4.6, 4.7, and 4.8. Two compounds, chlorothalonil and butylate, had no pK_a values that resulted in a low correlation coefficient in Figure 4.6 (A), 4.7 (A), and 4.8 (A). However, we obtained almost linear correlation coefficient (R^2 > 0.99) when we removed the two compounds without pK_a (Figure 4.6 (B), 4.7 (B), and 4.8 (B)). pK_a was one of the most important predictors among several predictors to create our QSAR model because we observed low correlation coefficient (R^2 and adj. R^2 < 0.3) when we didn't consider pK_a in our model.
Table 4. Estimated coefficients by stepwiselm regression model for dD/dt at t = 0 hr

<table>
<thead>
<tr>
<th>Variable</th>
<th>Predictor</th>
<th>Coefficient</th>
<th>SE</th>
<th>t Stat</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>a0</td>
<td>Constant</td>
<td>739.396</td>
<td>26.149</td>
<td>28.277</td>
<td>0.023</td>
</tr>
<tr>
<td>a1</td>
<td>pK_a, x1</td>
<td>-11.150</td>
<td>0.839</td>
<td>-13.295</td>
<td>0.048</td>
</tr>
<tr>
<td>a2</td>
<td>C_w, x2</td>
<td>-0.271</td>
<td>0.014</td>
<td>-18.970</td>
<td>0.034</td>
</tr>
<tr>
<td>a3</td>
<td>log K_{ow}, x3</td>
<td>-78.829</td>
<td>2.951</td>
<td>-26.715</td>
<td>0.024</td>
</tr>
<tr>
<td>a4</td>
<td>M.W., x4</td>
<td>-0.825</td>
<td>0.038</td>
<td>-21.744</td>
<td>0.029</td>
</tr>
<tr>
<td>a5</td>
<td>Polar surface area, x5</td>
<td>2.882</td>
<td>0.112</td>
<td>25.636</td>
<td>0.025</td>
</tr>
<tr>
<td>a6</td>
<td>Molar volume, x6</td>
<td>-1.033</td>
<td>0.036</td>
<td>-28.533</td>
<td>0.022</td>
</tr>
<tr>
<td>a7</td>
<td>#H bond donors, x7</td>
<td>-192.410</td>
<td>7.348</td>
<td>-26.186</td>
<td>0.024</td>
</tr>
<tr>
<td>a8</td>
<td>x1:x7</td>
<td>21.654</td>
<td>0.945</td>
<td>22.911</td>
<td>0.028</td>
</tr>
</tbody>
</table>
Figure 4. 6 Relationship between modeled response and observed response for dD/dt using coefficients by stepwiselm function at 0 hr
Table 4. 7 Estimated coefficients by stepwiselm regression model for increase rate of diameter at t = 0 hr

<table>
<thead>
<tr>
<th>Variable</th>
<th>Predictor</th>
<th>Coefficient</th>
<th>SE</th>
<th>t Stat</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>a0</td>
<td>Constant</td>
<td>8.608</td>
<td>0.282</td>
<td>30.549</td>
<td>0.021</td>
</tr>
<tr>
<td>a1</td>
<td>$pK_a$, x1</td>
<td>-0.115</td>
<td>0.009</td>
<td>-12.755</td>
<td>0.050</td>
</tr>
<tr>
<td>a2</td>
<td>$C_w$, x2</td>
<td>-0.003</td>
<td>0.000</td>
<td>-18.210</td>
<td>0.035</td>
</tr>
<tr>
<td>a3</td>
<td>log $K_{ow}$, x3</td>
<td>-0.812</td>
<td>0.032</td>
<td>-25.541</td>
<td>0.025</td>
</tr>
<tr>
<td>a4</td>
<td>M.W., x4</td>
<td>-0.009</td>
<td>0.000</td>
<td>-20.823</td>
<td>0.031</td>
</tr>
<tr>
<td>a5</td>
<td>Polar surface area, x5</td>
<td>0.030</td>
<td>0.001</td>
<td>24.556</td>
<td>0.026</td>
</tr>
<tr>
<td>a6</td>
<td>Molar volume, x6</td>
<td>-0.011</td>
<td>0.000</td>
<td>-27.126</td>
<td>0.023</td>
</tr>
<tr>
<td>a7</td>
<td>#H bond donors, x7</td>
<td>-1.983</td>
<td>0.079</td>
<td>-25.042</td>
<td>0.025</td>
</tr>
<tr>
<td>a8</td>
<td>x1:x7</td>
<td>0.223</td>
<td>0.010</td>
<td>21.915</td>
<td>0.029</td>
</tr>
</tbody>
</table>
Figure 4. 7 Relationship between modeled response and observed response for increase rate of diameter using coefficients by stepwiselm function at 0 hr
Table 4. 8 Estimated coefficients by stepwiselm regression model for $k_1$ at $t = 0\ hr$

<table>
<thead>
<tr>
<th>Variable</th>
<th>Predictor</th>
<th>Coefficient</th>
<th>SE</th>
<th>t Stat</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>a0</td>
<td>Constant</td>
<td>147.487</td>
<td>5.095</td>
<td>28.950</td>
<td>0.022</td>
</tr>
<tr>
<td>a1</td>
<td>$pK_a$, x1</td>
<td>-2.209</td>
<td>0.163</td>
<td>-13.522</td>
<td>0.047</td>
</tr>
<tr>
<td>a2</td>
<td>$C_w$, x2</td>
<td>-0.054</td>
<td>0.003</td>
<td>-19.403</td>
<td>0.033</td>
</tr>
<tr>
<td>a3</td>
<td>log $K_{ow}$, x3</td>
<td>-15.715</td>
<td>0.575</td>
<td>-27.337</td>
<td>0.023</td>
</tr>
<tr>
<td>a4</td>
<td>M.W., x4</td>
<td>-0.165</td>
<td>0.007</td>
<td>-22.276</td>
<td>0.029</td>
</tr>
<tr>
<td>a5</td>
<td>Polar surface area, x5</td>
<td>0.575</td>
<td>0.022</td>
<td>26.266</td>
<td>0.024</td>
</tr>
<tr>
<td>a6</td>
<td>Molar volume, x6</td>
<td>-0.207</td>
<td>0.007</td>
<td>-29.286</td>
<td>0.022</td>
</tr>
<tr>
<td>a7</td>
<td>#H bond donors, x7</td>
<td>-38.350</td>
<td>1.432</td>
<td>-26.789</td>
<td>0.024</td>
</tr>
<tr>
<td>a8</td>
<td>x1:x7</td>
<td>4.315</td>
<td>0.184</td>
<td>23.431</td>
<td>0.027</td>
</tr>
</tbody>
</table>
Figure 4. 8 Relationship between modeled response and observed response for $k_1$ using coefficients by stepwiselm function at 0 hr
Table 4.9, 4.10, and 4.11 show the results of the stepwiselm model developed at 12 hr. Table 4.9 and 4.11 presented the model that only considered two predictors, x1 (pK\textsubscript{a}) and x6 (molar volume) with p value < 0.05. From these results, the correlation coefficients (R\textsuperscript{2} and adj. R\textsuperscript{2}) in Table 4.9 and 4.11 were 0.68 and 0.58, respectively. Using coefficients in models, relationship between modeled response and observed response was obtained in Figure 4.9 and 4.11. Relationship output showed that model predictions were not well correlated with observed values for both models (R\textsuperscript{2} = 0.39). The QSAR model in Table 4.10 used four predictors that were x1 (pK\textsubscript{a}), x2 (C\textsubscript{w}), x6 (molar volume), and x7 (#H bond donor) with high correlation coefficients (R\textsuperscript{2} = 0.84 and adj. R\textsuperscript{2} = 0.71). Using coefficients in models from Table 4.10, relationship between modeled response and observed response was obtained in Figure 4.10. Relationship output showed that model predictions were good correlation with observed values (R\textsuperscript{2} = 0.76). Here, we could observe that some predictors such as x1 (pK\textsubscript{a}) and x6 (molar volume) were statistically-significant predictors, which was same as models in Table 4.9, 4.10, and 4.11.
Table 4. 9 Estimated coefficients by stepwiselm regression model for dD/dt at t = 12 hr

<table>
<thead>
<tr>
<th>Variable</th>
<th>Predictor (x)</th>
<th>Coefficient</th>
<th>SE</th>
<th>t Stat</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>a0</td>
<td>Constant</td>
<td>2.6307</td>
<td>0.8962</td>
<td>2.9355</td>
<td>0.0219</td>
</tr>
<tr>
<td>a1</td>
<td>pK_a, x1</td>
<td>0.1479</td>
<td>0.0433</td>
<td>3.4142</td>
<td>0.0112</td>
</tr>
<tr>
<td>a6</td>
<td>Molar volume, x6</td>
<td>-0.0115</td>
<td>0.0042</td>
<td>-2.7434</td>
<td>0.0288</td>
</tr>
</tbody>
</table>

\[ Y = a0 + a1 \cdot x1 + a6 \cdot x6 \ (R^2 = 0.68, \text{ Adj. } R^2 = 0.58) \]

Figure 4. 9 Relationship between modeled response and observed response for dD/dt using coefficients by stepwiselm function at 12 hr
Table 4. Estimated coefficients by stepwiselm regression model for increase rate of diameter at $t = 12$ hr

<table>
<thead>
<tr>
<th>Variable</th>
<th>Predictor (x)</th>
<th>Coefficient</th>
<th>SE</th>
<th>t Stat</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>a0</td>
<td>Constant</td>
<td>7.237</td>
<td>1.968</td>
<td>3.677</td>
<td>0.014</td>
</tr>
<tr>
<td>a1</td>
<td>$pK_a$, x1</td>
<td>0.294</td>
<td>0.075</td>
<td>3.908</td>
<td>0.011</td>
</tr>
<tr>
<td>a2</td>
<td>$C_w$, x2</td>
<td>0.003</td>
<td>0.002</td>
<td>2.016</td>
<td>0.100</td>
</tr>
<tr>
<td>a6</td>
<td>Molar volume, x6</td>
<td>-0.031</td>
<td>0.010</td>
<td>-3.080</td>
<td>0.027</td>
</tr>
<tr>
<td>a7</td>
<td>#H bond donors, x7</td>
<td>-0.411</td>
<td>0.204</td>
<td>-2.019</td>
<td>0.099</td>
</tr>
</tbody>
</table>

$Y = a0 + a1 \cdot x1 + a2 \cdot x2 + a6 \cdot x6 + a7 \cdot x7 \ (R^2 = 0.84, \ Adj. \ R^2 = 0.71)$

Figure 4. Relationship between modeled response and observed response for increase rate of diameter using coefficients by stepwiselm function at 12 hr
Table 4. Estimated coefficients by stepwiselm regression model for $k_2$ at $t = 12$ hr

