

The Fate, Transport, Transformation and Toxicity of Manufactured Nanomaterials in Drinking Water

Arizona State University Investigators

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Project Objectives

Goal: To understand the fate and significance of nanomaterials in drinking water

The objectives of this project are:

- 1) to characterize the fundamental properties of nanomaterials in aquatic environments
- 2) to examine the interactions between nanomaterials and pollutants and pathogens
- 3) to evaluate the removal efficiency of nanomaterials by drinking water unit processes
- 4) to test the toxicity of nanomaterials in drinking water using cell culture model system of the epithelium.

Project Timetable

Tasks	Scheduled Time
Literature preparation	January 2005
Nanomaterial Detection Methods	Oct 2004 – Mar 2005
Characterization of Nanomaterials in Water	Jan 2005 – Dec 2005
Adsorption of Dissolved Pollutants onto Nanoparticles	July 2006 – June 2007
Aggregation and Coagulation of Nanomaterials	Jan 2005 – Mar 2007
Nanoparticle Adsorption and Disinfectant Shielding of Virus	June 2006 – Mar 2007
Nanoparticle Toxicity Screening for Drinking Water	Jan 2005 – Dec 2006
Final Report	July 2007 - Oct 2007

I. Nanoparticle Characterization

Interesting note: Several papers investigate environmental applications of nanoparticles, but use 0.45 μm filtration to remove the “nano”particles.

We have found for metal oxide nanoparticles:

- Nanoparticles placed in water are aggregated
- Aggregation due to electrostatics of dry powders, manufacturing process, and/or aggregation in solution
- Sonication temporarily dis-aggregates some nanoparticles
- Surfactants and/or solvents promote some dis-aggregation
- Nanoparticles purchased in solutions are less, but still, aggregated
- Solution = Producing nanoparticles in laboratory rather than commercial sources

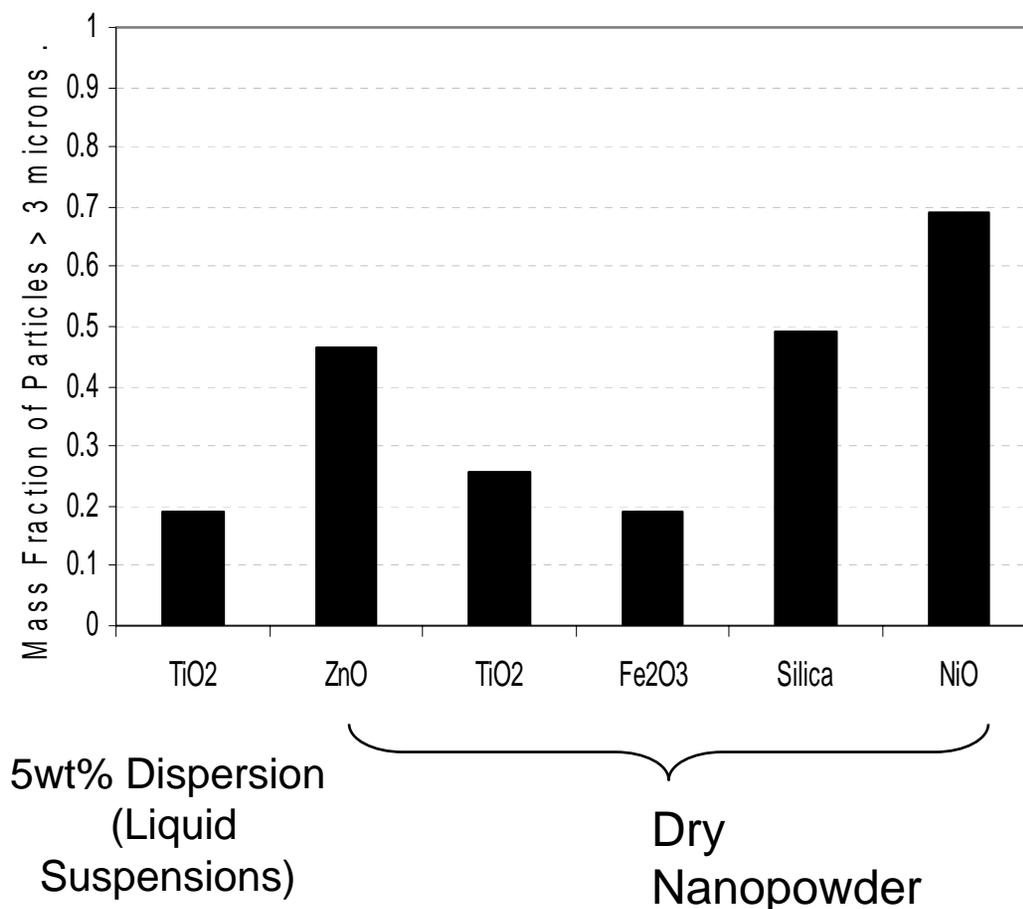
Challenge for all nanoparticle research: commercial nanoparticles in water are NOT nanoparticles ($< 100 \text{ nm}$ in at least one dimension)

Nanomaterials in Study

Nanoparticle	Vendor Reported Mean Particle Size	Density	Source
Titanium dioxide	< 40 nm	3.9g/mL	5wt% Dispersion in water
Aluminum oxide	< 20 nm	3.97g/mL	5wt% Dispersion in water
Zinc Oxide	50 ~ 70 nm	5.61g/mL	Nanopowder
Titanium dioxide	15 nm	3.9g/mL	99.7% Nanopowder
Iron (III) Oxide	5 ~ 25 nm	5.24g/mL	Nanopowder
Nickel Oxide	10 ~ 20 nm	6.67g/mL	99.8% Nanopowder
Silica	10 nm	2.6g/mL	99.5% Nanopowder

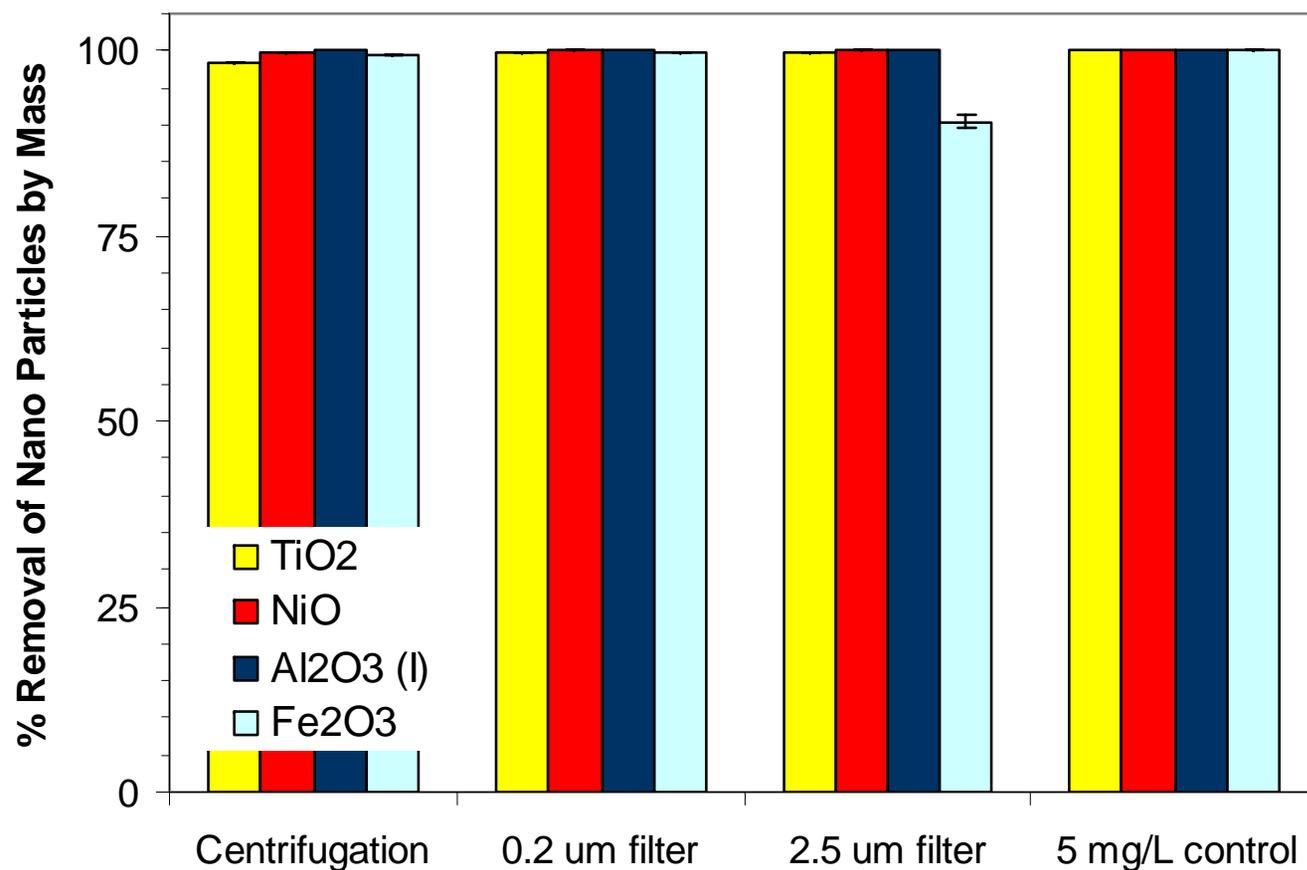
- All nanoparticles purchased as powders and liquid suspensions
- All values in above table are reported by Vendor.
- Other nanomaterials currently in use:
 - Several other commercial metal oxide nanoparticles
 - Carbon nanotubes and fullerenes
 - TiO₂ nanotubes and nanoparticles – fabricated in ASU laboratory
 - Gold and/or cadmium quantum dots

