# AGGREGATION STATE OF FULLERENE NANOPARTICLES: IMPLICATIONS FOR REACTIVITY, TRANSPORT, AND MICROBIAL TOXICITY

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## ABSTRACT

The production and use of engineered nanomaterials (ENMs) will likely lead to environmental exposure with unknown consequences. In particular, carbon-based nanomaterials (CNMs) such as fullerene and carbon nanotubes (CNTs) have been of great interest across a wide array of fields. However, due to the extremely low solubility of non-functionalized CNMs, individual molecules of CNMs are not typically present in water. Rather, CNMs tend to form colloidal aggregates. In this work, we show that variation in characteristics of the colloidal aggregates is attributed to difference in electrophoretic mobility, relative affinity for an organic solvent (i.e., toluene), photoreactivity, and microbial toxicity of the fullerene nanoparticles. This implies that studies evaluating potential environmental impacts of released ENMs should take into account the aggregation states of nanoparticles.

**Keywords:** Engineered nanomaterials, fullerene, carbon nanotubes, colloidal aggregates, photoreactivity, microbial toxicity.

## INTRODUCTION

Nanomaterials are becoming more available in the products we use daily and an extensive number of consumer end-products are being manufactured with engineered nanomaterials (ENMs). As of March 2011, more than 1,300 products were reported as nanomaterial-based consumer products and devices (Nanotechnology Consumer Product Inventory, http://www.nanotechproject.org).

In particular, carbon-based nanomaterials including fullerene $C_{60}$, single-wall (SW) and multi-wall (MW) carbon nanotubes (CNTs) show unique physical, chemical, and photochemical properties, that have potential applicability for water treatment and energy production as well as biomedical drug carriers (Mauter and Elimelech, 2008, Bosi et al., 2003).

Due to the rapid growth of nanotechnology, it is clear that the substantial production and use of ENMs will likely lead to environmental exposure with unknown consequences. However, transformation and alteration of original properties of ENMs in the environment are largely unknown. For example, pristine fullerene nanomaterials are in micron size but they tend to form aggregates in aqueous suspensions. Colloidal aggregates of fullerene ($nC_{60}$) have been reported to produce little to no detectable reactive oxygen species (ROS) in water (Hotze et al., 2008, Lee et al., 2007). However, we recently found that when the $nC_{60}$ suspension was fractionated by successive membrane filtration to enrich it in smaller aggregates, the net ROS production increased associated with bacterial and viral inactivation (Chae et al., 2010a).

Also, it was previously thought that non-functionalized CNTs were not dispersed in aqueous suspensions. However, we reported that CNTs could form colloidal aggregates in water after sonication. Colloidal aggregates of CNTs (i.e., *n*SWCNT or *n*MWCNT) are observed to be considerably more photoactive than underivatized $nC_{60}$ (Chae et al., 2011).

Therefore, it is important to study alteration of the aggregates' properties for better understanding of their risk in the environment. In this work, we select four fullerene nanomaterials and explore the effects of aggregation states of nanoparticles on photocatalytic reactivity, mobility, and bacterial toxicity.

## MATERIAL AND METHODS

### Fullerene nanomaterials

Spherical fullerenes such as $C_{60}$ (99.9%) and $C_{60}(OH)_{24}$ were purchased from MER (Tucson, AZ). Tubular fullerenes such as SWCNT and MWCNT were obtained from BuckyUSA (+99.5%, Huston, TX). The initial diameter of the CNTs as reported by the supplier was 0.5 - 4 µm for the SWCNTs and about 50 µm for the MWCNTs.

### Preparation of fullerene nanoparticle suspensions

All fullerene suspensions were prepared in deionized (DI) water that had a resistivity of 18.2 MΩ -cm and dissolved organic carbon concentration was less than 30 ppb (Nanopure, Barnstead, Dubuque, IA). A stock suspension of $C_{60}(OH)_{24}$ was prepared by adding pristine powder form to DI water and stirred at 200 rpm for 24 hours. Aqueous suspensions of $C_{60}$, SWCNT, and MWCNT were prepared by sonication with a high-energy probe (S-4000, Misonix, Qsonica, LLC, Newtown, CT) for 10 h without any organic solvent addition. All suspensions were stored in a refrigerator at $4^{o}C$ before use.