<table>
<thead>
<tr>
<th>Variable</th>
<th>Predictor (x)</th>
<th>Coefficient</th>
<th>SE</th>
<th>t Stat</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>a0</td>
<td>Constant</td>
<td>0.526</td>
<td>0.179</td>
<td>2.938</td>
<td>0.022</td>
</tr>
<tr>
<td>a1</td>
<td>$pK_a$, x1</td>
<td>0.030</td>
<td>0.009</td>
<td>3.418</td>
<td>0.011</td>
</tr>
<tr>
<td>a6</td>
<td>Molar volume, x6</td>
<td>-0.002</td>
<td>0.001</td>
<td>-2.746</td>
<td>0.029</td>
</tr>
</tbody>
</table>

$Y = a0 + a1 \cdot x1 + a6 \cdot x6$ ($R^2 = 0.68$, Adj. $R^2 = 0.58$)

Figure 4. Relationship between modeled response and observed response for $k_2$ using coefficients by stepwiselm function at 12 hr
4.3.3 MLR QSAR models for the 1 mg/L n-TiO₂ + 0.2 mg/L of Cₜ of OWCs

4.3.3.1 MLR QSAR models using fitlm function

Table 4.12 and 4.13 showed the summary about three observed responses between 0 hr and 12 hr to evaluate a QSAR model. Details in diameter and zeta potential of n-TiO₂ with OWCs were shown in Figure 4.3. Table 4.14 showed results of QSAR models for three responses that were evaluated by fitlm function, which provided correlation coefficients (R² and Adj. R²) and p-value of each coefficient. Here, we assumed that all predictors strongly were related in a QSAR model. The correlation coefficients for three responses showed high correlation values (R²) at 0 hr and 12 hr that ranged 0.75 and 0.86, respectively. However, adj. R² were low values both 0 hr (-0.1) and 12 hr (0.35). Additionally, we observed high p-value of coefficients both 0 hr and 12 hr that reflect little statistically significant between predictors of OWCs and aggregation.

We evaluated relationship between modeled responses and observed responses using coefficients obtained from linear regression in Table 4.14. Figure 4.12 and 4.13 showed the relationship between model data and observed data for dD/dt and increase rate of diameter at 0 hr and 12 hr. The R² values were 0.55 (0 hr) and 0.24 (12 hr). From the correlation values, correlation value at 0 hr showed higher than at 12 hr. Additionally, we observed correlation values in 1 mg/L TiO₂ + 0.2 mg/L OWCs were lower than in 5 mg/L TiO₂ + 80% Cₜ of OWCs (Figure 4.4 and 4.5) that may indicate the effect of the ratio between n-TiO₂ and OWCs on a predictable QSAR model.
Table 4. Calculation of responses at $t = 0$ hr for the 1 mg/L n-TiO$_2$ + 0.2 mg/L of C$_w$ of OWCs

<table>
<thead>
<tr>
<th>Predictors</th>
<th>X</th>
<th>x2</th>
<th>x3</th>
<th>x4</th>
<th>x5</th>
<th>x6</th>
<th>x7</th>
<th>Diameter [nm], $t = 0$h</th>
<th>Diameter [nm] w/o chemicals</th>
<th>$\frac{dD}{dt}$</th>
<th>$k_1$, (nm·L/(min·mg)), $t = 0$h</th>
<th>Increase rate of diameter, $t = 0$ h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Estrone</td>
<td>10.77</td>
<td>3</td>
<td>3.13</td>
<td>270.37</td>
<td>37.3</td>
<td>232.1</td>
<td>1</td>
<td>594</td>
<td>381</td>
<td>23.667</td>
<td>23.667</td>
<td>1.559</td>
</tr>
<tr>
<td>17\text{b}-estradiol</td>
<td>10.71</td>
<td>3.6</td>
<td>4.01</td>
<td>270.39</td>
<td>40.46</td>
<td>232.6</td>
<td>2</td>
<td>552.9</td>
<td>381</td>
<td>19.1</td>
<td>19.1</td>
<td>1.451</td>
</tr>
<tr>
<td>Azithromycin</td>
<td>8.74</td>
<td>7.09</td>
<td>4.02</td>
<td>748.99</td>
<td>180.08</td>
<td>169.9</td>
<td>5</td>
<td>559.9</td>
<td>381</td>
<td>19.874</td>
<td>19.874</td>
<td>1.469</td>
</tr>
<tr>
<td>Cyanazine</td>
<td>0.87</td>
<td>170</td>
<td>2.22</td>
<td>240.7</td>
<td>86.52</td>
<td>179.4</td>
<td>2</td>
<td>465.4</td>
<td>381</td>
<td>9.374</td>
<td>9.374</td>
<td>1.221</td>
</tr>
<tr>
<td>Chlorothalonil</td>
<td>-</td>
<td>0.6</td>
<td>3.05</td>
<td>265.9</td>
<td>47.58</td>
<td>154.6</td>
<td>0</td>
<td>739.7</td>
<td>381</td>
<td>39.852</td>
<td>39.852</td>
<td>1.941</td>
</tr>
<tr>
<td>17\text{a}-Ethinylestradiol</td>
<td>10.33</td>
<td>11.3</td>
<td>3.67</td>
<td>296.41</td>
<td>40.46</td>
<td>244.47</td>
<td>2</td>
<td>515.7</td>
<td>381</td>
<td>14.963</td>
<td>14.963</td>
<td>1.353</td>
</tr>
<tr>
<td>Ibuprofen</td>
<td>4.91</td>
<td>21</td>
<td>3.97</td>
<td>206.28</td>
<td>37.3</td>
<td>200.3</td>
<td>1</td>
<td>597.9</td>
<td>381</td>
<td>24.1</td>
<td>24.1</td>
<td>1.569</td>
</tr>
<tr>
<td>Atrazine</td>
<td>1.3</td>
<td>34.7</td>
<td>2.61</td>
<td>215.69</td>
<td>62.73</td>
<td>169.9</td>
<td>2</td>
<td>545.5</td>
<td>381</td>
<td>18.281</td>
<td>18.281</td>
<td>1.432</td>
</tr>
<tr>
<td>Alachlor</td>
<td>0.62</td>
<td>240</td>
<td>3.52</td>
<td>269.77</td>
<td>29.54</td>
<td>240.9</td>
<td>0</td>
<td>640.3</td>
<td>381</td>
<td>28.815</td>
<td>28.815</td>
<td>1.681</td>
</tr>
<tr>
<td>Lincomycin</td>
<td>7.6</td>
<td>927</td>
<td>0.56</td>
<td>406.54</td>
<td>147.79</td>
<td>313.3</td>
<td>5</td>
<td>735.8</td>
<td>381</td>
<td>33.137</td>
<td>33.137</td>
<td>1.783</td>
</tr>
<tr>
<td>Pendimethalin</td>
<td>2.8</td>
<td>0.3</td>
<td>5.18</td>
<td>281.31</td>
<td>103.67</td>
<td>231.5</td>
<td>1</td>
<td>533.9</td>
<td>381</td>
<td>16.993</td>
<td>16.993</td>
<td>1.401</td>
</tr>
<tr>
<td>Butylate</td>
<td>-</td>
<td>45</td>
<td>4.15</td>
<td>217.41</td>
<td>45.61</td>
<td>211.945</td>
<td>0</td>
<td>554.6</td>
<td>381</td>
<td>19.289</td>
<td>19.289</td>
<td>1.456</td>
</tr>
</tbody>
</table>
Table 4. Calculation of responses \( t = 12 \text{ hr} \) for the 1 mg/L n-TiO$_2$ + 0.2 mg/L of C$_w$ of OWCs

<table>
<thead>
<tr>
<th>Predictors</th>
<th>x1</th>
<th>x2</th>
<th>x3</th>
<th>x4</th>
<th>x5</th>
<th>x6</th>
<th>x7</th>
<th>Diameter [nm], ( t = 12 \text{ h} )</th>
<th>Diameter [nm] w/o chemicals</th>
<th>Response</th>
</tr>
</thead>
<tbody>
<tr>
<td>Compounds</td>
<td>pK$_a$</td>
<td>Cw</td>
<td>log K$_{ow}$</td>
<td>M.W. [g/mol]</td>
<td>Polar Surface Area ([\text{Å}^2])</td>
<td>Molar volume ([\text{cm}^3])</td>
<td>#H bond donors</td>
<td>dD/dt</td>
<td>k$_c$, (nm·L)/(min·mg), ( t = 12 \text{ hr} )</td>
<td>Increase rate of diameter, ( t = 12 \text{ h} )</td>
</tr>
<tr>
<td>Azithromycin</td>
<td>8.74</td>
<td>7.09</td>
<td>4.02</td>
<td>748.99</td>
<td>180.08</td>
<td>169.9</td>
<td>5</td>
<td>948.2</td>
<td>735.8</td>
<td>0.295</td>
</tr>
<tr>
<td>17b-estradiol</td>
<td>10.71</td>
<td>3.6</td>
<td>4.01</td>
<td>270.39</td>
<td>40.46</td>
<td>232.6</td>
<td>2</td>
<td>938.4</td>
<td>735.8</td>
<td>0.281</td>
</tr>
<tr>
<td>Estrone</td>
<td>10.77</td>
<td>3</td>
<td>3.13</td>
<td>270.37</td>
<td>37.3</td>
<td>232.1</td>
<td>1</td>
<td>1018</td>
<td>735.8</td>
<td>0.392</td>
</tr>
<tr>
<td>Ibuprofen</td>
<td>4.91</td>
<td>21</td>
<td>3.97</td>
<td>206.28</td>
<td>37.3</td>
<td>200.3</td>
<td>1</td>
<td>955.9</td>
<td>735.8</td>
<td>0.306</td>
</tr>
<tr>
<td>Atrazine</td>
<td>1.3</td>
<td>34.7</td>
<td>2.61</td>
<td>215.69</td>
<td>62.73</td>
<td>169.9</td>
<td>2</td>
<td>1016.3</td>
<td>735.8</td>
<td>0.39</td>
</tr>
<tr>
<td>Chlorothalonil</td>
<td>-</td>
<td>0.6</td>
<td>3.05</td>
<td>265.9</td>
<td>47.58</td>
<td>154.6</td>
<td>0</td>
<td>1105.7</td>
<td>735.8</td>
<td>0.514</td>
</tr>
<tr>
<td>Cyanazine</td>
<td>0.87</td>
<td>170</td>
<td>2.22</td>
<td>240.7</td>
<td>86.52</td>
<td>179.4</td>
<td>2</td>
<td>982.1</td>
<td>735.8</td>
<td>0.342</td>
</tr>
<tr>
<td>Alachlor</td>
<td>0.62</td>
<td>240</td>
<td>3.52</td>
<td>269.77</td>
<td>29.54</td>
<td>240.9</td>
<td>0</td>
<td>1172.2</td>
<td>735.8</td>
<td>0.606</td>
</tr>
<tr>
<td>17a-Ethinylestradiol</td>
<td>10.33</td>
<td>11.3</td>
<td>3.67</td>
<td>296.41</td>
<td>40.46</td>
<td>244.467</td>
<td>2</td>
<td>882.1</td>
<td>735.8</td>
<td>0.203</td>
</tr>
<tr>
<td>Lincomycin</td>
<td>7.6</td>
<td>927</td>
<td>0.56</td>
<td>406.54</td>
<td>147.79</td>
<td>313.3</td>
<td>5</td>
<td>972.6</td>
<td>735.8</td>
<td>0.329</td>
</tr>
<tr>
<td>Pendimethalin</td>
<td>2.8</td>
<td>0.3</td>
<td>5.18</td>
<td>281.31</td>
<td>103.67</td>
<td>231.5</td>
<td>1</td>
<td>999.8</td>
<td>735.8</td>
<td>0.367</td>
</tr>
<tr>
<td>Butylate</td>
<td>-</td>
<td>45</td>
<td>4.15</td>
<td>217.41</td>
<td>45.61</td>
<td>211.945</td>
<td>0</td>
<td>788.5</td>
<td>735.8</td>
<td>0.073</td>
</tr>
</tbody>
</table>
Table 4. Estimated coefficients and p-values created by MLR using `fitlm` function at 0 hr and 12 hr

