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PHOTOCHEMICAL PROPERTIES AND CYTOTOXICITY OF TIO_2 NANOPARTICLES DEPENDING ON THE DEGREE OF AGGLOMERATION

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Abstract: The purpose of this paper is to evaluate the photocatalytic activity and the cytotoxicity of the primary TiO_2 nanoparticles (P-TiO_2 NPs) comparing with aggregated TiO_2 nanoparticles (A-TiO_2 NPs). A-TiO_2 NPs were suspended in pH 8 phosphate buffer and stabilized after magnetic stirring. P-TiO_2 NPs were isolated from A-TiO_2 NPs by using centrifuge method. The photocatalytic activity was measured by observing the photocatalytic oxidation rate of NADH and the cytotoxicity assessment was evaluated by using the MTT assay. The oxidation rate of NADH in A-TiO_2 NP suspension was slightly higher than that in P-TiO_2 NP suspension. P-TiO_2 NP showed about 20% higher cytotoxicity than A-TiO_2 NP at the concentration of 0.3 mg/ml.

Keywords: agglomerated TiO_2 NP, primary TiO_2 NP, photocatalytic activity, cytotoxicity

1. INTRODUCTION

TiO_2 NPs have been widely used as cosmetic ingredients for sunscreen and photocatalysts for the decomposition of wastewater by releasing ROS species under UV irradiation. The research interest on TiO_2 NPs has been increased along with the increase of industrial use. On the precedent study, TiO_2 NPs are reported to be easily aggregated in aqueous solution. Karla and David reported that although primary particle size of commercial TiO_2 NPs is usually less than 50 nm, the NPs are observed in the form of aggregates or agglomerates with the size of 100 ~ 1,000 nm in solution[1,2]. For this reason, researchers have tried to disperse NPs as a stable suspension and analyze the surface area of agglomerated TiO_2 NPs in aqueous solution. Particle size affects the photocatalytic activity. As the size of the TiO_2 NPs becomes smaller, the specific surface area is increased resulting in the increased photocatalytic activities. So the large surface area of NPs can be a profit to chemical industry providing efficient photocatalytic materials. However the small size of NPs can give adverse effect in the aspect of safety. Because small particles are usually known to penetrate into the inner part of the cells, they are regarded as more hazardous than the larger ones. Karin *et al.* observed that NPs less than 100 nm in size posed a significant health risk to human. Michael *et al.* reported that the reactivity of small size NPs is higher than that of the micron size particles. However, the study on the difference between agglomerated and primary NPs has not been yet performed in respect of photocatalytic activity and nanotoxicity.

In this study, we observed the photocatalytic activity and cytotoxicity of aqueous suspension of TiO_2 NPs (P-TiO_2 NPs) in the form of well-dispersed primary particles comparing with that of TiO_2 NPs in agglomerated forms (A-TiO_2 NPs).

2. METERIALS AND METHODS

Stabilized A-TiO_2 NP suspension was obtained in pH 8 phosphate buffer solution by stirring with a magnetic bar for 24 h. After sonication of A-TiO_2 NP suspension, stabilized P-TiO_2 NP suspension was isolated as the supernatant of centrifuged A-TiO_2 NP suspension.

The concentration of P- and A-TiO_2 NP suspension was determined by measuring the weight of dried powders after dehydration of 1 mL suspension in an oven until the weight change was lower than 0.1 % for one day. As the maximal concentration of P-TiO_2 NP suspension was 2 mg/ml, the concentration of A-TiO_2 NP suspension was adjusted to be the same value for comparison with P-TiO_2 NPs.

DLS size of TiO_2 NP in aqueous suspension was measured using a particle size analyzer (Ostuka Electronics, Photal ELS-Z). The UV-visible absorption spectrum of TiO_2 NP in aqueous suspension was measured using a spectrophotometer (Shimadzu UV-1700). The size and shape of P-TiO_2 NPs were measure using a scanning electron microscope (SEM, S-4800, HITACHI). The individual sizes of the P-TiO_2 NPs were analyzed on the image displayed by graphic program. The Photocatalytic activities of both NPs were compared by using NADH as the substrate for photocatalytic degradation.

3. RESULTS AND DISCUSSION

Fig. 1 shows the SEM image of the P-TiO_2 NPs. The average size of P-TiO_2 NPs was 24 ± 0.9 nm and the NPs exhibited elliptical in morphology.