## Characterisation of fullerene nanoparticle suspensions

Total carbon (TC) concentrations of the fullerene suspensions were measured by a total organic carbon (TOC) analyzer (TOC-5050A, Shimadzu, Columbia, MD). Hydrodynamic diameter ($d_h$) of nanoparticle aggregates was measured by DLS (dynamic laser scattering) and electrophoretic mobility (EPM) was measured by Zetasizer Nano (Malvern Instrument, Bedford, MA).

Surface morphology of pristine nanomaterials was examined by scanning electron microscopy (SEM) (FEI XL30 SEM-FEG, Hillsboro, OR). High magnification images of the suspensions are obtained by TEM (FEI Tecnai G2 Twin, Hillsboro, OR). 10 μL of each sample was dropped on a lacey carbon/Cu grid (300 meshes, Electron Microscopy Sciences, Hatfield, PA) and dried in air before TEM measurement.

Hydrophobicity coefficient ($K_{tw}$) of the fullerene suspensions was determined as basis for quantifying the relative affinity of these materials for the aqueous phase as described in the previous study (Chae et al., 2010b). The $K_{tw}$ was calculated by the following equation.

$$K_{tw} = \frac{C_t}{C_w} \qquad (1)$$

where $C_t$ is the concentration of fullerene in toluene and $C_w$ is the concentration of fullerene in water.

## Degradation of 2-chlorophenol by fullerene nanoparticles

2-Chlorophenol (2CP), which was purchased from Sigma-Aldrich (St. Louis, MO), was selected as a model organic compound for its sensitivity to singlet oxygen ($^1O_2$) (Gryglik et al., 2007, Chae et al., 2009). The fullerene-enabled degradation of 2CP was studied in a glass beaker (90 mm, O. D. x 115 mm, H) with a water jacket connected to a water circulator for temperature control as previously described (Chae et al., 2009). The light source was two 15 W fluorescent ultraviolet bulbs (Philips TLD 15W/08) in an UV/Cryo chamber (Electron Microscopy Science, Hatfield, PA). These bulbs had an output spectrum ranging from 310 to 400 nm and a total irradiance of 24.1 $W/m^2$ with a peak at 365 nm (UV-A). Water samples were collected from the suspension every five minutes for 30 min for further analyses. Concentration of 2CP was monitored over time in fullerene suspensions using a high-performance liquid chromatography (HPLC) (ProStar, Varian, Palo Alto, CA) equipped with a reverse phase column (Ultra aqueous C18, 5 μm, 150 x 4.6 mm, RESTEK, Bellefonte, PA) and a photodiode array (PDA) detector with a detection wavelength of 210 nm as previously described (Chae et al., 2009). Assuming that the probe substance (here, 2CP) reacts primarily with singlet oxygen ($^1O_2$) and negligibly with the excited sensitizers, we can use a simplified reaction scheme to describe this system (Chae et al., 2009). All experiments were performed in triplicate. Student's t-test was used to assess the significance of the results with a 95% confidence interval.

The pseudo-first-order rate constants of 2CP degradation by direct photolysis ($k_{dp}$, which was measured without fullerene nanoparticles under UV irradiation) and by overall reaction ($k_{overall}$, which was measured with fullerene nanoparticles under UV irradiation) were obtained from the experimental results. Then, the net pseudo-first-order rate constant, $k_{net}$ ($s^{-1}$) is calculated by subtracting $k_{dp}$ from $k_{overall}$ (Chae et al., 2009, Chae et al., 2011).

$$k_{net} = k_{overall} - k_{dp} \qquad (2)$$

## Microbial inactivation by fullerene nanoparticles

To explore bacterial inactivation by fullerene nanoparticles, exponential phase *E. coli* K12 grown in tryptone-broth at 37°C were harvested by centrifugation at 5000 *g* for 10 minutes at 4°C and resuspended in 1.6 mM PBS buffer (pH 7.3 adjusted) to obtain a stock suspension. The initial concentration of bacteria in all the test solutions is $1\text{-}1.5 \times 10^6$ colony forming unit (CFU)/mL (Chae et al., 2010b). Only 30 - 150 CFU from each plate were used for counting. Inactivation of *E. coli* K12 by the fullerene nanoparticles was calculated by the following equation.

$$\text{Inactivation} = -\log (N_t/N_o) \qquad (3)$$

where $N_t$ is the cell number of *E. coli* K12 per mL after 60 minutes and $N_o$ is the initial cell number of *E. coli* K12 per mL at time zero.