<table>
<thead>
<tr>
<th>Coefficient</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \frac{dD}{dt} )</td>
</tr>
<tr>
<td>0 hr</td>
</tr>
<tr>
<td>Coefficient</td>
</tr>
<tr>
<td>( \frac{dD}{dt} )</td>
</tr>
<tr>
<td>p-value</td>
</tr>
<tr>
<td>Increase rate of diameter</td>
</tr>
<tr>
<td>p-value</td>
</tr>
<tr>
<td>( k_1 )</td>
</tr>
<tr>
<td>p-value</td>
</tr>
<tr>
<td>12 hr</td>
</tr>
<tr>
<td>Coefficient</td>
</tr>
<tr>
<td>( \frac{dD}{dt} )</td>
</tr>
<tr>
<td>p-value</td>
</tr>
<tr>
<td>Increase rate of diameter</td>
</tr>
<tr>
<td>p-value</td>
</tr>
<tr>
<td>( k_2 )</td>
</tr>
<tr>
<td>p-value</td>
</tr>
</tbody>
</table>
Figure 4. Relationship between modeled response and observed response for \( \frac{dD}{dt} \) (A) and increase rate in diameter (B) using coefficients by \texttt{fitlm} function at 0 hr.
Figure 4. 13 Relationship between modeled response and observed response for $dD/dt$ (A) and increase rate in diameter (B) using coefficients by fitlm function at 12 hr
4.3.3.2 MLR QSAR models using stepwiselm function

Table 4.15, 4.16, 4.17, and 4.18 showed models estimated by stepwiselm algorithms for dD/dt and increase rate of diameter. Figure 4.14, 4.15, 4.16, and 4.17 presented relationship between modeled response and observed response based on coefficients in Table 4.15, 4.16, 4.17, and 4.18, respectively. As a result of stepwiselm model for 0 hr, p values of each predictor in Tables (4.15 and 4.16) both dD/dt and increase rate of diameter used in a predicted QSAR model that the relationship between modeled response and observed response resulted in a low correlation coefficient ($R^2 = 0.1$) (Figure 4.14 and 4.15).

For 12 hr, a predicted QSAR model was developed by four predictors that were pK$_a$, log K$_ow$, M.W., and #H bond donor, which were shown in Table 4.17 and 4.18. Particularly, p-value of log K$_ow$, M.W., and #H bond donor showed statistically significant ($p < 0.05$) and $R^2$ of a QASR model was 0.8, while adj.$R^2$ was 0.64. Using coefficients developed in Table 4.17 and 4.18, we evaluated the relationship between modeled response and observed response that were shown in Figure 4.16 and 4.17. The correlation coefficient showed low correlation coefficient with 0.25.

From results of QSAR models, we obtained different predictable QSAR models and relationship between modeled response and observed response according to the ratio of [n-TiO$_2$]/[OWCs], which may be an important key to predict a QSAR model of nanoparticle stability in the presence of organic compounds in aquatic systems. In addition, generally QSAR models at 0 hr showed higher $R^2$ values than $R^2$ values at 12 hr, which may show the direct effect of descriptors of OWCs on the aggregation of n-TiO$_2$. 
Table 4. 15 Estimated coefficients by stepwiselm regression model for dD/dt at t = 0 hr

<table>
<thead>
<tr>
<th>Variable</th>
<th>Predictor (x)</th>
<th>Coefficient</th>
<th>SE</th>
<th>t Stat</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>a0</td>
<td>Constant</td>
<td>17.1275</td>
<td>3.4737</td>
<td>4.9307</td>
<td>0.0017</td>
</tr>
<tr>
<td>a1</td>
<td>pK_a, x1</td>
<td>0.2610</td>
<td>0.4632</td>
<td>0.5636</td>
<td>0.5906</td>
</tr>
<tr>
<td>a2</td>
<td>C_w, x2</td>
<td>0.0153</td>
<td>0.0068</td>
<td>2.2425</td>
<td>0.0599</td>
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</tbody>
</table>

Y = a0 + a1·x1 + a2·x2 (R^2 = 0.43, Adj. R^2 = 0.26)

Figure 4. 14 Relationship between modeled response and observed response for dD/dt using coefficients by stepwiselm function at 0 hr
Table 4. Estimated coefficients by stepwiselm regression model for increase rate of diameter at $t = 0$ hr

<table>
<thead>
<tr>
<th>Variable</th>
<th>Predictor (x)</th>
<th>Coefficient</th>
<th>SE</th>
<th>t Stat</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>a0</td>
<td>Constant</td>
<td>1.404</td>
<td>0.082</td>
<td>17.092</td>
<td>5.76E-07</td>
</tr>
<tr>
<td>a1</td>
<td>$pK_a, x_1$</td>
<td>0.006</td>
<td>0.011</td>
<td>0.561</td>
<td>5.92E-01</td>
</tr>
<tr>
<td>a2</td>
<td>$C_w, x_2$</td>
<td>0.000</td>
<td>0.000</td>
<td>2.243</td>
<td>5.98E-02</td>
</tr>
</tbody>
</table>

$Y = a_0 + a_1 \cdot x_1 + a_2 \cdot x_2 \ (R^2 = 0.43, \text{ Adj. } R^2 = 0.26)$

Figure 4. Relationship between modeled response and observed response for increase rate of diameter using coefficients by stepwiselm function at $0$ hr
Table 4. 17 Estimated coefficients by stepwiselm regression model for dD/dt at t = 12 hr

<table>
<thead>
<tr>
<th>Variable</th>
<th>Predictor (x)</th>
<th>Coefficient</th>
<th>SE</th>
<th>t Stat</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>a0</td>
<td>Constant</td>
<td>0.5980</td>
<td>0.0870</td>
<td>6.8702</td>
<td>0.0010</td>
</tr>
<tr>
<td>a1</td>
<td>pH, x1</td>
<td>-0.0096</td>
<td>0.0056</td>
<td>-1.7100</td>
<td>0.1480</td>
</tr>
<tr>
<td>a3</td>
<td>log K_{ow}, x3</td>
<td>-0.0688</td>
<td>0.0267</td>
<td>-2.5719</td>
<td>0.0499</td>
</tr>
<tr>
<td>a4</td>
<td>M.W., x4</td>
<td>0.0008</td>
<td>0.0003</td>
<td>2.8282</td>
<td>0.0368</td>
</tr>
<tr>
<td>a7</td>
<td>#H bond donor, x7</td>
<td>-0.1052</td>
<td>0.0322</td>
<td>-3.2615</td>
<td>0.0224</td>
</tr>
</tbody>
</table>

\[ Y = a_0 + a_1 \cdot x_1 + a_3 \cdot x_3 + a_4 \cdot x_4 + a_7 \cdot x_7 \quad (R^2 = 0.8, \text{ Adj. } R^2 = 0.64) \]

Figure 4. 16 Relationship between modeled response and observed response for dD/dt using coefficients by stepwiselm function at 12 hr
Table 4. Estimated coefficients by stepwiselm regression model for increase rate of diameter at $t = 12$ hr

<table>
<thead>
<tr>
<th>Variable</th>
<th>Predictor (x)</th>
<th>Coefficient</th>
<th>SE</th>
<th>t Stat</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>a0</td>
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<td>1.585</td>
<td>8.49E-02</td>
<td>18.658</td>
<td>0.00001</td>
</tr>
<tr>
<td>a1</td>
<td>pK_a, x1</td>
<td>-0.009</td>
<td>5.48E-03</td>
<td>-1.716</td>
<td>0.147</td>
</tr>
<tr>
<td>a3</td>
<td>log $K_{ow}$, x3</td>
<td>-0.067</td>
<td>2.61E-02</td>
<td>-2.578</td>
<td>0.050</td>
</tr>
<tr>
<td>a4</td>
<td>M.W., x4</td>
<td>0.001</td>
<td>2.76E-04</td>
<td>2.839</td>
<td>0.036</td>
</tr>
<tr>
<td>a7</td>
<td>#H bond donor, x7</td>
<td>-0.103</td>
<td>3.15E-02</td>
<td>-3.269</td>
<td>0.022</td>
</tr>
</tbody>
</table>

$Y = a0 + a1 \cdot x1 + a3 \cdot x3 + a4 \cdot x4 + a7 \cdot x7$ ( $R^2 = 0.8$, Adj. $R^2 = 0.64$)

Figure 4. Relationship between modeled response and observed response for increase rate of diameter using coefficients by stepwiselm function at 12 hr.
4.4 References


CHAPTER 5 DEPOSITION OF n-TiO$_2$ ON ROUGH SURFACES USING QUARTZ CRYSTAL MICROBALANCE WITH DISSIPATION MONITORING (QCM-D)

5.1 Introduction

Nanoparticles (NPs) such as TiO$_2$ nanoparticles (n-TiO$_2$) have recently been widely used in various consumer products [1]. Due to large uses of NPs, detections of NPs in the aquatic environment as well as the effluent from wastewater treatment plants (WWTPs) have been reported in literatures [2-5]. In order to evaluate their potential toxicity in the ecosystem [6-8], it is essential to understand the transport and fate of NPs after they were released into environment.