Size of nanoparticles in water



- 10mg/L and 5mg/L nanoparticles in Nanopure water sonicated for 15 min
- Filter paper with 3 μm pore size and 110nm diameter
- 100ml and 50ml suspensions for filtration
- Concentrations of particles analyzed by digestion/AAS.
- Conclusion:
 - Significant mass of “nanoparticles” are aggregated and > 3 μm
 - DLS particle size instrument only measures particles < 3 μm

At high nanoparticle concentration (1g/L) more aggregation occurs



C(NP) = 1 g/L

10 mM NaHCO₃

pH = 8.4 ± 0.2

Analysis by:

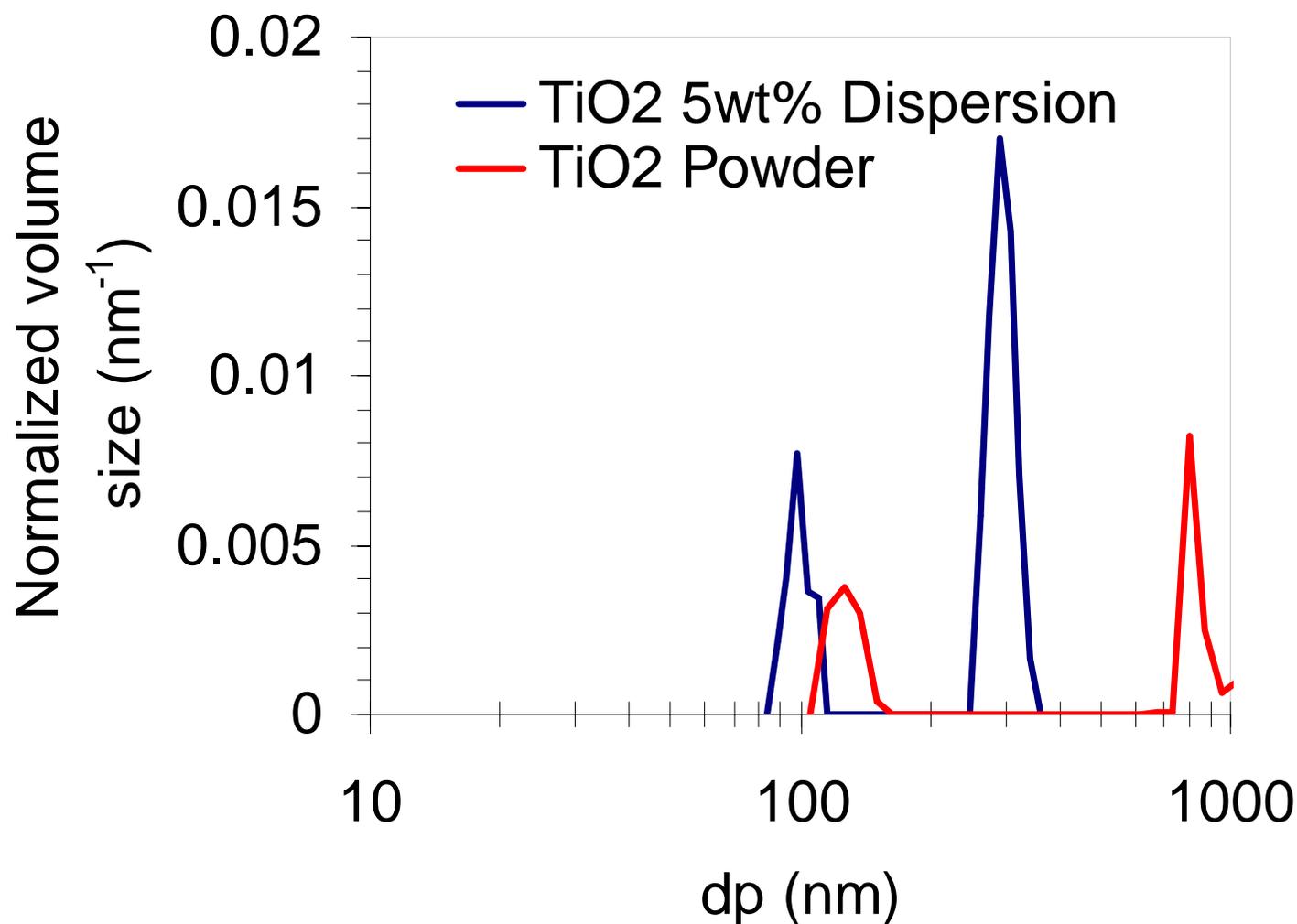
Digestion/GF-AAS

Centrifugation:

G > 1300

10 mg/L TiO₂ in Water

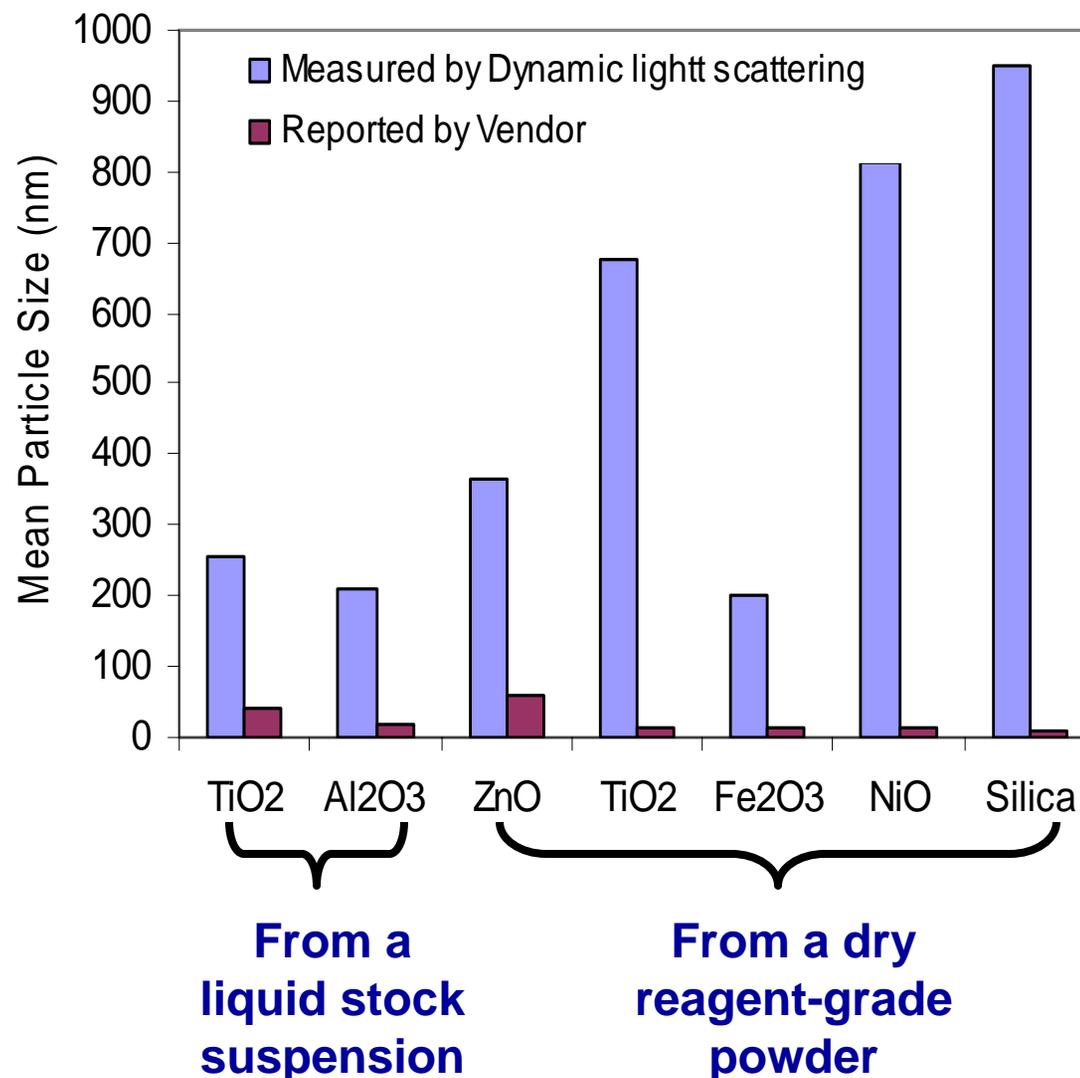
(ZetaPALS dynamic light scattering; < 3 μ m only)



