

Dispersion and Stability Optimization of TiO₂ Nanoparticles in Cell Culture Media

Supporting Information

by

Zhaoxia Ji[†], Xue Jin[‡], Saji George^{†#}, Tian Xia^{†#}, Huan Meng^{†#}, Xiang Wang^{†#}, Elizabeth Suarez^{†¶}, Haiyuan Zhang^{†#}, Eric M.V. Hoek^{†‡}, Hilary Godwin^{†¶}, André E. Nel^{†#}, and Jeffrey I. Zink^{†§*}

[†]California NanoSystems Institute, [‡]Department of Civil & Environmental Engineering, [#]Department of Medicine-Division of NanoMedicine, [¶]Department of Environmental Health Sciences, [§]Department of Chemistry and Biochemistry, University of California at Los Angeles, Los Angeles, CA 90095, USA

*Corresponding author: Department of Chemistry and Biochemistry, University of California, Los Angeles, 405 Hilgard Ave., Los Angeles, CA 90095; phone: (310) 825-1001; fax: (310) 206-4038
email: zink@chem.ucla.edu

Submitted to

The Bill Glaze Tribute Issue

Environmental Science & Technology

February 05, 2010

No. of pages: 8
No. of Figures: 2
No. of Tables: 1

Materials and Methods

Nanoparticles and Chemicals. Titanium dioxide P25 nanoparticles were purchased from Evonik (Germany). Bronchial Epithelial Growth Medium (BEGM) was obtained from Lonza. Dulbecco's Modified Eagle's Medium (DMEM) with high glucose was purchased from Invitrogen. Both Luria Bertani Broth (LB) and Tryptic Soy Broth (TSB) were from Fisher Scientific. Synthetic Defined medium (SD) and Difco™ Yeast Extract Peptone Dextrose medium (YPD) were supplied by Clotech and BD Bioscience, respectively. Low-endotoxin bovine serum albumin (BSA) and BenchMark® fetal bovine serum (FBS) were from Gemini Bio-Products. All nanoparticle stock solutions were prepared using pure deionized water (resistivity >18 MΩ-cm).

Physicochemical Characterization of TiO₂ Nanoparticles. Transmission electron microscopy (TEM, JEOL 1200 EX, accelerating voltage 80 kV) was used to determine the primary size and morphology of TiO₂ nanoparticles. Samples were prepared by placing a drop of the TiO₂ aqueous suspension on a carbon-coated TEM grid and waiting till all the water evaporates. X-ray powder diffraction (XRD, Panalytical X'Pert Pro diffractometer, CuKα radiation) was utilized to identify the crystalline phases present in the TiO₂ sample. The XRD pattern (Figure 1c) was collected with a step size of 0.02 ° and counting time of 0.5 s per step over a range of 20-80° 2θ. All peaks were indexed to either anatase (A) or rutile (R) and no other phase was detected. The mass fraction of rutile in the sample was determined using the empirical equation [1]: $W_R = 1/[1+0.79(I_A/I_R)]$. Where W_R is the mass fraction of rutile; I_A and I_R are the diffraction peak intensities for

anatase 101 reflection (the first peak in Figure 1c) and rutile 110 reflection (the second peak in Figure 1c), respectively. The results showed that TiO₂ P25 is composed of 81 wt.% anatase and 19 wt.% rutile.