The deposition of nanoparticles onto soil or sediment grain surfaces is an important process related to their environmental fate and transport [9]. The transport of sub-micron particles is governed by convection and diffusion, whereas transport of micron-sized particles is dominated by the gravitation force [9, 10]. In case of nanoparticles, Brownian diffusion is the main mechanism for transport [11]. The attachment of nanoparticles is governed by interactions between particles and collector surfaces. The Derjaguin-Landau-Verwey-Overbeek (DLVO) theory, which accounts the sum of van der Waals interactions and electrical double layer interactions, was used to estimate the interaction energy [9, 11]. However, a few studies demonstrated discrepancies of measured and theoretically calculated attachment efficiency. Ignoring the surface roughness of particle and collectors in the DLVO theory was considered as an important reason that led to the discrepancy [9, 12, 13]. Under unfavorable condition (i.e. particles and surfaces are oppositely charged), they showed that the height of the energy
barrier will decrease with the increase of surface roughness, due to the decreased the radii of curvature of the protrusions. In addition, surface roughness on collectors could provide more surface areas that enhance the interaction between particles and collectors, which could lead to a higher deposition rate of nanoparticles [14].

To investigate the transport and attachment of nanoparticles in porous media, the quartz crystal microbalance with dissipation (QCM-D) have been previously used to measure the deposition of nanoparticles onto solid surfaces [15-19]. The QCM-D technique is able to elucidate the interaction mechanism between solid and liquid phases using Sauerbrey relationship and dissipation energy [20].

In this study, we use n-TiO$_2$ as a model nanoparticle to study deposition and transport of nanoparticles in aquatic environment. The purpose is to examine the deposition of n-TiO$_2$ on collector surfaces with various physical (e.g. surface roughness) and chemicals (e.g. Al$_2$O$_3$ or SiO$_2$) properties, which represent important components of soil and sediment surfaces. To generate different surface roughness, we modified the QCM cell surface with 100 nm Si slanted columnar thin films (Si-SCTFs) coated with Al$_2$O$_3$ or SiO$_2$ atomic layer deposition (ALD, approximately 4 nm thickness). Particularly, we use a new technique that combines the spectroscopic ellipsometry (SE) with QCM-D experiment. In this equipment, QCM-D can determine the total amount of attached particles, including attached particles and water. SE can differentiate the refraction index between attached nanoparticles and water, so that it can determine an effective thickness parameter representing only the attached nanoparticles. Therefore, the QCM-D/SE would provide not only a deposition rate as typical QCM-D, but also surface properties (e.g. volume fraction) of the attached m-TiO$_2$ particle film.
5.2 Material and Methods

5.2.1 Preparation of n-TiO₂ suspensions

Two different types of TiO₂ nanoparticles (n-TiO₂) were used to prepare n-TiO₂ suspensions. n-TiO₂ suspensions of 5 mg /L were prepared using n-TiO₂ powder (rutile, 10×40 nm, Nanostructured & Amorphous Material Inc.). The second type of n-TiO₂ suspensions were prepared using a 1.5 mg/ml solution of n-TiO₂ anatase crystals stabilized in sodium polyacrylate (Sciventions Inc.).

To make 5 mg/L n-TiO₂ suspension, desirable amount of n-TiO₂ (1.5 mg n-TiO₂ powder; 1 ml n-TiO₂-sodium polyacrylate) was added into 300 ml electrolyte stock solution (DI water, 10 mM NaCl, and 100 mM NaCl). Both suspensions were immediately stirred on a magnetic plate for 30 s followed by sonication in an ultrasonic water bath (FS 60, 100 W, 42 kHz, Fisher Scientific, Pittsburg, PA) for 1 hr. After sonication, the flask was cooled to 25 °C and 0.01 M NaOH was added to adjust pH to 7 ± 0.1. In addition, to modify the surface of n-TiO₂, humic acid stock solution with 1000 mg/L was prepared in DI water and was diluted into n-TiO₂ suspension with 8.4 mg/L in final concentration. Humic acid was purchased from Fisher Scientific.

The particle size and zeta potential were measured in triplicates. Particle size was measured using a 90Plus Particle Size Anlayzer (Brookhaven Instruments Corporation, Holtsville, NY) based on a dynamic light scattering (DLS) principle. Zeta potential was obtained using a ZetaPALS zeta potential analyzer (Brookhaven Instruments Corporation, Holtsville, NY) that utilizes phase analysis light scattering (PALS) technique, an extension of electrophoretic light scattering, to measure the electrophoretic mobility, which was converted to zeta potential base on a Smoluchowski equation.
5.2.2 Combined quartz crystal microbalance with dissipation (QCM-D) and spectroscopic ellipsometry (SE) Experiments

The combination of QCM-D and SE is an emerging analytical technique, which consists of two commonly used noninvasive surface characterization techniques operated in tandem. In particular, SE is sensitive to the mass of adsorbed molecules only, whereas the mass determined by QCM-D also includes solvent that is coupled to the film. Thus, a comparison of the mass measured by the two techniques provides information about the amount of solvent in film. Therefore, properties of attached film, including thickness, surface density, and volume or mass fraction, can be obtained in situ. QCM-D/SE at UNL was assembled using a commercially-available liquid cell equipped with a QCM-D apparatus (Q-Sense E1 SE) with an optical access window configured for a spectroscopic ellipsometer (M-2000-UV, J.A. Woollam Company) at 60 - 65° angle of incident. The novel analytical approach, called QCM-D/SE can provide crucial insight into formation and conformation kinetics of surface deposition.

Prior to QCM-D/SE experiments, QCM crystal sensor surface was first equilibrated with background buffer solution (DI water, 10 mM NaCl, and 100 mM NaCl) at a flow rate of 0.1 ml/min until base line reaches stable. Then, n-TiO₂ suspension in buffer solution was injected into sensor chamber at a flow rate of 0.1 ml/min [15]. Finally, background solution was injected into sensor chamber at 0.1 ml/min and some cases 10 mM HCl was injected into sensor chamber for at least 10 min. QCM-D is used to determine the mass of absorbate on an oscillating piezoelectric material by measuring shifts of vibrational frequency odd overtones (3,5,…,13). When the adsorbed materials are assumed rigid, Sauerbrey equation [21] is generally used to explain a linear
relationship between frequency shifts and the surface density $\Gamma_{QCM} \left( \frac{ng}{cm^2} \right)$ of deposited n-TiO$_2$:

$$\Gamma_{QCM} \left( \frac{ng}{cm^2} \right) = \rho_{eff} d_{QCM} = -\Delta f_n C \quad (5-1)$$

where $\rho_{eff}$ is the average effective density of thickness of absorbate, $d_{QCM}$ is the total thin thickness included ambient liquid, $-\Delta f_n$ is the frequency shift [Hz], and $C$ is the crystal constant (17.7 ng/Hz·cm$^2$). Additionally, dissipation shift is measured to determine the viscoelasticity for rigid film [22]. The frequency and dissipation shift are monitored at odd overtone ($n = 3$) that is the fundamental resonance.

Simultaneously, measurements can sensitively detect the polarization state of light from a surface that that data are expressed by tow parameters, $\tan \Psi$ and $\Delta$, for relative amplitude ratio and relative phase shift, respectively [22, 23]. The general equation is:

$$F = \frac{R_p}{R_s} = \tan \Psi \cdot \exp (i\Delta) \quad (5-2)$$

where $F$ is the complex ratio, $R_p$ (p-polarized electrical field) and $R_s$ (s-polarized electrical field) are Fresnel-reflection-coefficients and $i$ is the number of layers (0, 1, 2 …). $F$ is consisted with complex parameters (angle of incident $\Phi_o$, the wavelength $\lambda$, the optical constants of the substrate $N_s$, the ambient medium $n_o$, the layers $n_i$ and $k_i$, and the layer thickness $d_i$).

$\delta \Psi$ and $\delta \Delta$ are related with the Fresnel reflection parameters $R_p$ and $R_s$ that are generated by the formation of ultra-thin film on the surface in the bulk, which is related with the constituent adsorbate materials [24]. SE data was converted using a basis–spline (B-spline) function in an optical box model to identify the refraction of thickness by
constant \( n \) and \( k \) [24-26]. The surface density of nanoparticles within and on SCTFs was obtained by mueller matrix measurements based on anisotropic Bruggeman effective medium approximation (AB-EMA). The details about SE data analysis are discussed in the literature [22]. From the AB-EMA model, surface density of nanoparticles in bulk solution, \( \Gamma_{SE} \) can be estimated as [22-25]:

\[
\Gamma_{SE} = \rho_{n-TiO_2} f_{EMA} SF
\]  (5-3)

where \( \Gamma_{SE} \) [ng/cm\(^2\)] is the surface density of n-TiO\(_2\) suspension within SCTFs, \( \rho_{n-TiO_2} \) is the density of n-TiO\(_2\) (4.26 g/cm\(^3\)), \( f_{EMA} \) is the EMA buffer fraction of n-TiO\(_2\), and \( SF \) is the geometric scaling factor (59.5) for SCTFs. Finally, the EMA buffer fraction on SCTFs could be obtained from AB-EMA using the dispersion relation for dry SCTFs based on equation 1.

Finally, mass fraction of surface density was calculated as a simple ratio of \( [\Gamma_{SE}] / [\Gamma_{QCM}] \) based on the relationship between SE and QCM-D measurements which indicated porosity as a mass fraction parameter [27].

5.2.3 Preparation of surfaces

Deposition of n-TiO\(_2\) onto both flat and rough surfaces was investigated. To simulate different types of aquifer material surface properties, flat gold QCM sensors (Q-Sense Inc.) were coated with either Al\(_2\)O\(_3\) or SiO\(_2\) by atomic layer deposition (ALD, Fiji 200 Cambridge Nanotech) (Figure 5.1(A)). To simulate roughness aquifer material surfaces, Si-slanted columnar thin films (Si-SCTFs) with a length of 100 nm and a slant angle of 65° angles was deposited onto the flat sensor surfaces using a glancing angle electron beam deposition (GLAD) [28, 29], a physical vapor deposition technique.
facilitated by electron beam evaporation. This approach results in SCTFs that possess uniform, highly-ordered, highly coherent and controllable architectures ranging in size and shape from nano- to micro-scales, depending on the vapor flux angle, time, and surface diffusion [28, 30]. These nanostructures are aligned with respect to a single characteristic axis and may be arranged in regular or irregular patterns. As parallel to flat surface, SCTF surfaces were subsequently-coated with Al₂O₃ or SiO₂ using ALD (Figure 5.1(B)) to simulate various properties of aquifer materials.
Figure 5. (A) Flat surface: Al$_2$O$_3$ or SiO$_2$ ALD and (B) Roughness surface: Si-SCTF with Al$_2$O$_3$ or SiO$_2$ ALD on QCM gold crystal sensor with a schematic and SEM of SCTF by NOVA NANOSEM 450.
Figure 5. 2 Picture of QCM-D/SE experimental setup
<table>
<thead>
<tr>
<th>Surface</th>
<th>Ionic Strength</th>
<th>Type of TiO₂</th>
<th>Humic Acid [HA, 8.4 mg/L]</th>
<th>Average Zeta Potential [mV]</th>
<th>Average Diameter by Number Intensity [nm]</th>
</tr>
</thead>
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<tr>
<td>Flat Al₂O₃</td>
<td>DI water</td>
<td>Stock solution (a)</td>
<td>-</td>
<td>-19.6</td>
<td>2.9</td>
</tr>
<tr>
<td></td>
<td></td>
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<td>HA</td>
<td>-18.5 (w/o HA)</td>
<td>117.4 (w/o HA)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Powder (b-2)</td>
<td>HA</td>
<td>-27.4 (w/ HA)</td>
<td>43.5 (w/ HA)</td>
</tr>
<tr>
<td>Flat SiO₂</td>
<td>DI water</td>
<td>Stock solution (c)</td>
<td>-</td>
<td>-20.5</td>
<td>12.6</td>
</tr>
<tr>
<td>Rough Surface</td>
<td>DI water</td>
<td>Stock solution (d)</td>
<td>-</td>
<td>-19.6</td>
<td>9.8</td>
</tr>
<tr>
<td>Si-STF 100 nm</td>
<td>10 mM NaCl</td>
<td>Stock solution (e)</td>
<td>-</td>
<td>-23.6</td>
<td>12.3</td>
</tr>
<tr>
<td>Al₂O₃ ALD</td>
<td>100 mM NaCl</td>
<td>Stock solution (f)</td>
<td>-</td>
<td>-5.8</td>
<td>42.8</td>
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<tr>
<td>Rough Surface</td>
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<td>Stock solution (h)</td>
<td>-</td>
<td>-7.3</td>
<td>12.5</td>
</tr>
<tr>
<td>Si-STF 100 nm</td>
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<td>Stock solution (i)</td>
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<td>-8.6</td>
<td>59.3</td>
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<tr>
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<td>Stock solution (i)</td>
<td>-</td>
<td>-8.6</td>
<td>59.3</td>
</tr>
</tbody>
</table>
5.3 Results