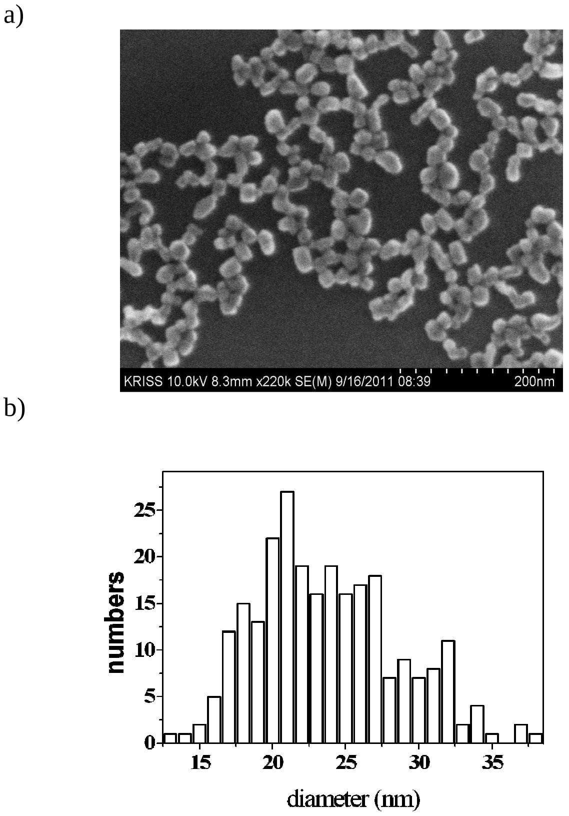

Figure 1. a) The SEM image of the P-TiO_2 sample, b)Particle

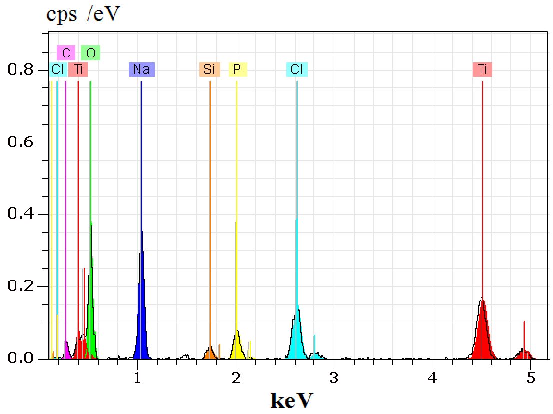

Figure 2. SEM-EDX spectrum of P-TiO_2 NPs dispersed in pH 8 phosphate buffer solution

The EDS spectrum (Fig. 2) shows the characteristic K_α and K_β peak of titanium (energy of K_α and K_β: 4.511 keV and 4.931 keV). And other peaks were identified as phosphorous (K_α: 2.013 keV, K_β: 2.139 keV), chlorine (K_α: 2.622 keV, K_β: 2.815 keV), chloride (K_α: 2.622 keV, K_β: 2.815 keV) and silicon (K_α: 1.739 keV, K_β: 1.835 keV). Phosphorous and chlorine species came from the Na_2HPO_4, which was the composition of pH 8 buffer solution. And chloride and silicon species are associated with silicon substrate.

Table 1. SEM-EDX spectra elements of P-TiO_2 dispersed in pH 8 phosphate buffer solution

Element	Atom (%)
Titanium	21.09
Oxygen	48.18
Sodium	14.82
Chlorine	6.37
Carbon	5.45
Phosphorus	2.71
Silicon	1.38

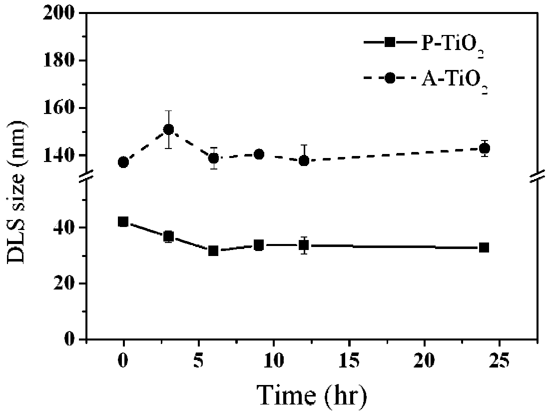

Figure 3. The average hydrodynamic diameter of P-TiO_2 and A-TiO_2 NPs in cell culture media as injection time

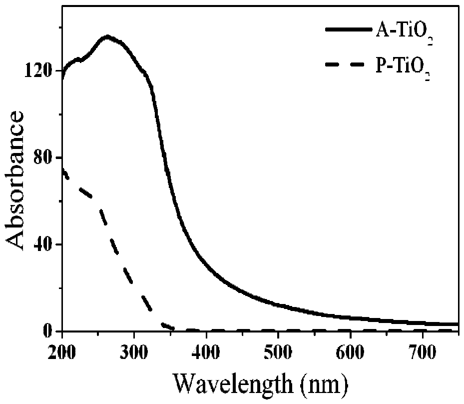

Figure 4. UV-vis absorption spectra of TiO_2 NPs at 2 mg/ml

Fig. 3 shows the DLS size of TiO_2 NPs in RPMI containing 10% FBS media. The hydrodynamic size was maintained to be 30 ± 10 nm and 140 ± 10 nm for 24 h in cell culture media with TiO_2 NP concentrations of 20 µg/ml. Fig. 4 shows UV-vis absorption spectra of aqueous TiO_2 NP suspensions. The absorption band maximum of P-TiO_2 was blue shifted compared with of A-TiO_2. The optical density of A-TiO_2 NP suspension was higher than