## RESULTS AND DISCUSSION

### Characteristics of fullerene nanoparicles

From SEM analysis, it was found that size of pristine powders of four fullerene namomaterials ranges from micron to millimetre (Fig. 1). However, the size of colloidal aggregates in DI water measured by DLS ($d_h$) is in sub-micron scale ($nC_{60}$ = 254.0 ± 33.9 nm, $nC_{60}(OH)_{24}$ = 193.8 ± 14.9 nm, *n*SWCNT = 215.5 ± 47.0 nm, and *n*MWCNT = 341.9 ± 66.2 nm). As shown in Figure 2, it is also confirmed by TEM images of the fullerene aggregates. The initial pH of four fullerene suspensions ranges from 4.1 to 5.9. EPMs (i.e., ζ-potentials) of $nC_{60}$, $nC_{60}(OH)_{24}$, *n*SWCNT, and *n*MWCNT suspensions averaged $-2.76 \pm 0.23 \times 10^{-8}$, $-3.35 \pm 0.19 \times 10^{-8}$, $-2.93 \pm 0.18 \times 10^{-8}$, and $-2.08 \pm 0.66 \times 10^{-8}$ $m^2/Vs$, respectively.

Table 1. Characteristics of various fullerene nanoparticles in water

| Aggregate of nanomaterial | $d_h$ (nm) | Initial pH | EPM (x $10^{-8}$ $m^2$/Vs) |
|---|---|---|---|
| $nC_{60}$ | 254.0 ± 33.9 | 4.9 | -2.76 ± 0.23 |
| $nC_{60}(OH)_{24}$ | 193.8 ± 14.9 | 5.9 | -3.35 ± 0.19 |
| *n*SWCNT | 215.5 ± 47.0 | 4.1 | -2.93 ± 0.18 |
| *n*MWCNT | 341.9 ± 66.2 | 5.3 | -2.08 ± 0.66 |