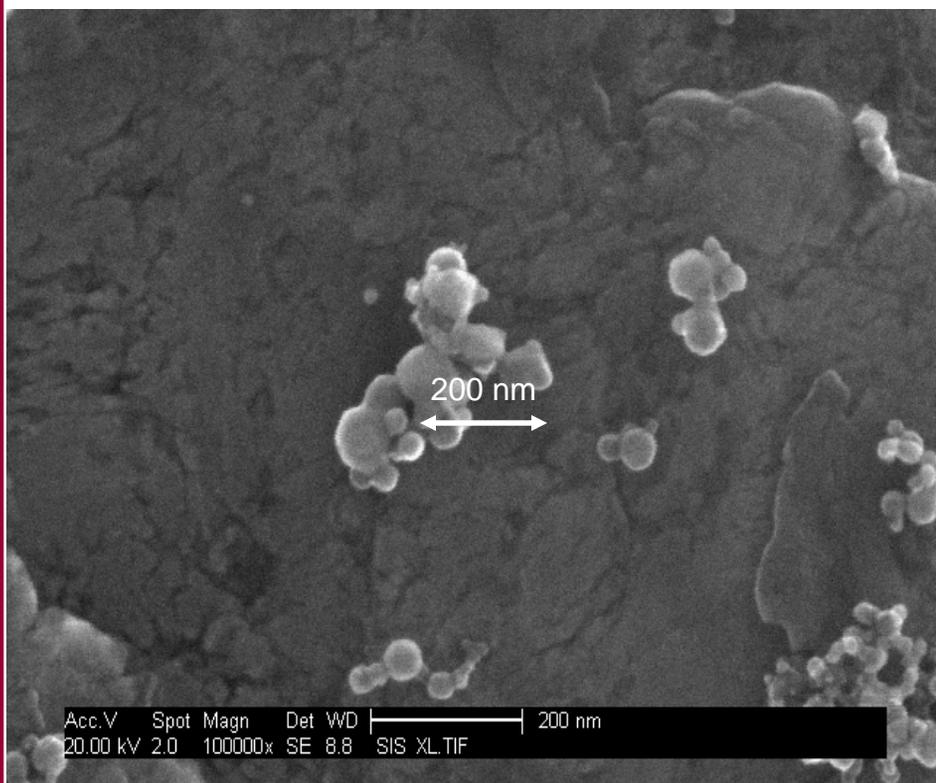
Conclusion: Nanoparticles do not appear to be discrete NP in water

Size of nanoparticles in water

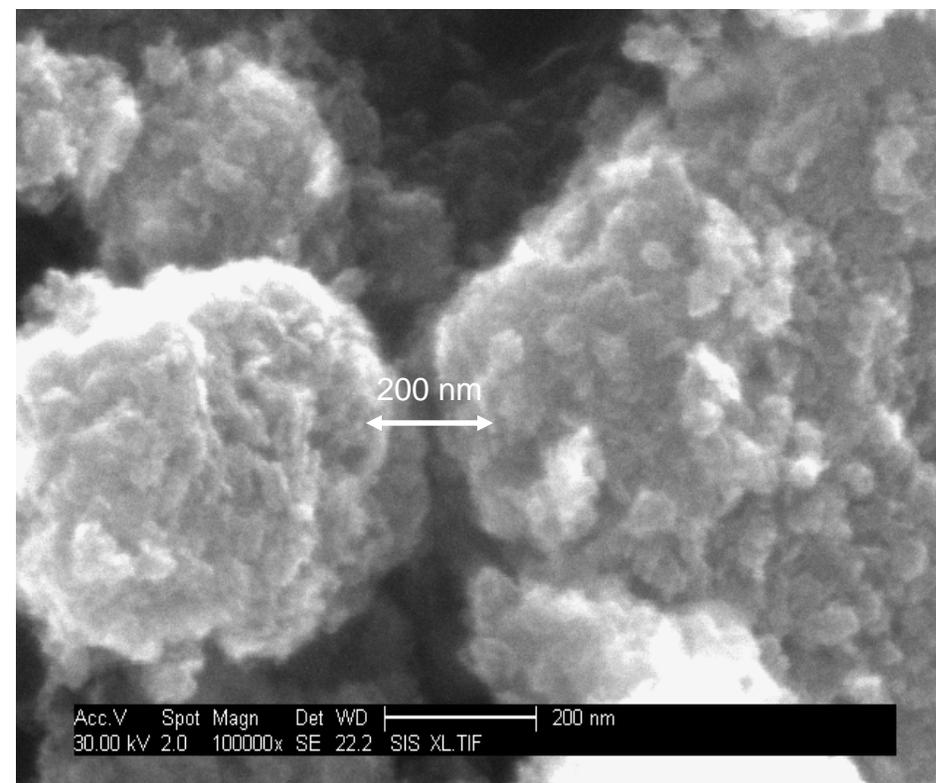
- Particle Size of nanoparticles were analyzed by Dynamic Light Scattering.
 - 10mg/L nanoparticles in Nanopure water
 - Sonicated for 15 min at 200 W/L and 20 kHz.
 - Instrument range: 2nm ~ 3 μ m.
- Mean particle size is greater than reported by manufacturer.



SEM Analysis of Commercial TiO₂ NPs



TiO₂ NPs from **5wt% Dispersion in water**



TiO₂ NPs from a **dry powder**

Nanoparticles do not appear to be discrete NPs

Dispersion of aggregated NPs in water

- Sodium hexameta phosphate, Sodium dodecyl sulfate, Isopropanol, Acetone, Butonanone, Methanol, and Ethylene Glycol selected as a dispersant solvent.
- All suspensions sonicated for 15 mins
- With all above dispersants, mean particle sizes of TiO₂ nanoparticles in Nanopure water still were much more than size of discrete nanoparticles.
- It is very difficult to disaggregate these NPs and obtain homogenous discrete nanoparticles.

