Biomolecular interaction and kinematics differences between P25 and E171 TiO₂ nanoparticles

Ridhwan Yusoff; Kathawala, Mustafa Hussain; Nguyen, Luong Thi Hien; Setyawati, Magdiel
Inggrid; Chiew, Paul; Wu, Yuansheng; Ch'ng, Ai Lee; Wang, Zheng Ming; Ng, Kee Woei

2018

Ridhwan Yusoff., Kathawala, M. H., Nguyen, L. T. H., Setyawati, M. I., Chiew, P., Wu, Y. et al.
(2018). Biomolecular interaction and kinematics differences between P25 and E171 TiO₂
nanoparticles. Nanoimpact, 12, 51-57. doi:10.1016/j.impact.2018.10.001

<https://hdl.handle.net/10356/136963>

<https://doi.org/10.1016/j.impact.2018.10.001>

© 2018 Elsevier B.V. All rights reserved. This paper was published in Nanoimpact and is
made available with permission of Elsevier B.V.

Downloaded on 26 Apr 2023 19:47:12 SGT

Highlights

- Comparative study of P25 and E171 TiO₂ NPs' interaction with model food ingredients.
- TiO₂ NPs' properties dictated their interactions with the food ingredient components.
- E171 were better dispersed in BSA than P25.
- In sucrose, E171 showed agglomerate size reduction while P25 agglomerated extensively.
- P25 attracted more corona formation per unit mass of material compared to E171.

NPs - food biomolecules interaction

**Biomolecular Interaction and Kinematics Differences between P25 and E171 TiO₂
Nanoparticles**

Ridhwan Yusoff^a, Mustafa Hussain Kathawala^a, Luong T. H. Nguyen^a, Magdiel I. Setyawati^a, Paul Chiew^b, Yuansheng Wu^b, Ai Lee Ch'ng^b, Zheng Ming Wang^{b *}, and Kee Woei Ng^{a, c, d *}

^aSchool of Materials Science and Engineering, Nanyang Technological University, 50 Nanyang Avenue, Singapore 639798

^bLaboratories Group, Agri-Food & Veterinary Authority of Singapore, 10 Perahu Road, Singapore 718837

^cEnvironmental Chemistry and Materials Centre, Nanyang Environment & Water Research Institute, 1 Cleantech Loop, CleanTech One, Singapore 637141

^dSkin Research Institute of Singapore, 8A Biomedical Grove, Singapore 138648

*Corresponding authors:

Zheng Ming Wang, Laboratories Group, Agri-Food & Veterinary Authority of Singapore, 10 Perahu Road, Singapore 718837. E-mail : WANG_Zheng_Ming@ava.gov.sg

Kee Woei Ng, School of Materials Science and Engineering, Nanyang Technological University, 50 Nanyang Avenue, Singapore 639798. E-mail: kwng@ntu.edu.sg

Abstract

Titanium dioxide (TiO₂) nanoparticles (NPs) are used abundantly as food additives (E171). For the purpose of risk assessment, it is imperative to understand the behavior of these nanoparticles in a food relevant environment, and their consequent toxicology impacts. However, most of such studies use model TiO₂ NPs (P25) as substitutes for E171. To understand the suitability of this approach, we investigated the functional behavior of E171 and P25 in solutions of bovine serum albumin (BSA) and sucrose as model food ingredients. Our data showed that E171 were better dispersed in BSA than P25. In sucrose, E171 displayed a reduction in agglomerated size while P25 agglomerated extensively. Adsorption studies showed that P25 attracted more pronounced corona formation per unit mass of material compared to E171. *In vitro* sedimentation, diffusion and dosimetry (ISDD) results demonstrated that the time-weighted dosage of E171 was more than two-folds higher than P25, implying that any test performed using P25 to model E171 would underestimate actual dosage and potential toxicity. Taken collectively, this study demonstrated the specificity of TiO₂ nanoparticle interaction with food ingredients, and the importance of using food-grade E171 TiO₂ for food-relevant toxicological assessments.