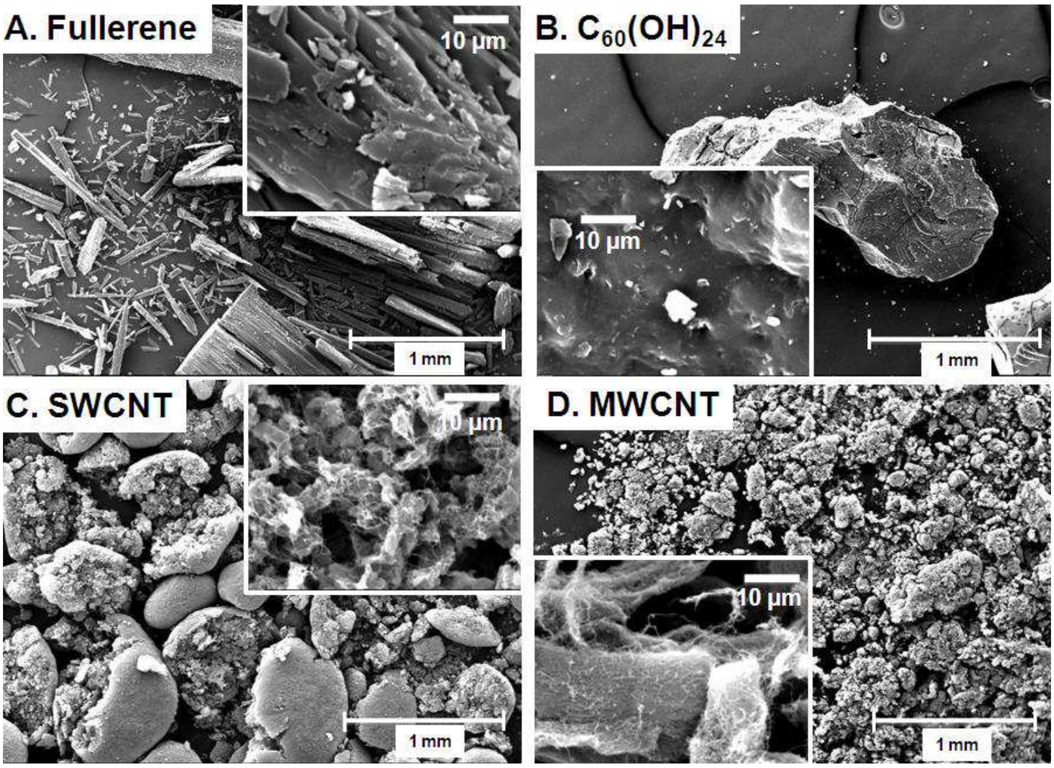

Fig. 1: SEM images of pristine fullerene-based nanomaterials.

Variation in characteristics of fullerene nanoparticle suspensions implies that each nanoparticle has the potential to participate in different states of aggregates. For example, as shown in Fig. 3, the absolute value of EPM of $nC_{60}$ decreases as hydrodynamic diameter (i.e., $d_h$) of the aggregates increased while hydrophobicity coefficient (i.e., $K_{tw}$) increased. A lower absolute value of EPM and higher $K_{tw}$ value are consistent with a less degree of hydroxylation on nanoparticle surfaces and higher affinity for an organic solvent such as toluene.

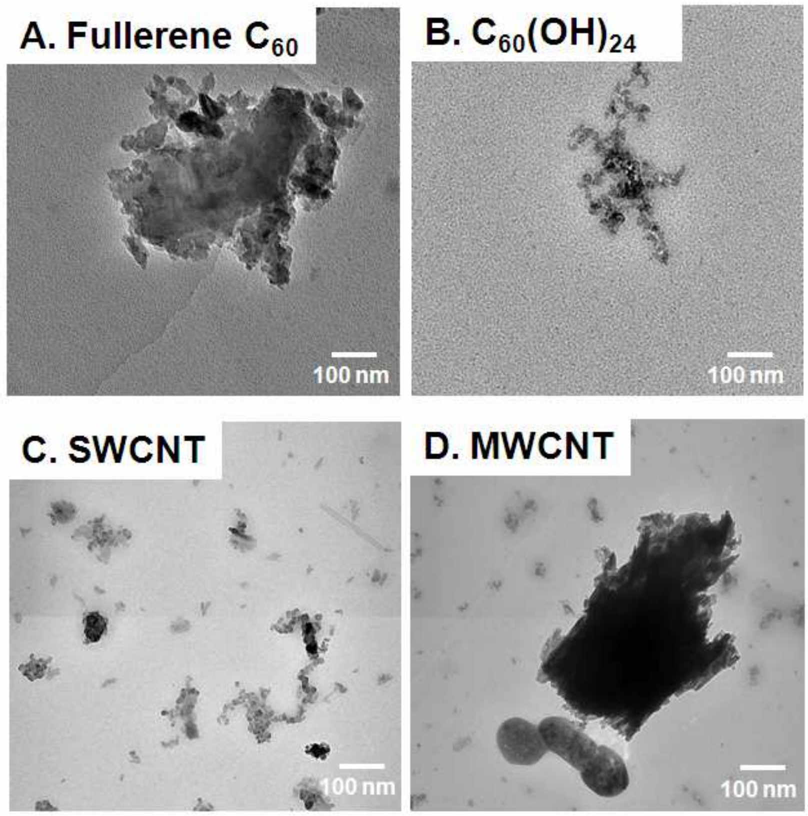

Fig. 2: TEM images of fullerene-based nanomaterials in DI water (A, C, D: after 10 hour sonication, B: after 24 hour mixing at 200 rpm).