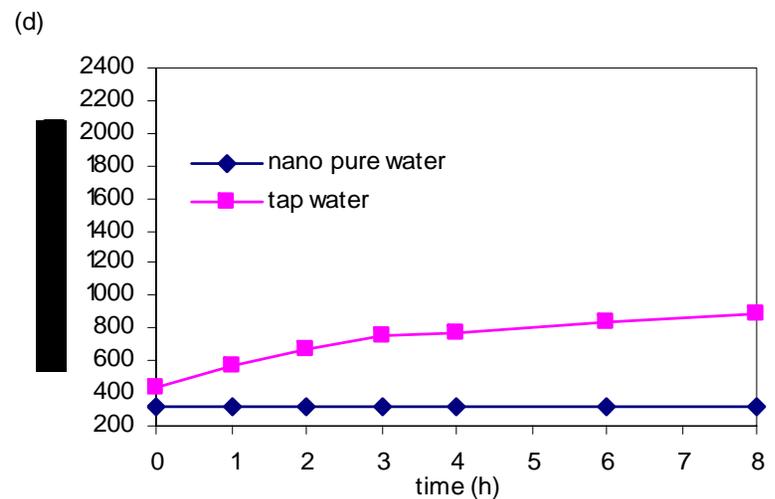
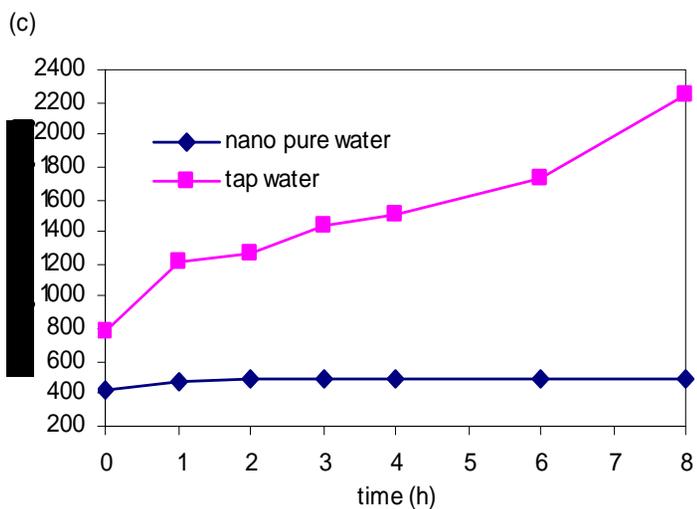
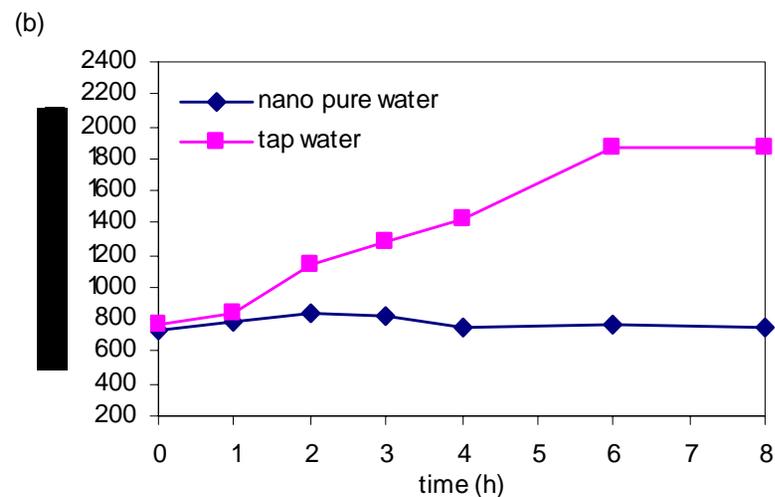
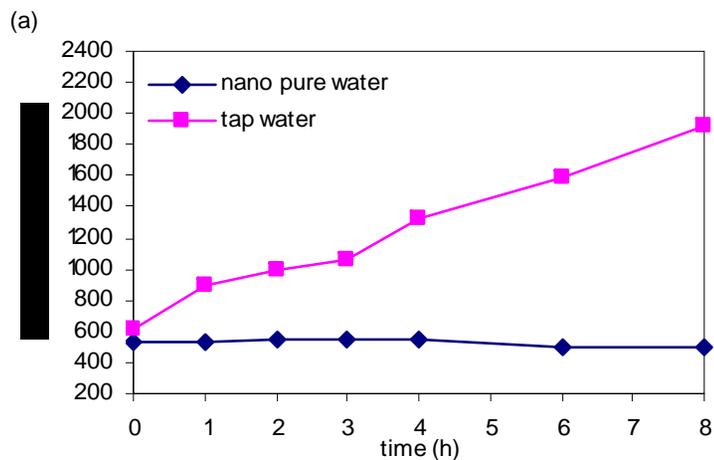
Dispersant	Mean Particle Size
SDS (Sodium dodecyl sulfate)	530~570nm
(NaPO ₃) ₆ (Sodium hexameta phosphate)	560~580nm
Ethylene Glycol (10~100%)	300~550nm
Isopropanol (10%)	550~1050nm
Acetone (10%)	560~580nm
Butonanone (10%)	580~640nm
Methanol (10%)	550~570nm
Nanopure water (no dispersant)	540~600nm

Mean particle size of TiO₂ by Dynamic Light Scattering in Nanopure water w/o dispersants

II. Removal of Nanoparticles in Simulated Water Treatment Systems

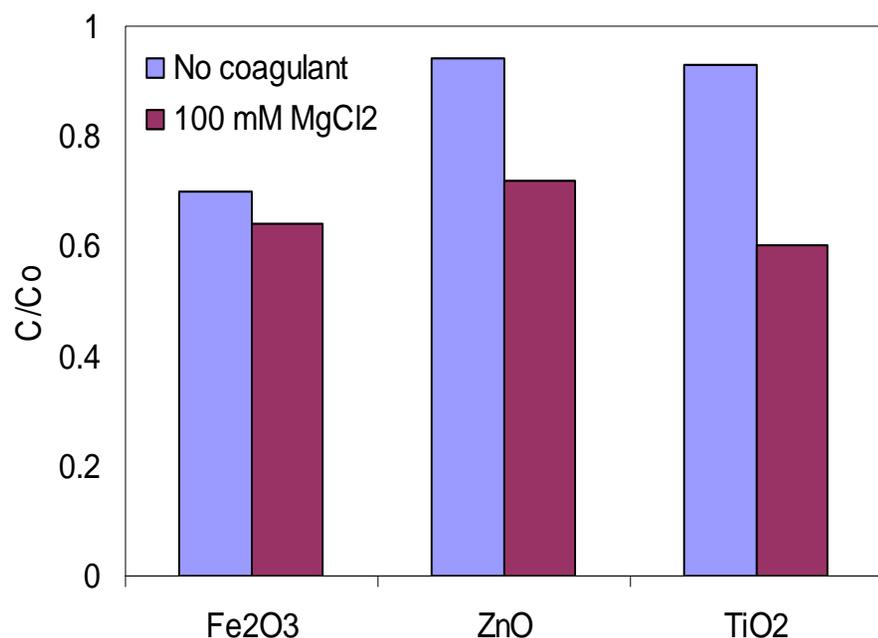


Further aggregation of nanoparticles in water

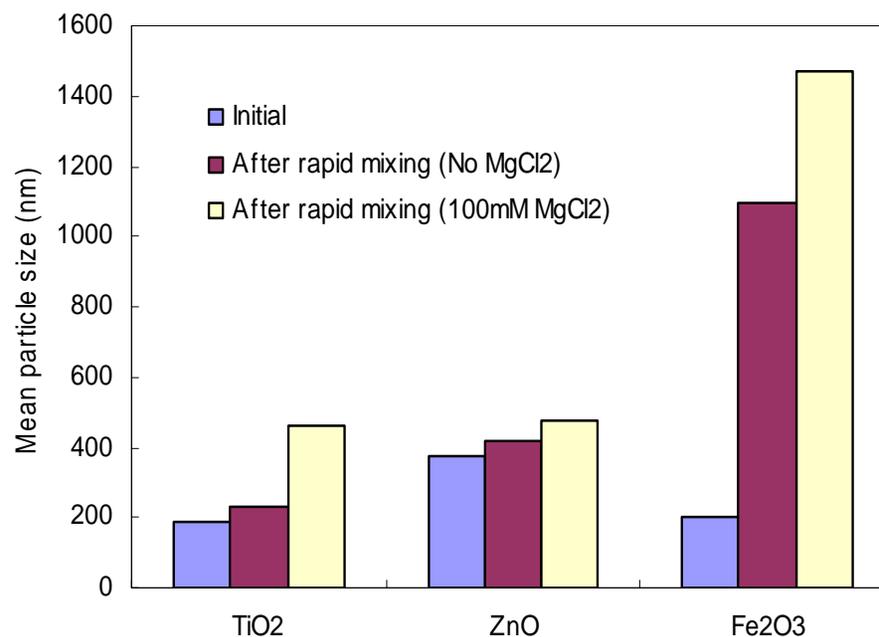


Mean particle size of 10mg/L nanoparticle suspensions with time. a. TiO_2 b. NiO c. Fe_2O_3 d. ZnO

Electric Double Layer Compression



Removal of nanoparticles in nano pure water without coagulant and with 100mM MgCl₂