Keywords

Nanoparticles, Titanium dioxide, Protein, Sucrose, Corona, ISDD

1. Introduction

Titanium dioxide (TiO₂) is a metal oxide used as a white pigment in a plethora of consumer products including paints, sunscreens, and food products. Sanctioned by the Food and Drug Administration (FDA) and assigned as food additive E171 in the European Union, TiO₂ has been used in large quantities to improve the color of food products such as candies, chewing gums, white sauces, and icing (Chaudhry et al. 2008). It is also added to low-fat products such as skimmed milk, pastries, and ice creams. It has been estimated that an average adult would ingest approximately 0.2-1 mg/kg of TiO₂ daily, while an average child's ingestion of TiO₂ was estimated to be 1-3 mg/kg (Weir et al. 2012). The prevalent use of TiO₂ did not raise any specific concern until nano-sized of TiO₂ were detected in food products. Studies have demonstrated that up to 40% of E171 contained nano-sized particles of dimensions lesser than 100 nm, with primary particles displaying broad size distributions of diameters ranging from 30 to 400 nm (Chen et al. 2013; Peters et al. 2014; Weir et al. 2012; Yang et al. 2014; Yusoff et al. 2018). Though absorption of bulk TiO₂ by the gastrointestinal tract (GIT) has been determined to be negligible, several studies have shown that TiO₂ particles were absorbed by the GIT in a size-dependent manner, where smaller particles were absorbed at a better rate than larger ones (Jovanovic 2015). Given the fact that consumers are intimately exposed to dietary nano-TiO₂ in foods, concerns have been raised over the unexpected adverse impact of these nanoparticles (NPs) on human health (Shi et al. 2013).

Despite efforts dedicated to catalogue the health implications of TiO₂ in consumer products and food, most studies utilized model TiO₂ NPs such as Aeroxide P25, which is referenced in the NP repository of the Joint Research Centre (JRC) as NM-105 (Relier et al. 2017). In fact, it was found in a recent review that only 21% of NP in food studies used food-grade nanomaterials for testing (Sohal et al. 2018). Notably, while it is known that there are significant differences between

1 P25 and E171 in terms of their physicochemical properties which could have striking impact on
2 the experimental outcomes, P25 remained the preferred choice of test material because the primary
3 particles are strictly nano-sized and homogeneous, allowing a reasonably straightforward
4 conclusion to be made (Brun et al. 2014; McCracken et al. 2016). These particles, through various
5 mechanisms, can interfere with endogenous cell signaling machinery to elicit a variety of
6 responses that are consistent with exposure to toxins (Setyawati et al. 2013; Zhao et al. 2013). To
7 date, relatively little toxicology data on E171 is publicly available. One previous study had shown
8 that TiO₂ NPs (E171 equivalent) extracted from chewing gums displayed higher cytotoxicity than
9 P25 NPs (Chen et al. 2013). In another study, E171 NPs were reported to have adverse effects on
10 human mesenchymal stem cells (hMSCs) by inducing oxidative stress-mediated cell death
11 (Athinarayanan et al. 2015). More recently, it was suggested that chronic exposure to these
12 particles could promote colon micro-inflammation and contribute to the development of colorectal
13 cancer in rats (Bettini et al. 2017). Specifically in the context of analysis of NPs in food samples,
14 it has to be taken into account that these NPs are not common chemicals but highly dynamic
15 physical objects of nano-sized dimensions and characterized by a heterogeneous or evolving nature
16 (Mattarozzi et al. 2017). The physicochemical properties of these NPs may depend on the
17 surrounding matrix and can change over time in response to perturbations of their environment
18 (Nel et al. 2009; Smolkova et al. 2015). It is widely accepted in the scientific community that the
19 formation of a bio-corona around NPs is highly dependent on the NPs properties such as size,
20 shape, crystal structure, porosity, surface area, and surface chemistry (Foroozandeh and Aziz 2015;
21 Kharazian et al. 2016). The inevitable interaction of food-borne NPs, such as E171, with various
22 organic biomolecules like proteins and sugars makes it improbable for them to remain as single
23 and pure in composition. Under such conditions, the physicochemical state of these E171 NPs may

be altered, such that the resulting toxicological outcomes derived from their exposure could be different from those that have been reported for P25. Similar concerns have been shared in recent reports that advocated greater efforts to evaluate and characterize the properties of actual NPs intended for or used in food formulations such as E171, as opposed to P25 reference NPs (Dudefoi et al. 2017; Weir et al. 2012; Yang et al. 2014). The importance of elucidating food matrix as well as gastrointestinal tract (GIT) effects on the properties of ingested NPs under simulated GI conditions have also been highlighted (DeLoid et al. 2017; McClements et al. 2016). Such fundamental studies would lead to a better understanding and prediction of the behavior of food-borne NPs within the human body, and therefore help establish their potential toxic effects. In spite of the fact that differences in physicochemical properties between P25 and E171 TiO₂ NPs have been described, specific studies aimed to understand how these differences may implicate particle behavior, especially in a food-relevant environment, remain scarce.