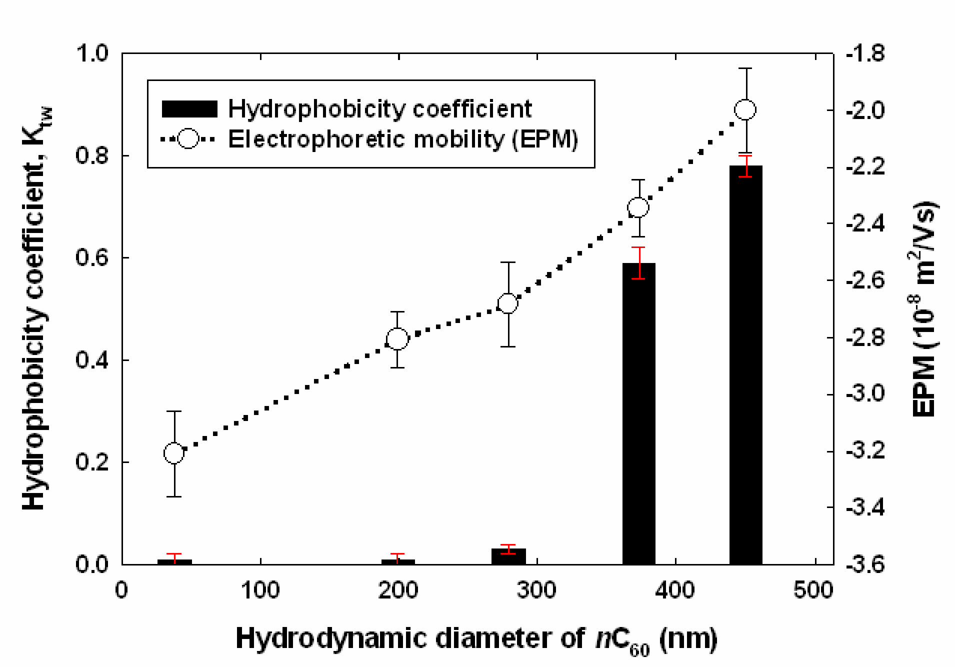

Fig. 3: Relative hydrophobicity and electrophoretic mobility (EPM) of fullerene nanoparticles ($nC_{60}$) with various sizes.

## Degradation of 2-chlorophenol by photosensitized fullerene nanoparticles

ROS production of fullerene nanoparticles was measured under UV-A irradiation. In the previous study, superoxide ($O_2^{\cdot-}$) production of the nanoparticles was negligible as measured by the reduction of XTT (2,3-bis(2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxanilide) due to the lack of an electron donor in the suspensions (Chae et al., 2010b, Chae et al., 2011).

In contrast, significant singlet oxygen ($^1O_2$) was produced by all fullerene aggregates with differences observed from one nanoparticle to another. Degradation of 2CP by the singlet oxygen produced from *n*$C_{60}$, *n*SWCNT, and *n*MWCNT is shown in Fig. 4. As shown in this figure, nanoparticles in smaller size were more efficient to degrade 2CP than those in larger size. Interestingly, the degradation rate constants for 2CP by the nanoparticle aggregates ranging between 200 and 600 nm was stable and about 7 times smaller than rates by the smaller aggregates (< 50 nm).

Despite its high quantum yield, colloidal aggregates (*n*$C_{60}$) of fullerene in water have been reported to produce little to no detectable ROS (Hotze et al., 2008, Lee et al., 2007, Lee et al., 2008). However, the sonication procedure employed to disperse or break up initial powder of fullerene and CNTs for preparation of stable suspensions has been observed to modify the surface chemistry of $C_{60}$ and CNTs. Labille *et al.* (2009) have shown Fourier Transform Infrared (FT-IR) absorption at wavelengths consistent with C-O stretching after sonication, pointing to the hydroxylation of fullerene due to the suspension mechanism (Labille et al., 2009). This may also lead to a larger fraction of the *n*$C_{60}$ present as small aggregates. Increasing the small fractions of *n*$C_{60}$ and *n*CNTs in aqueous suspension will minimize self-quenching and triplet-triplet annihilation, non-ROS producing pathways of energy dissipation, effectively improving the quantum yield of the aggregate and increasing the net ROS production observed (Hoffman et al., 2007).