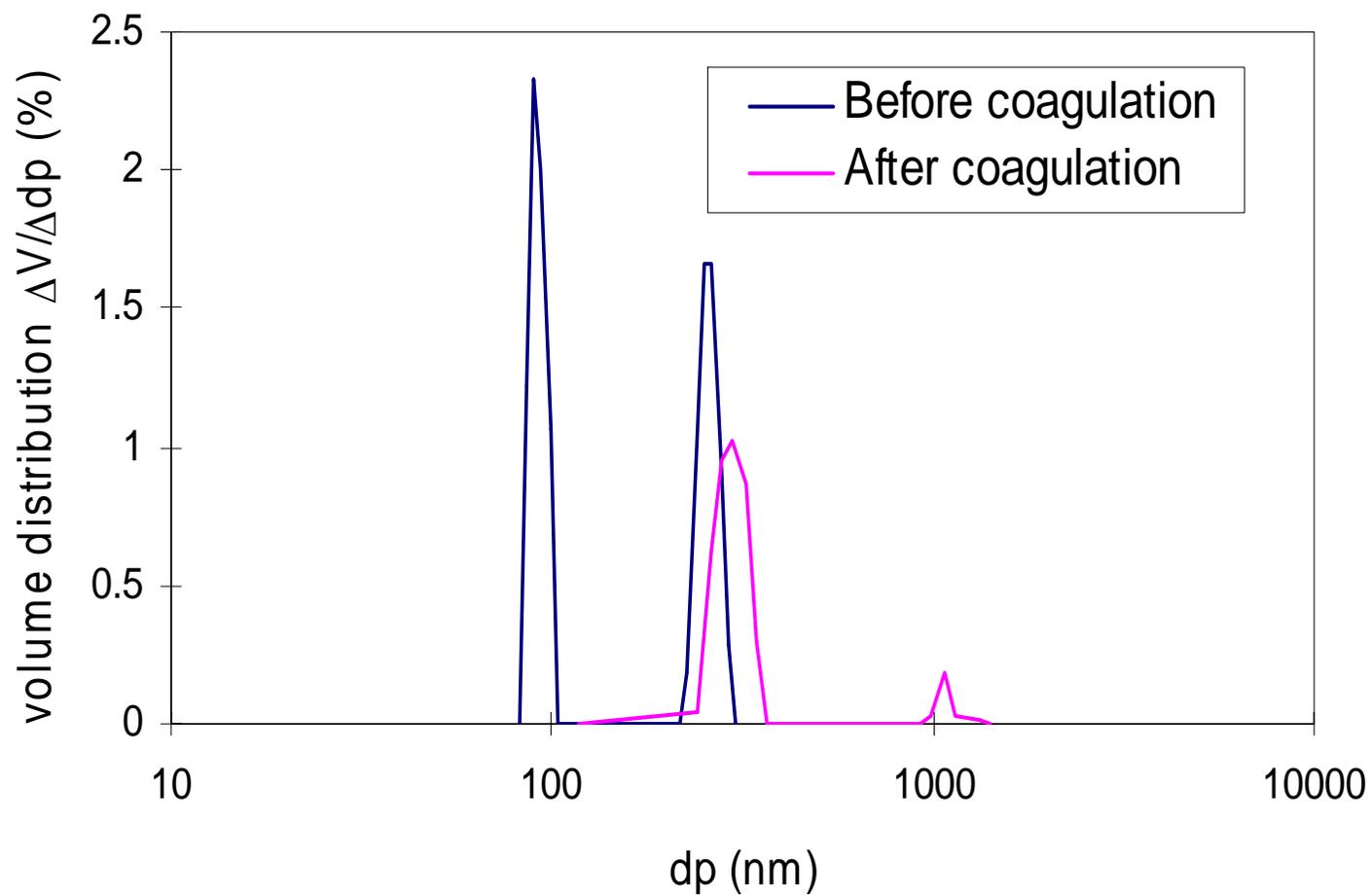
Mean nanoparticle size in nano pure water without coagulant and with 100mM MgCl₂

Test Conditions:

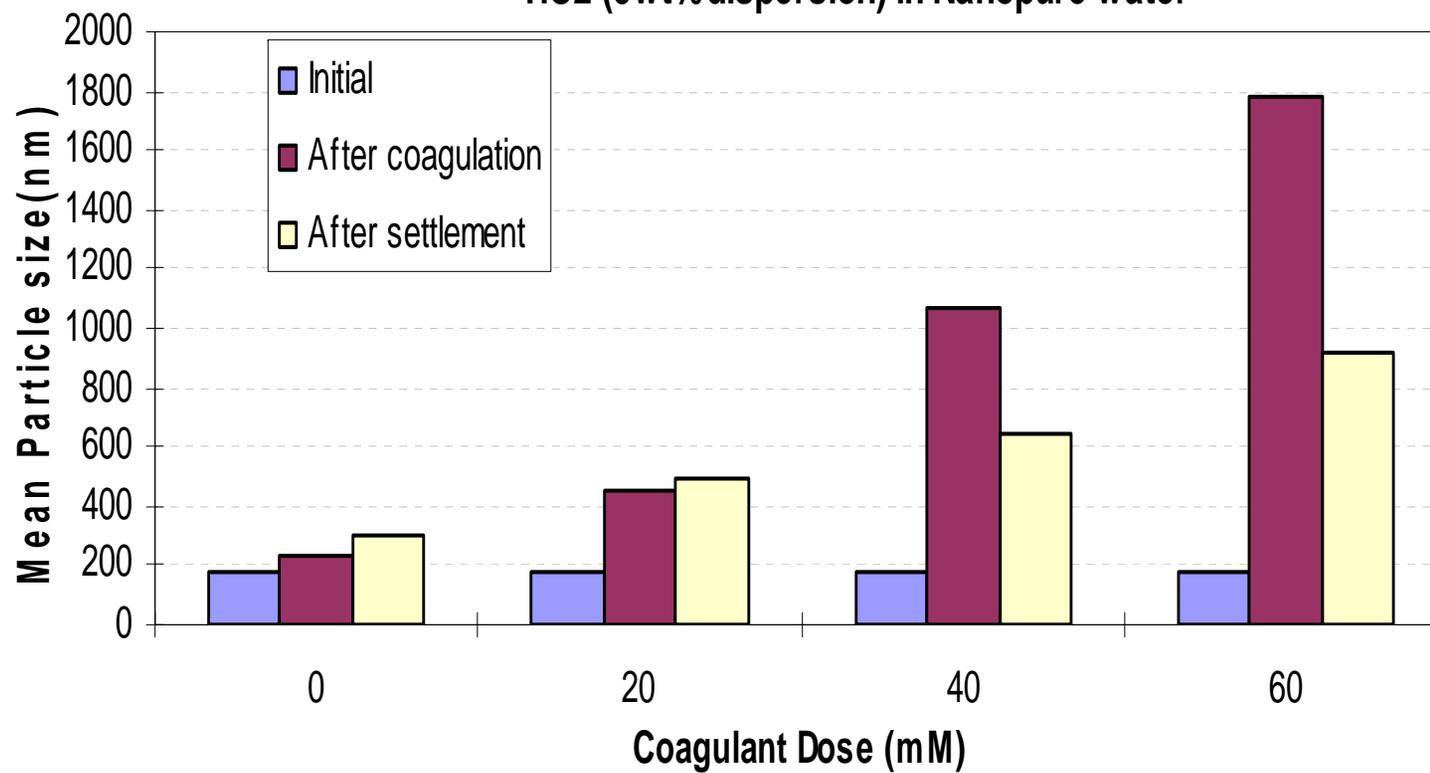
- 10 mg/L NPs in Nanopure water buffered by 10mM NaHCO₃ (pH=8.1± 0.2)
- 100 mM MgCl₂ for EDL compression and NP destabilization
- NPs in supernatant measured by digestion/AAS after sedimentation period

Conclusions:

- Sedimentation removes aggregated NPs
- EDL compression leads to more aggregation for some NPs
- > 40% of NPs remain after sedimentation