5.3.1 Deposition and release of n-TiO$_2$ on flat surfaces under favorable condition

The deposition of n-TiO$_2$ on flat surfaces coated with alumina surface (Al$_2$O$_3$) or silica surface (SiO$_2$) was conducted using QCM-D. Figure 5.3 (A) shows changing frequency ($f_3$) and dissipation ($D_3$) that are representative normalized QCM-D measurements as the deposition of n-TiO$_2$ occurred on the surfaces at pH 7 in DI water. This condition is favorable deposition condition, where n-TiO$_2$ is negatively charged and flat Al$_2$O$_3$ surface is positively charged. According to the DLVO theory, attractive interaction energy dominates the deposition process. When n-TiO$_2$ suspension was injected onto flat Al$_2$O$_3$ surface at a flow rate of 0.1 ml/min, we observed a decrease of frequency shifts and an increase dissipation due to the deposition of n-TiO$_2$. The frequency shift was dramatically changed linearly after injection n-TiO$_2$ solution within 20 min, and then little frequency shifts were observed after 20 min. The frequency shift changes over 20 min was almost -10 Hz. Simultaneously, the dissipation (D) also was changed by the deposition of n-TiO$_2$ (Figure 5.3 (A)). The dissipation shift was about 0.8 $\times$ 10$^{-6}$ while frequency shift occurred. The shift dissipation (D) accounts for amount of energy loss due to the deposition of particles on surfaces, therefore the dissipation can be used for monitoring the deposition process between particles and surfaces [16]. Our observation also showed the dissipation shifts according to the deposition of n-TiO$_2$ on the surface. Generally, the deposition mass of n-TiO$_2$ can be calculated by Sauerbrey relationships (equation 5-1). The deposition mass of n-TiO$_2$ on flat Al$_2$O$_3$ surface was proportional to the frequency shift, so the mass of deposition was calculated based on the frequency changing (Figure 5.3 (B)).
Once the frequency shift was stopped which indicated the saturation of n-TiO$_2$ on flat Al$_2$O$_3$ surfaces, DI water buffer solution was injected for 20 min to stabilize the frequency and dissipation. During this rinsing process, the release of n-TiO$_2$ was not observed from the flat Al$_2$O$_3$ surface because little frequency and dissipation shifts were observed. This indicates that deposition of n-TiO$_2$ on the flat Al$_2$O$_3$ surface is very stable. Following DI water, we injected 10 mM HCl (pH 2) solution on the surface, which led to observable frequency shift (Figure 5.3 (A)). The slight increase of dissipation in this stage is unexpected. Poitras and Tufenkji (2009) reports that dissipation is more useful sensor than frequency for bacteria detecting because dissipation shifts are sensitively responding on between bacteria and crystal surface due to high viscoelasticity of bacteria [31]. In case of n-TiO$_2$, it may be too rigid to the flat Al$_2$O$_3$ surfaces, so that dissipation was less sensitive than frequency. At the same time, we calculated the surface density of n-TiO$_2$ on flat Al$_2$O$_3$ surface using equation 5-1 and 5-3, which provided the deposition density of n-TiO$_2$ with water ($\Gamma_{QCM}$) and without water ($\Gamma_{SE}$), respectively. The results of surface density showed in Figure 5.3 (B). The maximum surface density of n-TiO$_2$ on flat Al$_2$O$_3$ surface was about 175 ng/cm$^2$ and 100 ng/cm$^2$ for $\Gamma_{QCM}$ and $\Gamma_{SE}$.

To investigate the effect of ionic strength on deposition of n-TiO$_2$ on flat Al$_2$O$_3$ surfaces, we used 10 mM NaCl as background electrolyte solution. Figure 5.4 (A) showed the frequency/dissipation shifts by the deposition of n-TiO$_2$ in 10 mM NaCl. A decrease of frequency and an increase of dissipation were observed due to the deposition of n-TiO$_2$. After the injection of n-TiO$_2$ for 20min, flat Al$_2$O$_3$ surface was flushed with background electrolyte of 10 mM NaCl for 20 min that led to slight increase of frequency and dissipation. Please note that no increased frequency was occurred in the first
experiment (Figure 5.3 (A)) when deposited n-TiO$_2$ was rinsed by DI water buffer solution, which indicated very stable deposition. Here, we observed the increase of frequency when injecting 10 mM NaCl. In spite of low ionic strength (10 mM NaCl), sodium cation could lead to reduce the deposition of n-TiO$_2$ (with negative charge) on Al$_2$O$_3$ surface (with positive charge) at pH 7 that resulted in some release of n-TiO$_2$ from Al$_2$O$_3$ surface.

In addition, the surface density of n-TiO$_2$ on flat Al$_2$O$_3$ surfaces was investigated in 10 mM NaCl (Figure 5.4 (B)). The surface density of $\Gamma_{QCM}$ and $\Gamma_{SE}$ were increased linearly and reached to the maximum deposition of n-TiO$_2$ within 20 min after injection of n-TiO$_2$ solution. Then, the injection of 10 mM NaCl caused the decrease of the surface density of n-TiO$_2$. Particularly, the maximum deposition of n-TiO$_2$ observed in DI water was 1.7 times and 2 times higher than in 10 mM for NaCl $\Gamma_{QCM}$ and $\Gamma_{SE}$. From the results, we observed that background ionic strength solution could influence on the deposition behavior of n-TiO$_2$ on surface. More deposition of n-TiO$_2$ in DI water than in 10 mM NaCl under favorable condition is expected and consistent with the DLVO theory.

Figure 5.3 (C) and 5.4 (C) presented the mass fraction of surface density between in DI water and in 10 mM NaCl, respectively. The mass fraction of surface density could be explained with porosity that was adsorbate fraction parameter for deposition growth [24]. The mass fraction of surface density increased as deposited mass of n-TiO$_2$ increased during n-TiO$_2$ injection until flat surface was occupied with n-TiO$_2$. Then, the mass fraction of surface density was stable at maximum deposition of n-TiO$_2$. The mass fraction of surface density was 0.6 in DI water and 0.4 in 10 mM NaCl.
5.3.2 Deposition and release of n-TiO$_2$ on flat surfaces under unfavorable condition

Figure 5.5 showed the frequency and dissipation shifts in DI water under unfavorable deposition between n-TiO$_2$ and flat SiO$_2$ surface at pH 7, where both n-TiO$_2$ and SiO$_2$ surface were negative charge. During the injection of n-TiO$_2$, no frequency or dissipation shift was observed, which implicated that no n-TiO$_2$ deposition occurred on flat SiO$_2$ surface due to unfavorable conditions. The results were consistent with previous studies [16, 19] and the DLVO theory. Under such condition, DLVO theory will predict a significant energy barrier between n-TiO$_2$ and that prevent deposition from happening. Johnson et al. (1996) demonstrated that the deposition rate of colloid was directly related with favorable surface charges as well as favorable surface fraction [32]. SiO$_2$ surface used in the QCM-D/SE experiment is pure and no favorable surface charge is expected. Therefore, it is not surprising that no deposition was observed. In conclusion, we observed no deposition of n-TiO$_2$ under unfavorable conditions. Under favorable conditions, deposition of n-TiO$_2$ is influenced by ionic strength. Less deposition was observed in 10 mM NaCl than in DI water.
Figure 5. 3 QCM-D/SE measurements on flat Al$_2$O$_3$ ALD in DI water (Table 5.1 (a)): (A) Frequency and dissipation, (B) surface density of n-TiO$_2$ both QCM and SE calculation, and (C) mass fraction as a ratio of surface density [SE/QCM].
Figure 5. 4 QCM-D/SE measurements on flat Al$_2$O$_3$ ALD in 10 mM NaCl (Table 5.1 (c)): (A) Frequency and dissipation, (B) surface density of n-TiO$_2$ both QCM and SE calculation, and (C) mass fraction as a ratio of surface density [SE/QCM].
Figure 5. QCM-D/SE measurements on flat SiO$_2$ ALD in DI water (Table 5.1 (g)):

Frequency and dissipation shifts.
5.3.3 n-TiO$_2$ deposition onto rough surfaces

5.3.3.1 Deposition under favorable condition

Deposition of n-TiO$_2$ on Al$_2$O$_3$ coated 100 nm SCTF rough surface was investigated under different IS conditions (DI water, Figure 5.6; 10 mM NaCl, Figure 5.7; and 100 mM NaCl, Figure 5.8) Figure 5.6 (A) (i.e. DI water condition) showed the significant frequency and dissipation shifts following injection of n-TiO$_2$. These conditions were favorable condition between n-TiO$_2$ and a rough surface, where n-TiO$_2$ was negatively charges and the rough SCTF Al$_2$O$_3$ surface was positively charged. According to DLVO theory, attractive interactions will dominate between them. When we compared these observations with the deposition onto the flat Al$_2$O$_3$ surface (Figure 5.3), it is very obvious that more n-TiO$_2$ particles were deposited onto the SCTF rough surface than the flat surface. The surface density of deposited n-TiO$_2$ on the rough surface were about 34 times and 15 times larger than in a flat surface for $\Gamma_{QCM}$ and $\Gamma_{SE}$, respectively. Recent studies have investigated the deposition of particles at rough surfaces, which also found that surface roughness played a critical role on the interactions between particles and collector surfaces [13, 14, 33, 34]. Darbha et al., (2012) showed that higher surface roughness ($R_q = 500$-$2000$ nm) resulted in more deposition of colloids compared to lower surface roughness ($R_q < 500$ nm). Eichenlaub et al., (2004) demonstrated that larger surface roughness presented larger adhesion forces. Shellenberger and Logan (2002) measured the collision efficiency of glass beads with different surface roughness, which demonstrated larger collision efficiency of bacteria on surfaces with larger roughness. In addition, Huang et al., (2009) investigated the effects of surface roughness in the aspect of interaction energy between particles and surface
roughness. They found that surface roughness may result in reduction of interaction energy. Here, our observation of larger deposition of n-TiO$_2$ further emphasizes the roughness effects on particle deposition, i.e. higher collision efficiency and larger adhesion forces.