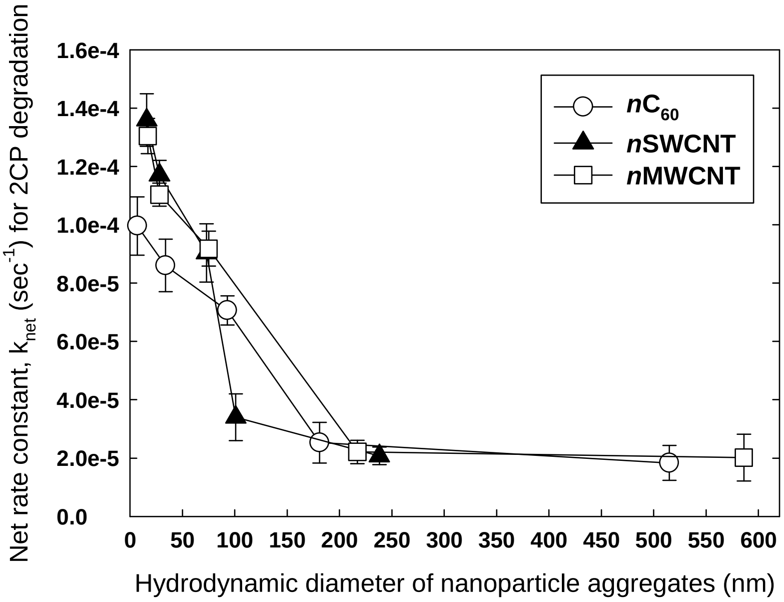

Fig. 4: Net rate constants of 2CP degradation by fullerene-based nanoparticles (TC concentrations of nanoparticle suspensions were normalized to 1 mg/L) with various sizes under UV-A irradiation at 20°C (pH = 7.0).

## Microbial disinfection by photosensitized fullerene nanoparticles

To study the effects of fullerene nanoparticles on microbial activity, we tested *E. coli* K12 with fullerene nanoparticles for 1 hour with and without UV-A irradiation. We observed less than 0.5 log inactivation of *E. coli* in the dark and under the UV irradiation without fullerene nanoparticles (data not shown). However, the ROS-mediated inactivation of *E. coli* by fullerene nanoparticles was related to the size of fullerene nanoparticles. The highest inactivation (4.5 log) occurred with the smallest size ($d_h$ = 37 nm) of $n\mathrm{C}_{60}$. However, the inactivation efficiency decreased as the size increased. Transmission electron micrographs of *E. coli* K12 confirmed that surface structure of the cell was severely damaged by $n\mathrm{C}_{60}$ and/or photosensitized $n\mathrm{C}_{60}$ (Fig. 6), while the cells had a normal appearance in all samples where bacteria were exposed to $n\mathrm{C}_{60}$ fractions in the dark (data not shown).