Preparation of TiO₂ Nanoparticle Suspension in Media. TiO₂ P25 nanoparticles, received as dry powder, were weighed on an analytical balance in the fume hood; and then suspended in deionized water at a concentration of 1 mg mL⁻¹ in a 36 mL glass sample vial. After that, the suspension was sonicated for 15 mins in a water bath sonicator (Branson, Model 2510, 100 W output power; 42 kHz frequency) and was used as the stock solution for further dispersion in cell culture media. All cell culture media were filtered through a 0.22 μm Millipore vacuum filter to remove dust and any non-sample contaminants. To the filtered cell culture medium, an appropriate amount of the stock solution was added to obtain the final nanoparticle suspension with a desired concentration. The diluted TiO₂ nanoparticle suspension in cell culture medium was vortexed for 15 s (fixed-speed vortex, 02-215-360, Fisher Scientific), sonicated for 15 mins, and followed by another 15 s vortex to obtain the final nanoparticle suspension. TiO₂ nanoparticle concentration in the final suspension was fixed at 50 μg mL⁻¹ unless otherwise specified. When dispersing agents such as BSA or FBS were used, they were added into cell culture medium before the stock solution was added. Figure S1 shows a schematic diagram of preparing TiO₂ nanoparticle suspension in cell culture media with or without dispersing agents.

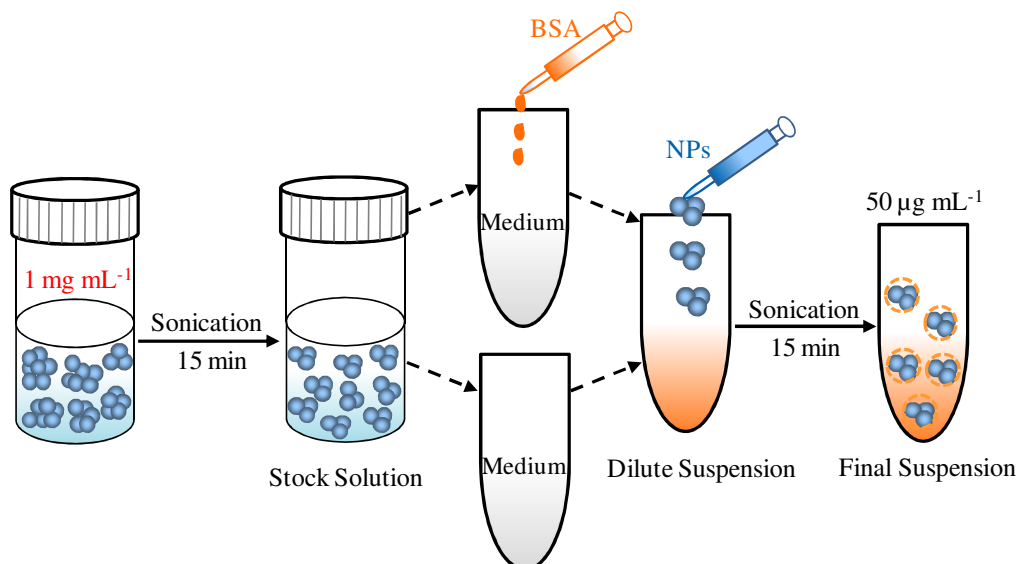


Figure S1. A schematic diagram of TiO₂ nanoparticle dispersion in cell culture media.

TiO₂ Nanoparticle Dispersion Characterization. High throughput dynamic light scattering (HT-DLS, Dynapro™ Plate Reader, Wyatt Technology) was performed to determine the particle size distribution and state of agglomeration/dispersion of the nanoparticles in water and all different cell culture media. This technique is based on the light scattering by particles that are undergoing random Brownian motion in the media. The scattered light fluctuates with time and can be related to the particle translational diffusion coefficient (D_t), from which hydrodynamic diameter (d_H , the diameter of a sphere that has the same diffusion coefficient as the particles) of nanoparticles can then be estimated using the Stokes-Einstein equation $d_H = kT/3\pi\eta D_t$; where k is the Boltzmann constant, T is the temperature, and η is the viscosity. All measurements were conducted in a 384-well plate at 298 K. Since this instrument offers a much more rapid measurement compared to the traditional DLS instruments, ten runs were collected for each well and the total measurement time for each well was 30-50 s. To ensure reproducibility, samples were loaded in triplicate. Regularization algorithm was carried

out using the software DYNAMICS v6 to determine the average hydrodynamic diameter and the polydispersity index (*PdI*, square of the polydispersity divided by the square of the mean hydrodynamic radius) of the TiO₂ nanoparticles.