To investigate the effects of background electrolyte on the deposition of n-TiO$_2$, we conducted the deposition experiments on a same rough surface in two different ionic strength, i.e 10 mM and 100 mM NaCl (Figure 5.7 and 5.8). In 10 mM NaCl, frequency and dissipation shifts were dramatically changed after injection of n-TiO$_2$, similar trends as in DI water (Figure 5.7 (A)). In 10 mM NaCl (Figure 5.8 (A)), both frequency and dissipation shifts reached more quickly to stable (i.e. saturation of deposition on the rough surface) than in DI water and 10 mM NaCl. In addition, the surface density in 10 mM NaCl and 100 mM NaCl are different compared to the surface density in DI water (Figure 5.7 (B)). The maximum surface density of n-TiO$_2$ on a rough surface in 10 mM and 100 mM NaCl detected in the deposition experiments were approximately 8000 ng/cm$^2$ and 850 ng/cm$^2$ for $\Gamma_{QCM}$ and 420 ng/cm$^2$ and 870 ng/cm$^2$ for $\Gamma_{SE}$ (Figure 5.7 (B) and 5.8 (B)), respectively.

From the frequency and dissipattion changes in different IS conditions, we observed the effect of IS on the deposition of n-TiO$_2$. The surface density of $\Gamma_{SE}$ is the largest in DI water than in the other ionic strength conditions, which is the expected and consisten with DLVO theory. The trend for the surface density of $\Gamma_{QCM}$ in 10 mM NaCl, however, is unexpected. It was likely that cation concentrations (Na$^+$) may enhance the surface density with increasing ionic strength. Elimelech and O’Mella (1990) indicated that maximum attachment of colloids in porous media occurred at 10 mM CaCl$_2$ but
attachment efficiency decreased when concentration exceed 10 mM CaCl₂ due to increased repulsion of cation concentrations.

Figure 5.6 (C), 5.7 (C), and 5.8 (C) presented the mass fraction of surface density in DI water, in 10 mM NaCl, and 100 mM NaCl under favorable condition, respectively. The highest mass fraction of surface density was 0.8 in 100 mM NaCl and the lowest mass fraction was 0.06 in 10 mM NaCl, while the mass fraction was 0.4 in DI water. However, little changes of mass fraction was observed when ΓSE was stable. These results presented that IS could influence on the mass fraction, however the highest surface deposition density of n-TiO₂ was in DI water than in 100 mM NaCl although 100 mM NaCl showed higher mass fraction of surface density than in DI water. Additionally, Figure 5.8 (C) showed the deposition of n-TiO₂ occurred under favorable condition according to DLVO theory. The mass fraction of surface density was decreased as 10 mM HCl was injected on the rough surface, which presented unfavorable condition between deposited n-TiO₂ and rough surface. Therefore, we observed the mass fraction ratio could be a good parameter about deposition of n-TiO₂ in rough surface under favorable condition, but the mass fraction ration could not demonstrate the actual surface deposition density of n-TiO₂ on surfaces.

5.3.3.2 Release under favorable condition

To quantity the release of n-TiO₂ from a roughness surface, the deposition n-TiO₂ were conducted in an Al₂O₃ roughness surface in DI water. Following the deposition experiment, background DI water and 10 mM HCl were injected to release the deposited n-TiO₂. Figure 5.9 (A) presented experiment phases, including injection of n-TiO₂, rinsing with background electrolyte of DI water, and injection of 10 mM HCl. Each phase
last about 1 hr. Frequency and dissipation shifts were shown in Figure 5.9 (A). During injection of n-TiO$_2$ suspension on a rough surface, deposition of n-TiO$_2$ resulted in the decrease of frequency and increase of dissipation for 1 hr. After the deposition of n-TiO$_2$, the injection of DI water led to no change of frequency and dissipation, indicating that no n-TiO$_2$ was release from the rough surface. When 10 mM HCl was injected, frequency was dramatically increased and reached to the initial frequency before injection of n-TiO$_2$, which indicated that all deposited particles were released from the surfaces. However, little change was observed in dissipation that was similar as in the flat surface experiment, which indicated dissipation was not sensitive for release of n-TiO$_2$. Additionally, surface density was calculated from the frequency shifts, which showed that all of deposited particles were released from a rough surface by unfavorable condition after injection of 10 mM HCl for 1 hour (Figure 5.9 (B)). Thus, the released of n-TiO$_2$ indicated that deposited n-TiO$_2$ in a rough surface could be released and transported if surrounding water chemistry was changed.

Figure 5.9 (C) showed the mass fraction of surface density between SE and QCM-D. Initially, the mass fraction increased with injection of n-TiO$_2$ due to increasing surface density of SE. Then, the mass fraction of surface density reached to the maximum of 0.4 and little change was observed under favorable condition. However, the mass fraction of surface density was decreased under unfavorable condition in 10 mM HCl, which showed the mass fraction of surface density decreased with decreasing of surface density according to DLVO theory.
5.3.3.3 Deposition under unfavorable condition

Under unfavorable condition, deposition of n-TiO$_2$ onto SiO$_2$ coated 100nm SCTF rough surface was investigated in DI water and 100 mM NaCl (Figure 5.10). Generally, both n-TiO$_2$ and SiO$_2$ coated 100nm SCTF rough surfaces were negatively charged in both DI water and 100 mM NaCl. No significant frequency or dissipation shifts was observed, which indicates no deposition of n-TiO$_2$ on a SiO$_2$ rough surface. Although previous work (Huang et al. 2009) has reported that surface roughness may reduce the electrostatic repulsion between particles and rough surfaces, and therefore may create relatively attractive spots in spite of unfavorable conditions, which did not occur under our experimental conditions. Our n-TiO$_2$ deposition results in unfavorable and favorable conditions were strongly following DLVO theory regardless of rough surfaces. However, our results showed that roughness is critically important and can lead to larger retention or deposition of n-TiO$_2$ than on the flat surface in favorable condition. Under favorable conditions, rough surfaces would provide larger surface areas for particles to interact with, which may increase the retention or deposition of particles [14].
Figure 5. 6 QCM-D/SE measurements (Table 5.1 (d)): (A) Frequency and dissipation, (B) surface density of n-TiO₂ both QCM and SE calculation, and (C) mass fraction as a ratio of surface density [SE/QCM].
Figure 5. 7 QCM-D/SE measurements (Table 5.1 (e)): (A) Frequency and dissipation, (B) surface density of n-TiO$_2$ both QCM and SE calculation, and (C) mass fraction as a ratio of surface density [SE/QCM].
Figure 5. 8 QCM-D/SE measurements (Table 5.1 (f)): (A) Frequency and dissipation, (B) surface density of n-TiO$_2$ both QCM and SE calculation, and (C) mass fraction as a ratio of surface density [SE/QCM].
Figure 5. 9 QCM-D/SE measurements for deposition and release behaviors during at each 1 hr in DI water: (A) Frequency and dissipation, (B) surface density of n-TiO$_2$ both QCM and SE calculation, and (C) mass fraction as a ratio of surface density [SE/QCM].
Figure 5. 10 QCM-D/SE measurements (Table 5.1 (h) and (i)): (A) Frequency and dissipation (DI water) and (B) Frequency and dissipation (100 mM NaCl).
5.3.4 Deposition of n-TiO$_2$ rutile particles with and without humic acid

In this part of study, n-TiO$_2$ suspension was developed using rutile TiO$_2$ nanopowder, as described in the Material and Method section. n-TiO$_2$ deposition on flat Al$_2$O$_3$ surface was investigated in DI water at pH 7, both in the absence (Figure 5.11) and the presence (Figure 5.12) of humic acid (HA). In the absence of HA, little frequency and dissipation shifts were observed corresponding to very small surface density of deposited n-TiO$_2$ in spite of the favorable condition between n-TiO$_2$ and Al$_2$O$_3$ surfaces (Figure 5.11). This is in big contrast with the previous experiment (Figure 5.3) where stock solution of n-TiO$_2$ was used, and significant deposition of n-TiO$_2$ onto Al$_2$O$_3$ surfaces in DI water. Although both are n-TiO$_2$ nanomaterial and with similar zeta potential (-19.6 mV and -18.5 mV), n-TiO$_2$ suspensions used in these two experiments are very different in the particle sizes. As listed in Table 5.1, n-TiO$_2$ used in Figure 5.3 has a mean diameter of 2.9 nm, while the mean diameter of n-TiO$_2$ used in Figure 5.11 is 117 nm. Different deposition behaviors in these two experiments indicated that sizes of particle may play a key role on deposition in QCM-D experiments. Particularly, Brownian diffusion transport process could be reduced with increasing particle sizes. In a similar way, recent study demonstrated decreased deposition of n-TiO$_2$ on silica surface because of aggregation of n-TiO$_2$ that limited convective-diffusive transport [16].

Contrary, we observed deposition of n-TiO$_2$ on Al$_2$O$_3$ surface in the presence of HA (8.7 mg/L) (Figure 5.12). In the presence of HA, n-TiO$_2$ has an average particle size of 43.5 nm, which is significantly smaller than the n-TiO$_2$ particle size in the absence of HA. The zeta potential of n-TiO$_2$ in the presence of HA is -27.4 mV, notably more negative than the zeta potential of n-TiO$_2$ in the absence of HA (Table 5.1). Recent
literatures reported that zeta potential of nanoparticles can be more negative due to the adsorption of natural organic matter (NOM) on nanoparticles, which can lead to enhance the stability due to electrostatic and steric repulsion [19, 35-37]. In particularly, a decreased diameter of n-TiO$_2$ was reported by Domingos et al. (2009) in the presence of fulvic acid (> 1.0 mg/L) due to increased steric effects between particles [36]. Thus, our observed more negative zeta potential and smaller diameter of n-TiO$_2$ may be attributed to the adsorption of HA on n-TiO$_2$. As a result of these surface modification, we observed different deposition behaviors of n-TiO$_2$ on QCM-D experiments (Figure 5.12 (A)). The shifts of frequency and dissipation linearly changed until deposition of n-TiO$_2$ stopped. Surface density calculated using frequency shifts that were nearly 100 ng/cm$^2$ and 40 ng/cm$^2$ for $\Gamma_{QCM}$ and $\Gamma_{SE}$, respectively. When we injected background electrolyte solution and little frequency and dissipation shifts were observed, however the surface density of $\Gamma_{SE}$ was slightly decreased during injection of background electrolyte.