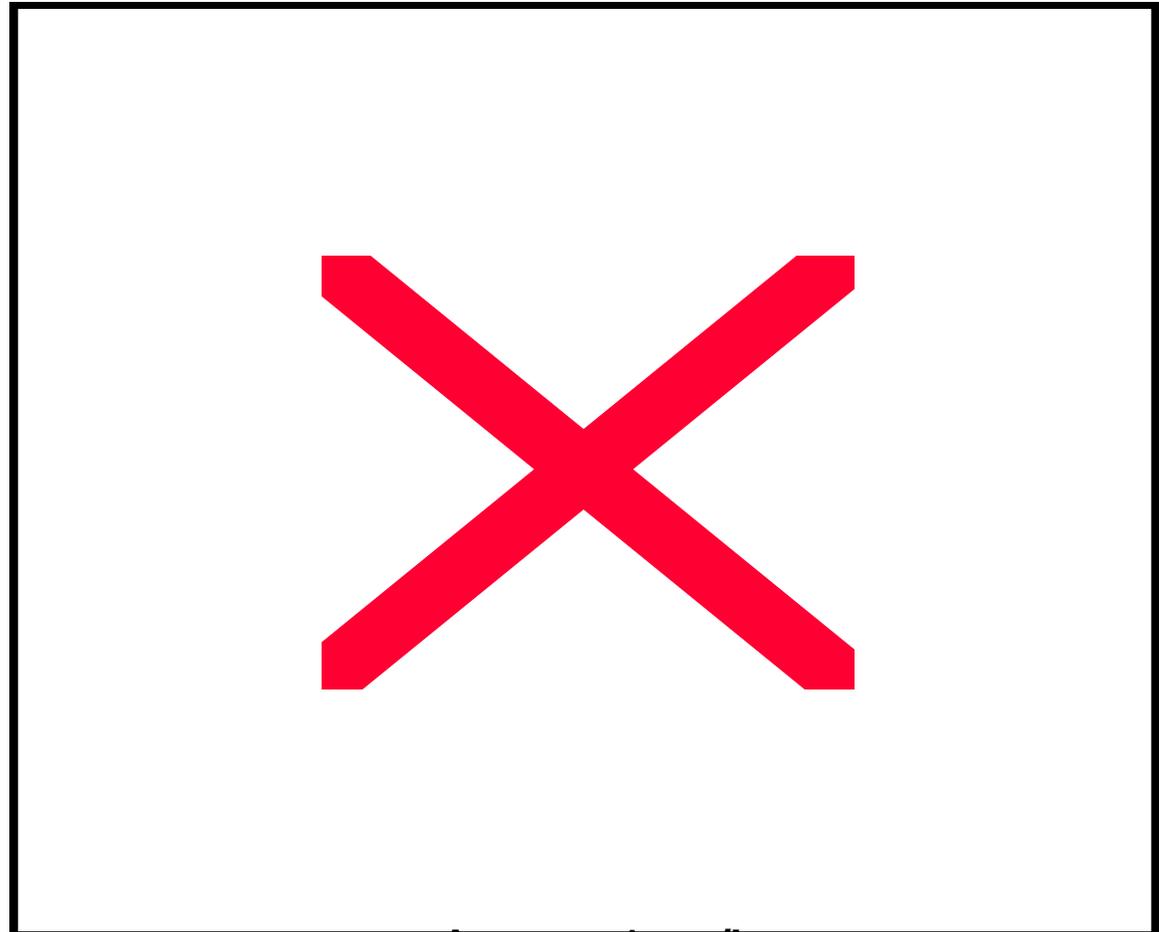
TiO₂ (5wt% Dispersion)

Mean particle size during coagulation experiment
TiO₂ (5wt% dispersion) in Nanopure water



III. Adsorption of Pollutants onto Nanoparticles

- Arsenic adsorption evaluated (see graphic)
- Adsorption of bacteriophage (MS2 & PRD1):
 - Adsorption occurs onto positively charged NPs
 - Charge based upon zeta potential measurements



$AS_{\text{Initial}} = 1 \text{ mg/L}$

$C(\text{NP}) = 1 \text{ g/L}$ $\text{pH} = 8.0 \pm 0.4$

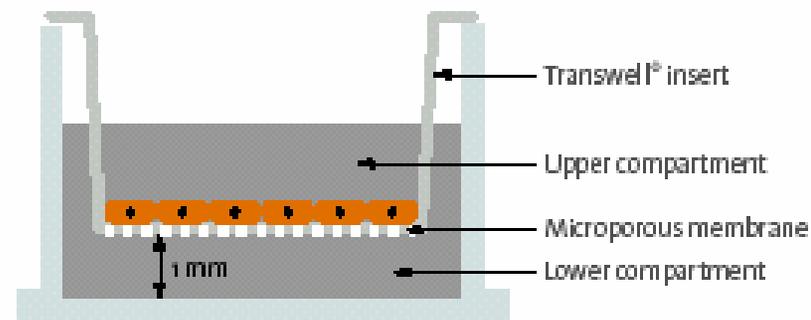
IV. Toxicity of Nanoparticles

Objectives:

- Evaluate transport and necrotic effects of nanoparticles across epithelial layers (esophageal & intestinal)
- Uniform cells are established on semi-permeable support. Continuous cell structure leads to conductivity gradient across biofilm. Disruption of conductivity gradient inferred as detrimental impact to cells or biofilm structure

Human intestinal tissue model (Caco-2BBE) – from ATCC:

- Cells were transferred to semi-porous membranes and allowed to anchor and form tight junctions (three days).
- Transepithelial electrical resistance (TEER) was utilized to monitor the density and junctional complex of the cell monolayer.
- Cells were maintained in DMEM containing 10% fetal calf serum, penicillin/streptomycin/fungizone, and transferrin at 37°C, humidified air containing 10% CO₂. Subsequently, the medium was changed every day after seeding onto the membrane.



Filter Inserts



Voltohmmeter

Testing Procedures

Rejected Methodology

- 5% CO₂
- Media absent of transferrin
- Membranes composed of polycarbonate
- Membranes composed of polyester
- 12mm membrane diameter
- 24mm membrane diameter
- 3.0 μm pore size in membrane
- Change media every three days
- Change media every two days

Adopted Methodology

- 10% CO₂
- Media containing 10 $\mu\text{g}/\text{mL}$ transferrin
- Pre-coated collagen membranes
- 6.5mm membrane diameter
- 0.4 μm pore size in membrane
- Change media daily