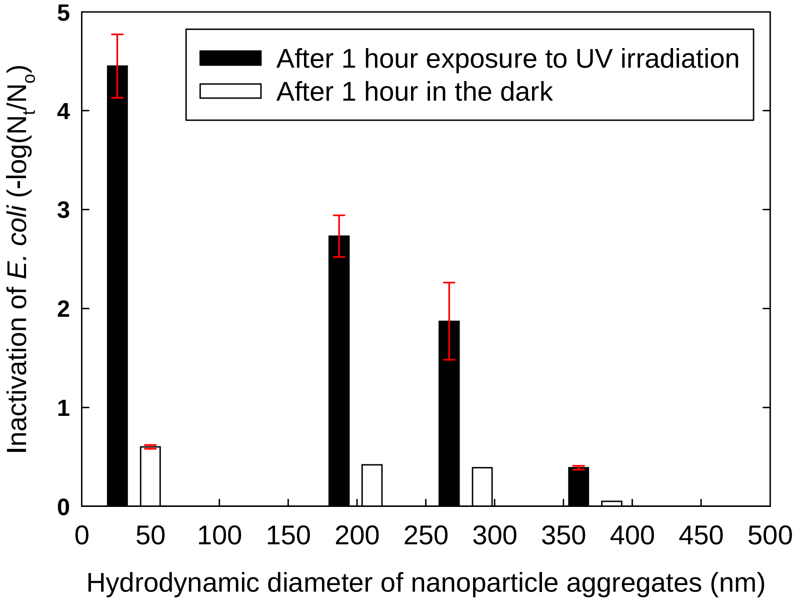

Fig. 5: Inactivation of *E. coli* K12 by fullerene aggregates ($n\mathrm{C}_{60}$, TC concentrations of nanoparticle suspensions were normalized to 1 mg/L) with various sizes (pH = 7.3).

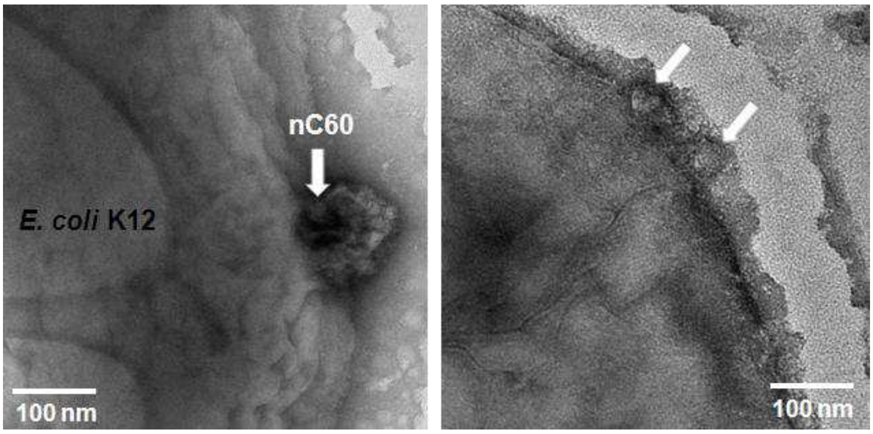

Fig. 6: TEM images of *E. coli* K12 with fullerene nanoparicles ($n\mathrm{C}_{60}$, $d_h$ = 37 nm) after 1 hour UV irradiation (pH = 7.3).

## CONCLUSIONS

Spherical and tubular fullerene nanomaterials such as $C_{60}$, $C_{60}(OH)_{24}$, SWCNT, and MWCNT formed colloidal aggregates in water with different sizes and surface properties. As the size of the colloidal aggregates measured by DLS decreased, affinity for an organic solvent (i.e., toluene) decreased while the absolute value of electrophoretic mobility increased. Variation in characteristics of the colloidal aggregates is attributed to differences in photoreactivity and microbial toxicity of the fullerene nanoparticles. We show that degradation efficiency of 2-chlorophenol by reactive oxygen species produced from fullerene nanoparticles increased as the size of the nanoparticle aggregates (especially, between 10 and 200 nm) decreased. The size effect of fullerene nanoparticles on photocatalytic inactivation of *E. coli* K12 was also clear. However, when the large nanoparticles (especially, > 300 nm) were introduced, the efficiencies of 2CP decay and ROS-mediated bacterial inactivation significantly reduced. As the quantity and influence of the more reactive fractions may increase in the aquatic environment with time, further study evaluating fate, transport, and toxicity of engineered nanoparticles should take into account the aggregation states of nanoparticles.

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Dr. Chae obtained his Ph.D 7 years ago from Korea Advanced Institute of Science and Technology (KAIST), Korea. Following his Ph.D, Dr. Chae was worked as a post-doctoral fellow in the Graduate School of Engineering at Hokkaido University (Japan) from 2004 to 2006. Since 2007, Dr. Chae was employed as a Research Associate in the CEINT (Center for the Environmental Implications of NanoTechnology) at Duke University. In December 2010, he joined in School of Chemical and Biomolecular Engineering at the University of Sydney as a lecture. Dr Chae's research interest is in the field of "membrane technology" associated with engineered nanomaterials to tackle current challenges on water and energy.