Table 2. Photo-oxidation rate constant (K_{app}) of NADH in the presence of TiO_2 nanoparticles

Concentration of TiO_2 (mg/ml)	K_{app} (µM/min) A-TiO_2 NPs	 P-TiO_2 NPs
0.46	0.75	0.70
0.23	1.30	1.02
0.11	1.81	1.32
0.06	2.87	28
0.03	3.34	1.94
0.01	3.44	1.37

that of P-TiO_2 NP suspension at the same concentration. In the range of near-UV wavelength (310 - 340 nm), the extinction coefficiency of A-TiO_2 NPs was five times higher than that of P-TiO_2 NPs.

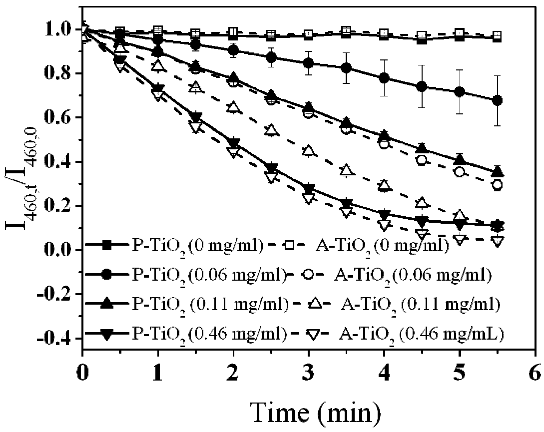

Figure 5. Relative fluorescence intensity [$I_{460,t}/I_{460,0}$] of NADH in the presence TiO_2 NPs

Fig. 5 shows the photocatalytic degradation of NADH in the presence of P-TiO_2 NPs and A-TiO_2 NPs in pH 8 phosphate buffer solution. The slope of curve is related the rate of NADH oxidation. The curve is represented with normalized value of fluorescence intensity at 460 nm. The normalization was done by using the initial fluorescence intensity of NADH before UV irradiation. ($I_{460,t}$/ $I_{460,0}$) In the absence of TiO_2 NPs, $I_{460,t}/I_{460,0}$ did not reduced maintaining unity for ten minutes by UV irradiation, which meant that NADH was not oxidized by the absorption of UV light itself but photo-oxidized by the catalytic activity of TiO_2 NPs.

k_{app} values of NADH photooxidation in the presence of P-TiO_2 NPs and A-TiO_2 NPs are summarized in Table 1.

It was calculated from the slope in the plot of $I_{460,t}/I_{460,0}$ *vs* UV irradiation time. When the concentration of TiO_2 NPs was 30 µg/ml, k_{app} value of P-TiO_2 NPs was 3.34 µM/min and that of A-TiO_2 NPs was 1.94 µM/min. When the concentration of TiO_2 NPs was 10 µg/ml, the k_{app} value of P-TiO_2 NPs was 3.44 µM/min and that of A-TiO_2 NPs was 1.37 µM/min. P-TiO_2 NPs exhibited higher k_{app} values than A-TiO_2 NPs in the concentration range of 10 ~ 460 µg/ml.

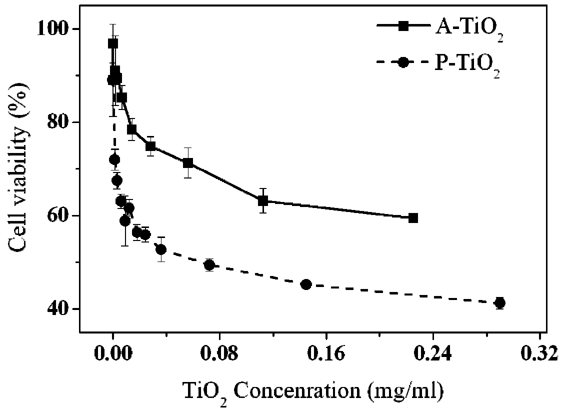

Figure 6. Cytotoxicity test of TiO_2 nanoparticles. RAW 264.7 cells were exposed 24 hr to nanoparticles, and cell mortality was assessed by MTT assay.

Fig. 6 shows the viability of RAW 264.cells according to the concentration of TiO_2 NPs. The cell viabilities in the presence of TiO_2 NPs were calculated from the normalized absorption of reduced MTT relative to negative control. The IC_{50} of P-TiO_2 NPs was 26.7 µg/ml and P-TiO_2 NPs exerted 20% higher cytotoxicity compared with A-TiO_2 NPs. Cell viability was dependent on NPs concentration. It was dramtically reduced until 20 ~ 40 µg/ml but slowly decreased above 40 µg/ml in both forms of TiO_2 NPs.

5. REFERENCES

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