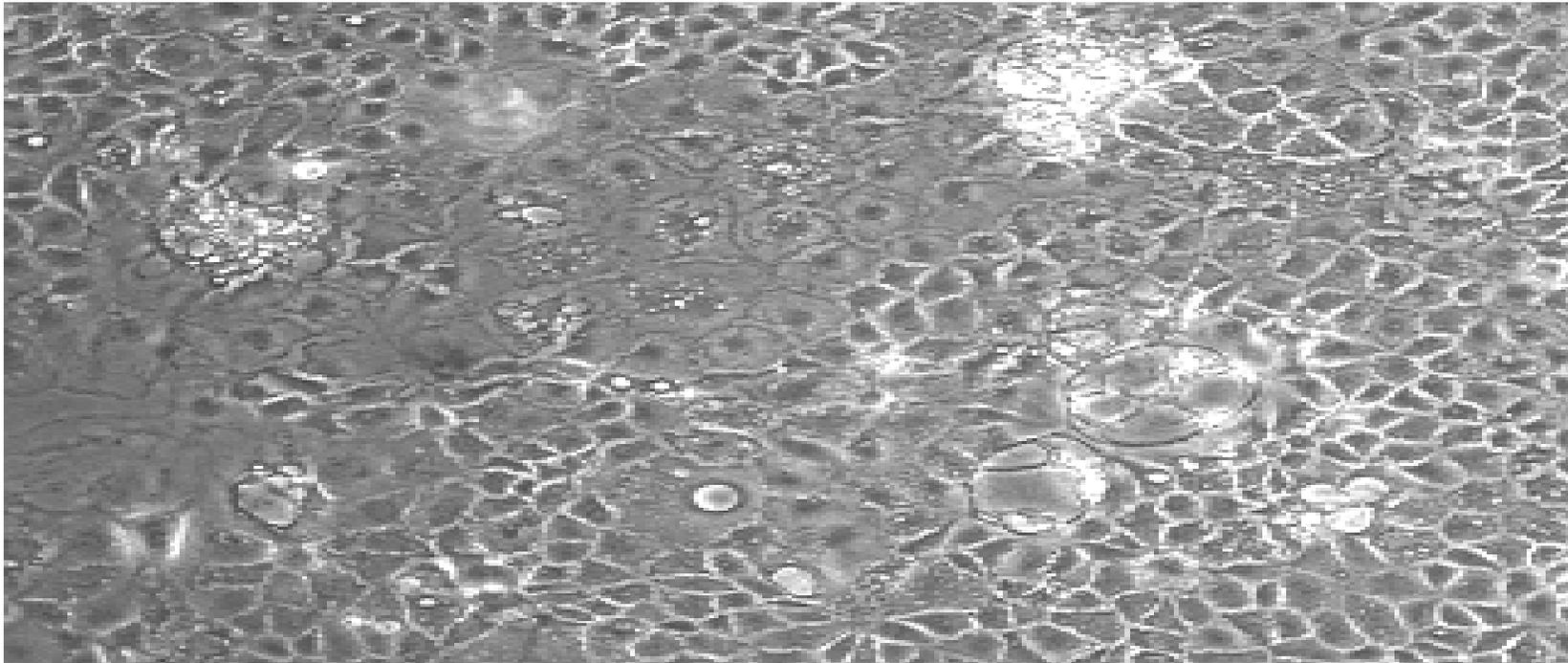
Optimization of Culture Conditions

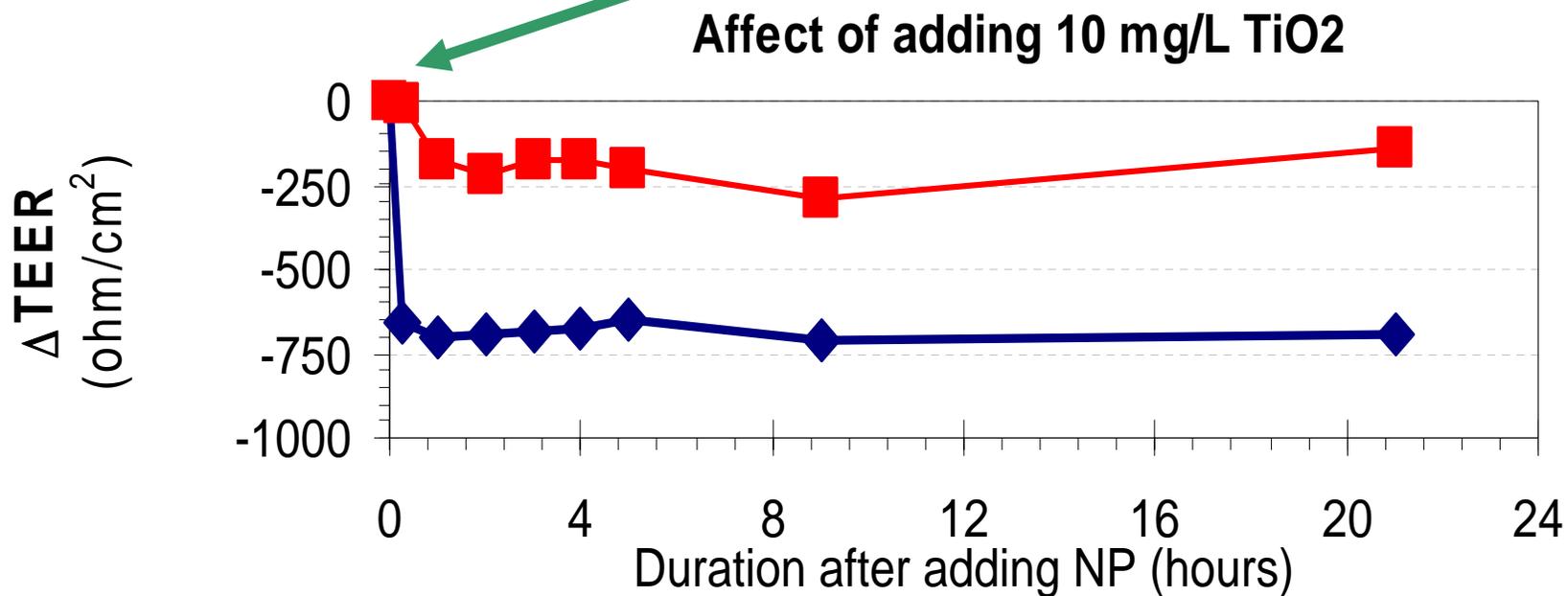
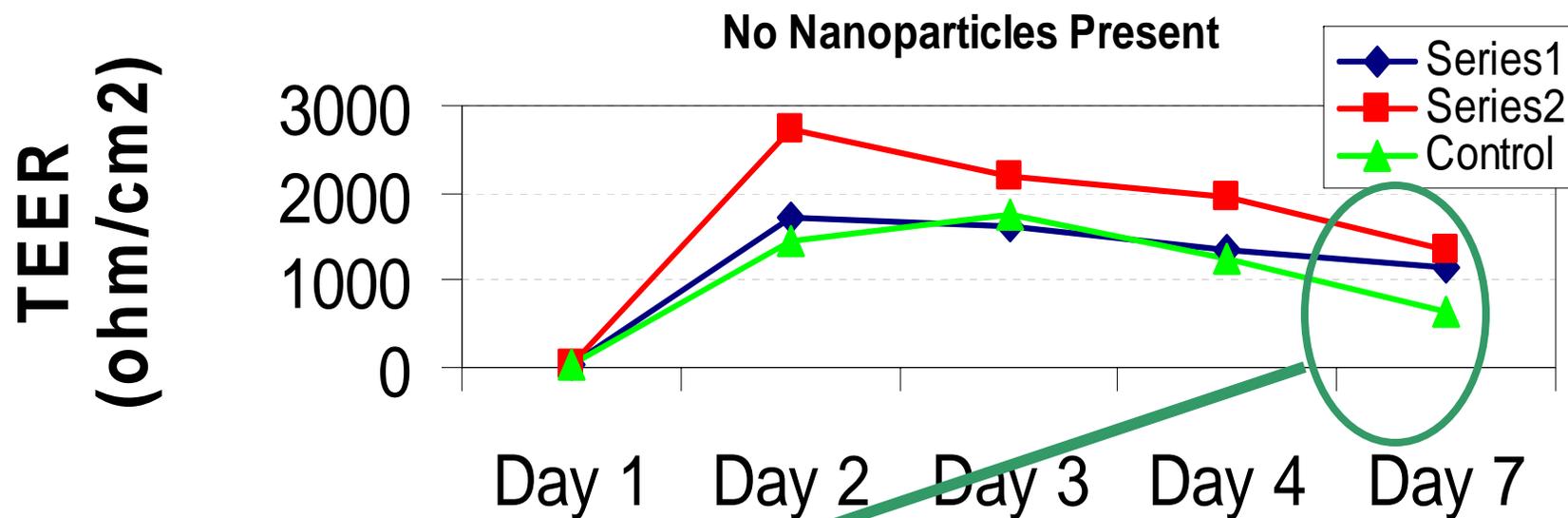
Condition	Relative Growth						
	0.05	1	2	3	4	5	
CO2 Level	5% 10%						
Transferrin ($\mu\text{g/mL}$)	0		10				
Filter diameter (mm)	24		12			6.5	
Filter pore size (μm)	3		0.4				
Filter material	Polycarb		Polyester				
Filter casing	Untreated				Collagated-PTFE		
Collogen coated	By hand		Precoated				
Frequency for media change-out (days)	3		2		1		

Optimization of Culture Conditions

- CO₂ Level: no difference with 5% or 10%
- Transferrin addition: 10 µg/L significantly better than no addition (costly but required)
- Filter diameter: 6.5 mm best (6.5 > 12 > 24 mm)
- Filter material: Polyester better than polycarbonate
- Filter coating:
 - Collagen coated better than untreated
 - Commercially precoated better than hand precoated
- Frequency for media changeout: 1 day better than 2 or 3 days