Figure 5.11 (C) and 5.12 (C) presented the mass fraction between without HA and with HA on flat Al$_2$O$_3$ surface was investigated in DI water at pH 7. No mass fraction was observed in without HA, while we observed a little change of mass fraction with HA. The trend of mass fraction followed in the change of surface density in SE. The mass fraction reached to 0.4 during injection of n-TiO$_2$ but decreased to 0.2 with rinsing with background electrolyte buffer. Only in this case, we observed decrease of mass fraction rinsing with background electrolyte, which could implicate there was weak reaction between n-TiO$_2$/HA and flat Al$_2$O$_3$ surface although HA enhanced the deposition of n-TiO$_2$ on flat surface comparing to without HA.
Figure 5. 11 QCM-D/SE measurements (Table 5.1 (b-1)): (A) Frequency and dissipation, (B) surface density of n-TiO$_2$ both QCM and SE calculation, and (C) mass fraction as a ratio of surface density [SE/QCM].
Figure 5. 12 QCM-D/SE measurements (Table 5.1 (b-2)): (A) Frequency and dissipation, (B) surface density of n-TiO$_2$ both QCM and SE calculation, and (C) mass fraction as a ratio of surface density [SE/QCM].
In conclusions, we observed the deposition of n-TiO$_2$ on both flat and rough surfaces follows the classical DLVO theory under favorable condition or unfavorable condition. Particularly, we observed significant deposition of n-TiO$_2$ on 100 nm SCTF rough surfaces than on flat surfaces under favorable condition that showed the effect of roughness of collector on deposition of n-TiO$_2$. In addition, surface modification of n-TiO$_2$ with HA showed increased deposition of n-TiO$_2$ while no deposition was observed under same favorable condition in the absence of HA, which is likely related to the size of particles and functional groups due to the adsorption of HA on the surface of n-TiO$_2$. Those deposition results may show us more environmentally relevant deposition behaviors of particle as well as collectors.
5.4 References


CHAPTER 6 CONCLUSIONS AND FUTURE RESEARCH WORKS

6.1 Conclusions

The research described in this dissertation provide a better understanding of the interaction between organic wastewater contaminants (OWCs) i.e. steroid hormones and/or pharmaceuticals and an engineered nanoparticle (NP), n-TiO$_2$, under natural environmental conditions. In Chapter 2 we evaluated the stability of n-TiO$_2$ as a function of 17β-estradiol (E2) concentrations (i.e. 0, 0.1, 0.5, 1, and 3 mg/L) under various environmentally-relevant ionic strength conditions (DI water, 10 mM NaCl, 20 mM NaCl, and 2 mM CaCl$_2$) at natural pH. In addition, we analyzed interaction between n-TiO$_2$ aggregates in aqueous suspensions according to Derjaguin, Landau, Verwey, and Overbeek (DLVO) theory and estimated the mobility of n-TiO$_2$ in porous media based on the clean bed filtration theory. In Chapter 3 we examined the aggregation of n-TiO$_2$ in the presence of natural organic matter (NOM) and 17β-estradiol (E2) under high ionic strength conditions simulating a landfill leachate. Humic acid and 17β-estradiol changed the stability of n-TiO$_2$ under different ionic strength conditions. In Chapter 4 we evaluated a statistical quantitative structure-activity relationship (QSAR) model for prediction of n-TiO$_2$ aggregation behavior by OWC molecular descriptors based on the information from Chapter 2 and 3. To predict a best QSAR model, we used a multiple linear regression (MLR) method evaluating seven descriptors for twelve OWCs compounds. The resulting QSAR models are useful to predict the fate and transport of nanoparticle/OWCs complexes in environment. Last, in Chapter 5 we evaluated the deposition and transport of n-TiO$_2$ under different collector surface properties using a new technique, quartz crystal microbalance with dissipation (QCM-D). As a result of
QCM-D experiments, we observed the prominent deposition and transport behaviors of n-TiO₂ according to surface roughness and surface composition.

Important observation from each chapter in this dissertation are summarized in the following paragraphs.

6.1.1 Effect of 17β-estradiol (E2) on stability and mobility of TiO₂ rutile nanoparticles

- Isotherm sorption experiment and Fourier Transform Infrared (FTIR) Spectrometer measurements showed that E2 was quickly sorbed onto the surface.

- The presence of E2 resulted in the larger aggregation of n-TiO₂ in high ionic strength, while little aggregation of n-TiO₂ was observed in low ionic strength.

- Interaction energy between n-TiO₂ in the presence of E2 showed that E2 caused steric repulsion on the surface of n-TiO₂, which contributed to the stability of n-TiO₂ in 10 mM NaCl. However, interaction energy of n-TiO₂/E2 in DI water showed unfavorable condition (repulsive energy), while interaction energy of n-TiO₂/E2 in 20 mM NaCl and 2 mM CaCl₂ was favorable condition (attractive energy).

- The effect of E2 on mobility of n-TiO₂ in porous media was minimal in comparison to the influence of ionic strength, while we observed that the mobility of n-TiO₂ slightly increased in the presence of E2.
6.1.2 Aggregation of n-TiO$_2$ in the presence of humic acid (HA) and 17$\beta$-estradiol (E2) under high ionic strength conditions

- Little changes in aggregation and zeta potential of n-TiO$_2$ was observed in the presence of HA (0.5 mg/L or 5 mg/L) under low ionic strength at pH 7 due to unfavorable conditions between HA and n-TiO$_2$.

- In the absence of HA, larger aggregation of n-TiO$_2$ was observed at increasing ionic strength concentrations due to screening effects of ions. Divalent cations were stronger at reducing electrical repulsion energy on the surface of n-TiO$_2$ than the monovalent cation, which led to larger aggregation.

- The presence of HA affected the aggregation of n-TiO$_2$ and the surface charge in 20 mM CaCl$_2$ due to the bridging effects of HA, but little effect of HA was observed in 100 mM NaCl.

- The presence of E2 increased the aggregation of n-TiO$_2$ in high ionic strength conditions compared to low ionic strength.

- Aggregation of n-TiO$_2$ in the presence of E2 between 2 mM CaCl$_2$ and 20 mM CaCl$_2$ implied that cation concentrations may influence on the heterogeneous surface of n-TiO$_2$, which could result in different aggregation of n-TiO$_2$.

- E2 enhanced the aggregation of n-TiO$_2$ in the presence of HA in 20 mM CaCl$_2$, while little aggregation of n-TiO$_2$ was observed in the presence of HA in 100 mM NaCl.
6.1.3 QSAR models to predict n-TiO$_2$ stability as a function of OWC molecular descriptors

- Aggregation of n-TiO$_2$ in the presence of OWCs was dependent on the ratio of [n-TiO$_2$]/[OWCs] as well as specific properties of OWCs.

- Predicted QSAR models by fitlm algorithms in MATLAB at 0 hr and 12 hr using seven descriptors for three responses showed higher R-squared values ($R^2 = 0.87 - 0.92$) for 5 mg/L TiO$_2$ + 80% of OWCs when compared to 1 mg/L TiO$_2$ + 0.2 mg/L of C$_w$ of OWCs (0.75 - 0.86).

- Predicted QSAR models by stepwiselm algorithms in MATLAB at 0 hr and 12 hr showed high R-squared values (> 0.99) at 0 hr and 0.68 - 0.84 at 12 hr in 5 mg/L TiO$_2$ + 80% of OWCs.

- We evaluated relationship between modeled response and observed response using coefficients calculated from QSAR models by fitlm algorithms between 0 hr and 12 hr. The R-squared were 0.55 and 0.24 for 0 hr and 12 hr in 1 mg/L TiO$_2$ + 0.2 mg/L of C$_w$ of OWCs, respectively.

- We evaluated relationship between modeled response and observed response using coefficients calculated from QSAR models by stepwiselm algorithms between 0 hr and 12 hr. The R-squared were 0.10 and 0.25 for 0 hr and 12 hr in 1 mg/L TiO$_2$ + 0.2 mg/L of C$_w$ of OWCs, respectively.
6.1.4 Deposition of n-TiO$_2$ on rough surfaces using quartz crystal microbalance with dissipation monitoring (QCM-D)

- Deposition of n-TiO$_2$ on flat surfaces follows the DLVO theory under favorable and unfavorable conditions. Under the favorable condition more deposition of n-TiO$_2$ was observed in DI water than in 10 mM NaCl.

- Deposition of n-TiO$_2$ on rough surfaces follows the DLVO theory under both favorable and unfavorable conditions. Significantly more deposition of n-TiO$_2$ was observed on the 100 nm SCTF rough surface than on the flat surfaces in the favorable condition. No deposition was observed on rough surfaces in unfavorable conditions.

- The release of n-TiO$_2$ from rough surfaces (SCTF coated with Al$_2$O$_3$) was observed under the unfavorable condition, when 10 mM HCl was injected after deposition experiments. This implies the possible release of n-TiO$_2$ due to the change of surrounding water chemistry.

- Surface modification of n-TiO$_2$ with HA increased the deposition of n-TiO$_2$ under favorable conditions.

6.2 Recommendations for Future Works

We conducted experiments between an engineered NP, TiO$_2$ and OWCs for predicting the stability of n-TiO$_2$ under natural environmental conditions. These stability results could be useful for predicting the fate and transport of nanoparticles/OWCs complexes in aquatic environment, therefore further future work is recommended.
6.2.1 Future works on the stability/mobility and QSAR model of n-TiO$_2$ in the presence of various OWCs as well as natural organic matter

- Conducting the stability of n-TiO$_2$ under various ratio of [n-TiO$_2$]/[OWCs] using under different environmentally relevant conditions (IS and pH).
- Developing the mobility of n-TiO$_2$/OWCs complexes by incorporating the steric repulsion energy on zeta potential that may give more realistic results of mobility.
- Improving a predicted QSAR model by increasing number of target compounds or by incorporating other descriptors of OWCs.
- Evaluating the bioavailability and toxicity of n-TiO$_2$/OWCs complexity.