Stability Evaluation of TiO₂ Nanoparticle Dispersion. In addition to the nanoparticle dispersion characterization, the stability of the nanoparticle dispersion with or without dispersing agents was also evaluated. For that, a sedimentation study was performed by monitoring the absorbance at 490 nm as a function of time (ELx808 Absorbance Microplate Reader, BioTek Instruments, Inc.). For each cell culture medium a calibration plot was generated, which showed a linear correlation between the absorbance at 490 nm and the TiO₂ nanoparticle concentration (1-50 µg mL⁻¹). Typically, a 5 mL TiO₂ nanoparticle suspension was prepared, 200 µL of the supernatant was then taken at 1 hr time intervals for absorbance reading, and the corresponding TiO₂ concentration was determined using the established correlation.

Zeta potential measurement of the TiO₂ nanoparticle suspension was performed using a ZetaSizer Nano-ZS instrument (Malvern Instruments). The technique used by this instrument is known as Laser Doppler Velocimetry (LDV), which basically measures the velocity of particles moving through a fluid in an electrophoresis experiment. The measured velocity is usually expressed in unit field strength and commonly referred to as its electrophoretic mobility (EPM). From electrophoretic mobility, zeta potential (ζ) is then calculated by applying the Henry equation. For each zeta potential measurement, 1 mL of sample was loaded in a folded capillary cell (DTS1060) and three samples were taken for each TiO₂ suspension.

A standard water quality analysis was conducted for all cell culture media. pH and conductivity of all cell culture media were measured using a pH/conductivity meter (Accumet, Fisher Scientific, Pittsburgh, PA). From the conductivity, ionic strength in each medium was also calculated. Concentrations of major cations were measured using an inductively coupled plasma mass spectroscopy (ICP-MS, Agilent 7500ce). Based on the concentrations of the two major divalent cations Ca^{2+} and Mg^{2+} , the total hardness expressed in terms of an equivalent concentration of calcium carbonate (mg/L CaCO_3) was calculated. Concentrations of major anions including Cl^- , SO_4^{2-} , PO_4^{3-} and NO_3^- were also determined by ion chromatography (Model DX 500, Dionex, Sunnyvale, CA). All other methods for the water quality analysis are included in the supporting information.

Supplementary Results and Discussion

Water Chemistry Analysis of Cell Culture Media. As shown in Table S1, all media had high ionic strength and high conductivity. The main cations were Na^+ and K^+ . The hardness appeared to be much higher in DMEM and SD media. PO_4^{3-} , SO_4^{2-} , and Cl^- were detected as the main anions in the media. The effects of water chemistries, especially multivalent cations (Ca^{2+} and Mg^{2+}) and phosphate ions on TiO_2 nanoparticle dispersion has been extensively discussed in the main context.

Stability Evaluation of TiO_2 Nanoparticle Dispersions. The sedimentation rate was determined by monitoring the absorbance at 490 nm as a function of time using an ELx 808 absorbance microplate reader. It should be noted that the absorbance reading at 490 nm was due to light scattering by TiO_2 nanoparticles since the wide bandgap ($E_g \sim 3.2$ eV,

UV-vis spectrum not shown) of this material does not allow it absorb at 490 nm. Figure S2 shows the temporal evolution of TiO₂ nanoparticle concentration in all six media. All nanoparticle suspensions exhibited much poorer stability compared to that in water. At a 50 µg mL⁻¹ initial concentration, only 1-7 µg mL⁻¹ of TiO₂ nanoparticles was left in the supernatant after 24 hour.

Table S1. A detailed water chemistry analysis of six different cell culture media including BEGM, DMEM, LB, TSB, SD, and YPD.