Caco-2 cells
9 days of growth in culture (10x)
Confluent cells are present

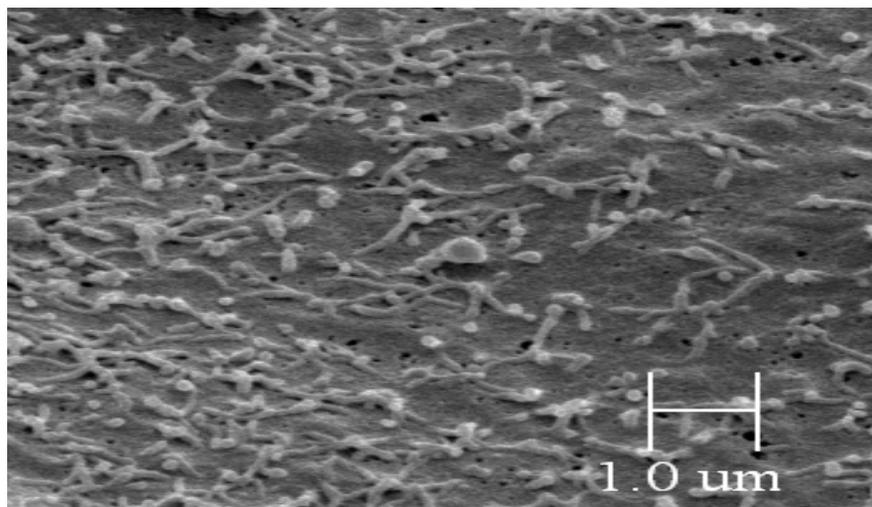
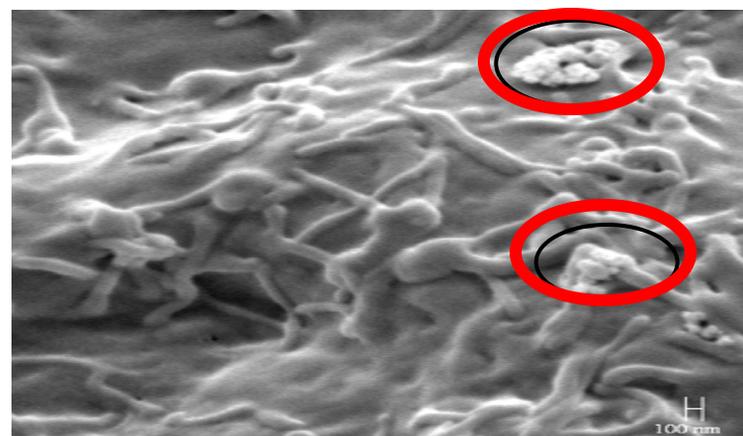
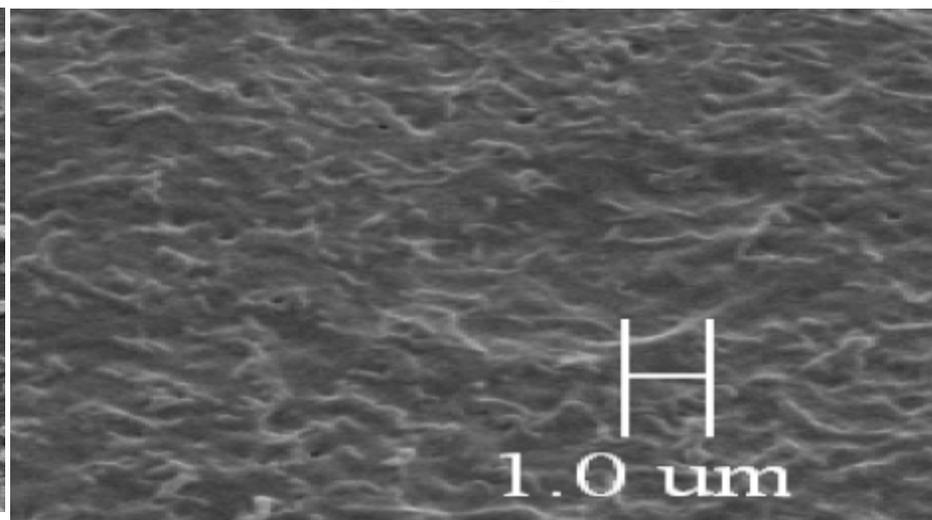




Scanning Electron Microscopy

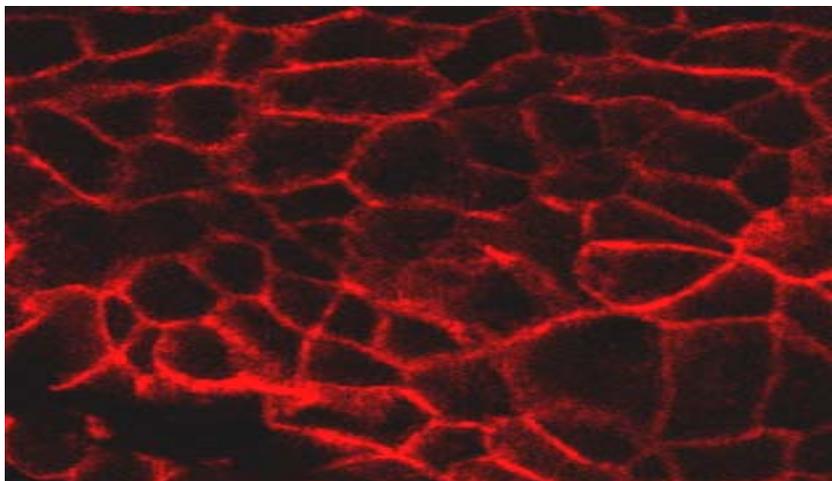
Nanoparticles may flatten down microvilli

Control

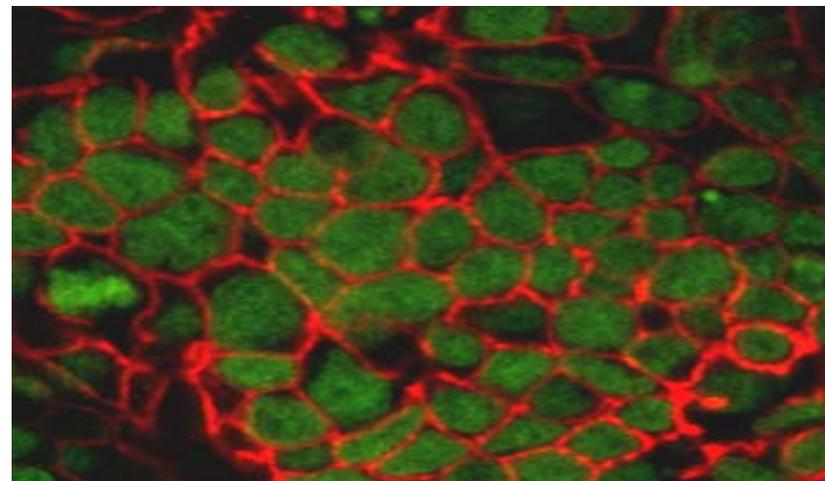
10ppm TiO₂

Confocal Scanning Laser Microscope

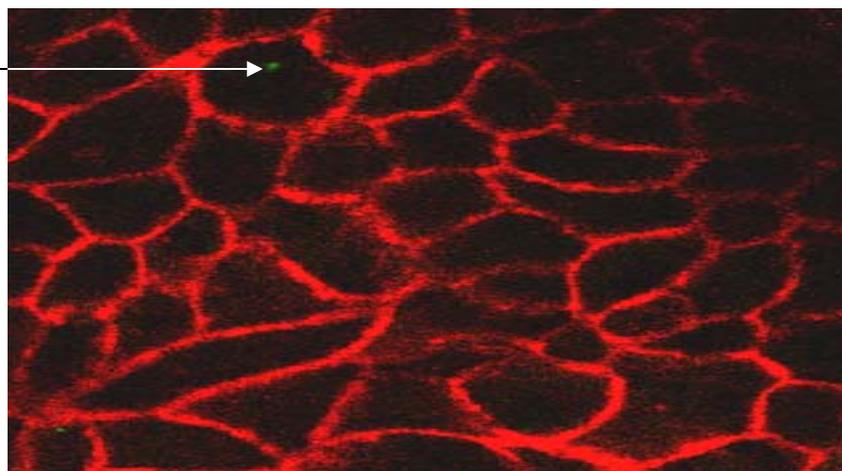
Red = gamma catenin



Green = Nuclei



Green =
Possible TiO_2
Particle
(no stain for nuclei)



Plan for Year 2

I. Nanoparticle Characterization

- Measure surface areas of commercial NPs
- Synthesize in-lab true NP and nanotubes
- Develop procedure to disaggregate commercial NPs
- Measure DLS on sample from WTP effluent

II. Nanoparticle removal during simulated water treatment

- Evaluate role of size, surface charge density, hydrophobicity, shape
- Removal after coag/sed and paper filter

III. Adsorption of pollutants onto nanoparticles

- Continue MS2/PRD1 work
- Evaluate adsorption of 1 hydrophobic and 1 hydrophilic SOC

IV. Nanoparticle Toxicity

- Test the toxicity of various nanoparticles
- Determine effects of chronic and acute exposure to nanoparticles
- Examine viability (live vs. dead) of the cells after treatment
- Design esophageal and stomach models