6.2.2 Future works on the deposition and transport of n-TiO$_2$ using a QCM-D technique

- Evaluating the deposition of n-TiO$_2$ on rough surfaces based on the surface heterogeneous sites (density or thickness of roughness) or surface modification of n-TiO$_2$ with OWCs.
- Evaluating the deposition and mobility of n-TiO$_2$ by changing water conditions, i.e. flow rate (frequency) and temperature considering hydrodynamic influences.
- Evaluating more accurate deposition process of NPs according to particle sizes by separating particle sizes using centrifugation or micro-channel methods.
SUPPLEMENTARY MATERIAL OF CHAPTER 2

EFFECT OF 17β-ESTRADIOL ON STABILITY AND MOBILITY OF TiO₂

RUTILE NANOPARTICLES
Estrogen Detection. A 12 mL sample was used for estrogen analysis. All samples were spiked with 100 μL of surrogate (androsterone, 10 ng/μl) prior to SPE extraction. The samples with 17β-estradiol were extracted with flow rate of 3 ml/min using solid phase extraction (SPE) onto polymeric reverse phase extraction cartridge (Oasis HLB-60mg) under vacuum. The extracted estrogen in SPE cartridge was then eluted with 3 ml of ethyl acetate with flow rate of 0.5 ml/min and dried to 0.5 ml by using nitrogen gas applied through SPE manifold and cover. 0.5 ml of ethyl acetate was added to the sample in the reaction vials. 100 μL of internal standard (13C3-17β-estradiol, 10 ng/μl) was added into the reaction vials before evaporation under nitrogen gas. When the reaction vials were dry completely, the derivatization agents of dimethylformamide (100 μL) and BSTFA+1% TMCS (100 μL) were added into each reaction vial and sealed tightly using clean Teflon-lined septa and vortexed for several seconds. Immediately, the vials were put in a block heater at 75 °C for 30 min. Then vials were cooled under room temperature and were analyzed by GC-MS (Hewlett Packard 5972). Gas chromatographic separation employed a DB-1MS column (30 m length × 0.25 mm diameter) and helium was used as a carrier with 7.5 psi at the head column head. The GC temperature program was used as following: an initial temperature of 80 °C (hold for 2 min), increased at 15 °C/min to 150 °C, and increased at 5 °C/min to 250 °C, then increased at 15 °C/min until 275 °C (hold for 10 min).

DLVO Interaction Energy. The classical Derjaguin-Landau-Verway-Overbeek (DLVO) was used, as a preliminary approach, to estimate the NP-NP interaction energies. Here, van der Waals attraction (VDW) energy and electrostatic double layer (EDL) repulsion energy were considered [1-4]. n-TiO2 was considered as sphere. The retarded van der
Waals attraction interaction energies for NP-NP and NP-sand systems were calculated using equation (1) [1, 5].

\[
\phi_{VDW} = -\frac{A a_{p1} a_{p2}}{6h(a_{p1} + a_{p2})} \left[ 1 - \frac{5.32h}{\lambda} \ln \left( 1 + \frac{\lambda}{5.32h} \right) \right] \quad (1)
\]

where, A is the Hamaker constants of n-TiO$_2$-n-TiO$_2$, $a$ is the radius of aggregated TiO$_2$ nanoparticle (nm), $a_{p1}$ and $a_{p2}$ are the average radii of two aggregated n-TiO$_2$ nanoparticles (nm), h is the distance between aggregated n-TiO$_2$ and the sands, and $\lambda$ is the characteristic wavelength of interaction (100 nm).

According to Ackler et al. (1996), Hamaker number for TiO$_2$ rutile were 26e-20J based on Tabor winterton approximation, 6.0e-20 J based on Atomic force microscope measurement, 6.0 – 9.4 e-20 J based on physical property measurement [6]. The value used in our previous calculation was based on Tabor Winterrton approximation, which is quite different from various physical measurement. Therefore, we modified our DLVO calculation by using an nTiO$_2$-water-nTiO$_2$ haymaker number of 6.0 e-20 J, which is a reported number with agreement based on various type of measurements (including atomic force microscope, simple spectral method, and full spectral method). Using this new haymaker number, DLVO predicts a sizable energy barrier for 10mM NaCl (Figure S.4), which is more consistent with our observation of a stable suspension under this condition.

The electrical double layer (EDL) repulsion energy between NP-NP was determined using equations below [1, 7]:

\[
\phi_{EDL} = \frac{2\pi a_{p1} a_{p2} n k_B T (\psi_{p1}^2 + \psi_{p2}^2)}{(a_{p1} + a_{p2})\kappa^2} \left( \frac{2\psi_{p1} \psi_{p2}}{(\psi_{p1}^2 + \psi_{p2}^2)} \ln \left[ \frac{1 + \exp(-kh)}{1 - \exp(-kh)} \right] + \ln[1 - \exp(-2kh)] \right) \quad (2)
\]
151

\[ \kappa = \left( \frac{2000 N_A e^2}{\varepsilon_0 \varepsilon_r k_B T} \right)^{0.5} \] (3)

Where \( \varepsilon_0 \) is the permittivity of a vacuum \((8.85 \times 10^{-12} \text{ F/m})\), \( \varepsilon_r \) is the relative dielectric constant of water \((78.5)\), \( \kappa \) is the inverse Debye length, \( k_B \) is the Boltzmann constant \((1.38 \times 10^{-23} \text{ m}^2 \text{ kg} \text{ s}^{-2} \text{ K}^{-1})\), \( T \) is temperature \((\text{K})\), \( n \) is the number concentrations of bulk solution, \( \psi \) is the reduced potentials \((\text{calculated by } z e \zeta/\kappa B T, \text{here, } z \text{ is the ion valence})\), \( N_A \) is the Avogadro constant \((6.02 \times 10^{23})\), \( I_c \) is the ionic strength \((\text{IS})\), and \( e \) is the elementary charge \((1.6 \times 10^{-19} \text{ C})\). The surface potentials \((\zeta_p)\) of aggregated n-TiO_2 were measured from our aggregation experiments.

**Steric Repulsion.** To further understand the repulsive forces by E2 adsorption on n-TiO_2, we determined the steric interaction energy of n-TiO_2- n-TiO_2 by Alexander-de Gennes equations below [2, 7, 8];

For n-TiO_2- n-TiO_2 and \( h \leq L \),

\[ F_{\text{steric}}(h) = 2\pi \frac{a_1 a_2}{a_1 + a_2} \left( \frac{\kappa g T}{s^3} \right) \left\{ \frac{4L}{5} \left[ \left( \frac{L}{h} \right)^{1.25} - 1 \right] + \frac{4L}{7} \left[ \left( \frac{h}{L} \right)^{1.75} - 1 \right] \right\} \] (4)

\[ Q_{\text{steric}}(h) = - \int_\infty^h F_{\text{steric}}(h) \, dh \] (5)

where \( F_{\text{steric}} \) and \( Q_{\text{steric}} \) are the steric forces and the steric interaction-energy, respectively. \( a_1 \) and \( a_2 \) are the radius of n-TiO_2 and \( k_B \) is the Boltzmann constant, \( T \) is the absolute temperature, and \( h \) is the separation distance between n-TiO_2. \( s \) is the distance of E2 chain on the n-TiO_2 surface, and \( L \) is the thickness of E2 on the n-TiO_2 surface. \( s \) and \( L \)
were both estimated as the thickness of E2 adsorbed on the surface n-TiO₂. The detailed estimation procedure is provided in the section below.

**Evaluation of the E2 Thickness adsorbed on n-TiO₂.** Although Ohshima soft particle theory has previously been applied to determine the thickness of sorbed layer on the surface of nanoparticles [8, 9], application of this theory to our data generated a negative thickness, probably due to the nonlinear response of zeta potential as a function of E2 concentration (Figure 4 in the main text). Therefore, we estimated the thickness of E2 layer sorbed on the surface of n-TiO₂ based on the equilibrium sorption data and the molecular structure of E2, as detailed below.

First, the mass of E2 required for monolayer coverage on an n-TiO₂ aggregate (QE1) is estimated:

\[
\text{QE1} \left( \frac{\text{mg E2}}{\# \text{ of TiO}_2} \right) = \frac{\text{nTiO}_2 \text{ Surface area m}^2}{\# \text{ of TiO}_2} \times 10^{20} \frac{\text{Å}^2}{1 \text{m}^2} \times \frac{\text{molec}}{80 \text{Å}^2} \times \frac{\text{mol}}{6.23 \times 10^{23} \text{molec}} \times 272.38 \frac{\text{g E2}}{\text{mol}} \times \frac{1000 \text{mg}}{1 \text{g}}
\]

Here, the molecular weight of E2 is 272.39 g/mol [10]. The surface area of E2 is assumed as 80 Å/molecule [11]. The hydrodynamic diameter of n-TiO₂ aggregate for 0.1, 0.5, and 1 mg/L E2 in 10mM NaCl is used. Then, total E2 mass sorbed on the surface of one n-TiO₂ aggregate can be estimated as:

\[
\text{QE2} \left( \frac{\text{mg E2}}{\# \text{ of TiO}_2} \right) = \rho_{\text{TiO}_2} \frac{g \text{TiO}_2}{cm^3} \times \frac{V_{\text{TiO}_2}}{\# \text{ of TiO}_2} \times q_e \frac{mg E2}{g \text{TiO}_2}
\]

where density of TiO₂ \( \rho_{\text{TiO}_2} \) was assumed as of 0.08 g/cm³. Each \( q_e \) for 0.1, 0.5, and 1.0 mg/L E2 was obtained from sorption isotherm data.

Finally, \( \frac{Q_{E2}}{Q_{E1}} \) provides the number of layers of E2 sorbed on the surface of n-TiO₂. The thickness of the sorbed E2 layer can be obtained by multiplying \( \frac{Q_{E2}}{Q_{E1}} \) with
the size of E2 (0.398 nm) [10]. The estimated thickness of each E2 of 0.1, 0.5, 1.0, and 3.0 mg/L on n-TiO$_2$ in DI water was approximately 0.03, 0.188, 0.418 nm, and 1.477 nm, respectively.
Figure S. 1 Sorption isotherms of 17β-estradiol (E2) on n-TiO₂ (5 mg/L) suspension in 10 mM NaCl at pH 7 and 25°C after 2 hr (n=3).
Figure S. 2 The particle diameter changing of n-TiO$_2$ (5mg/L) by various E2 concentrations at pH 7 during 12 hr: (A) 20 mM NaCl and (B) 2 mM CaCl$_2$. Data plots were triplicate measurements. * means p < 0.05 on unequal variances.
Figure S. 3 Zeta potential of n-TiO$_2$ at pH 7 under different E2 concentrations and under different ionic strength during 12 hr: (A) DI-water, (B) 10 mM NaCl, (C) 20 mM NaCl, and (D) 2 mM CaCl$_2$. Data plots were triplicate measurements.
Figure S. 4 DLVO interaction-energy for (A) DI water, (B) 10 mM NaCl, (C) 20 mM NaCl, and (D) 2 mM CaCl$_2$. 
Figure S. 5 FTIR Spectra band of pure E2
Table S. 1 Cumulative undersize distribution, C(d) and mobility distance of aggregated n-TiO$_2$ according to C(d) at 12hr.

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<th>Cumulative undersize distribution, C(d) [nm]</th>
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References (Supporting Material)