Parameter	unit	Medium					
		BEGM	DMEM	LB	TSB	SD	YPD
pH		7.7	7.5	6.7	7.4	4.6	6.0
TOC	[ppm]	3588	3831	6472	10028	8397	23781
UV254	[AU]	2.2	2.6	21.7	16.7	3.1	35.6
SUVA	[AU/ppm]	0.0	0.0	0.0	0.0	0.0	0.0
Turbidity	[NTU]	0.7	2.2	1.3	1.1	0.7	1.0
Conductivity	[ms/cm]	14.7	16.7	11.9	14.9	11.6	3.0
Ionic strength	[M]	0.24	0.27	0.19	0.24	0.19	0.05
TDS	[mg/L]	9408	10688	7616	9536	7424	1901
Alkalinity	[mg CaCO ₃ /L]	1376	2032	754	1700	24	664
Hardness	[mg CaCO ₃ /L]	69.2	254.4	43.0	78.7	476.2	52.1
Cl ⁻	[mg/L]	3985	4070	4708	5070	121	52
SO ₄ ²⁻	[mg/L]	15.1	99.9	41.0	87.0	N.D.	N.D.
PO ₄ ³⁻	[mg/L]	203	80	235	1078	528	781
Na ⁺	[ppm]	3041	3483	2635	2464	40	207
Mg ²⁺	[ppm]	13.8	19.2	3.7	13.1	94.1	6.1
Al ³⁺	[ppm]	0.03	0.04	0.09	0.24	0.06	0.15
K ⁺	[ppm]	51	191	419	1237	241	511
Ca ²⁺	[ppm]	5.0	70.3	11.1	10.0	35.6	10.8
Ti ⁴⁺	[ppm]	0.01	0.01	0.04	0.13	0.03	0.02
Mn ²⁺	[ppm]	0.001	0.005	0.017	0.025	0.199	0.028
Fe ^{2+/3+}	[ppm]	0.09	0.19	0.39	0.58	0.03	0.67
Cu ²⁺	[ppm]	0.003	0.017	0.012	0.018	0.014	0.039
Zn ²⁺	[ppm]	0.22	0.25	1.15	0.82	0.16	1.30
B	[ppm]	N.D.	0.02	0.06	0.30	0.08	0.06
P	[ppm]	66	26	77	352	172	255

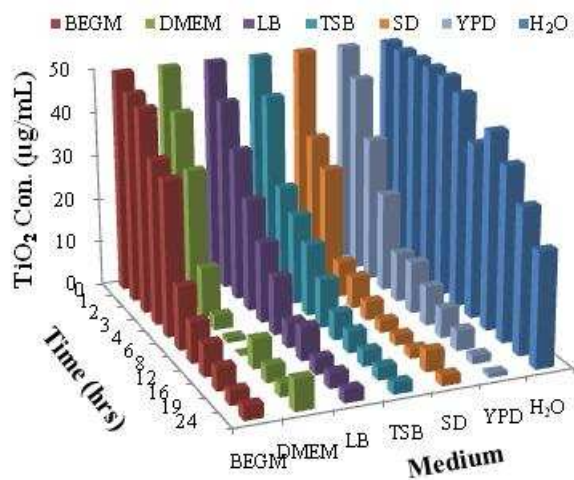


Figure S2. Temporal decrease in TiO₂ nanoparticle concentration in water and cell culture media in the absence of dispersing agents.

Literature Cited:

1. Scotti, R.; Bellobono, I. R.; Canevali, C.; Cannas, C.; Catti, M.; D'Arienzo, M.; Musinu, A.; Polizzi, S.; Sommariva, M.; Testino, A.; Morazzoni, F. Sol-Gel Pure and Mixed-Phase Titanium Dioxide for Photocatalytic Purposes: Relations between Phase Composition, Catalytic Activity, and Charge-Trapped Sites. *Chem. Mater.* **2008**, *20*, 4051–4061.