In order to better understand this, we characterized the differences in behaviors of P25 and E171 TiO₂ NPs following interaction with model food-relevant biomolecules bovine serum albumin (BSA) and sucrose. Physicochemical properties including primary and agglomerated particle size, surface charge, identity, and corona formation were determined. The sedimentation profiles of both particles were also evaluated using the *in vitro* sedimentation, diffusion and dosimetry (ISDD) model. We showed systematically that P25 NPs, although being homogenous and used as a reference material, do not behave similarly to E171 when interacting with BSA and sucrose. Therefore, we conclude that for hazard identification purposes in the context of food, using P25 as a substitute for E171 may not predict the latter's toxicity profile accurately.

2. Materials and methods

2.1. Materials

Photocatalytic (Aeroxide P25) and food-grade (E171) titanium dioxide (TiO₂) NPs were purchased from Evonik, Degussa (Essen, Germany) and Zhengzhou Sino Chemical Co. Ltd (China), respectively. Bovine serum albumin (BSA) was purchased from Sigma-Aldrich (USA) and sucrose was procured from Affymetrix (USA). All chemicals were of molecular biology or research grade and were used as received without any further purification unless otherwise stated. All aqueous solutions were prepared with deionized (DI) water.

2.2. Methods

2.2.1. Stability of P25 and E171 NPs in food ingredient solutions

NP dispersions were characterized for hydrodynamic diameter and surface charge (zeta potential) by dynamic light scattering (DLS) and electrophoretic light scattering (ELS) techniques, respectively, using a ZetaSizer Nano-ZS (Malvern Instruments, UK). P25 and E171 NPs were weighed and suspended in DI water at a concentration of 2 mg/mL, and were used as stock solutions for further dispersion in food ingredient solutions. New stock solutions were prepared on the day of each experiment. An appropriate amount of each NP stock solution was added to each of the food ingredient solution to obtain a final NP concentration of 100 µg/mL and 1 µg/mL for P25 and E171 NPs, respectively. BSA and sucrose, which are model protein and carbohydrates used in this study, respectively, were dissolved in DI water at a concentration of 200 mg/mL and further diluted to obtain various concentrations (Table 1). The concentrations of nanoparticle, protein, and sucrose were carefully chosen according to a market analysis that was conducted prior to designing the experiments. The ingredient lists of food products reportedly known to contain

1 food-grade E171 TiO₂ NPs (Chen et al. 2013; Dufouir et al. 2018) were consulted and the
2 respective representative ingredient concentrations were tabulated. The colloidal suspensions were
3 vortexed for 15 s and sonicated in a water bath for 30 min at 50 Hz. Each measurement was
4 performed in triplicates, and results were reported as means $\pm$ standard deviations. Three
5 independent experiments were run to confirm the trends observed. Control samples were also
6 prepared in the absence of food ingredients and similarly analyzed. All measurements were carried
7 out at 25 ± 0.1 °C. Preliminary tests showed that the final NP concentration did not confound the
8 DLS measurements.

9 2.2.2. P25 and E171 NP corona in food ingredient solutions

10 P25 and E171 NPs with a final concentration of 1 mg/mL were dispersed in different
11 concentrations of food ingredient solutions (Table 1). After incubation at 37 °C for 24 h, the
12 samples were collected by centrifugation at 17000 g for 15 min. The supernatant was aspirated,
13 and the remaining pellets were washed thrice with DI water to remove unbound and loosely bound
14 biomolecules. Several methods were used to analyze the corona qualitatively and quantitatively.

15 2.2.3. Fourier transform infrared spectroscopy

16 The identities of the NP-biomolecule corona were determined using fourier transform
17 infrared spectroscopy (FTIR, Perkin Elmer Spectrum GX, USA). Prior to analysis, the collected
18 food ingredient solution treated NPs were frozen at -80 °C for 24 h and lyophilized for 24 h. A
19 fraction of the samples were mixed well with potassium bromide (KBr) powder and compressed
20 to form pellets before measurement was carried out. Transmission spectra were recorded from
21 2000 cm⁻¹ to 400 cm⁻¹ at a resolution of 4 cm⁻¹. A total of 64 scans were performed to improve the
22 signal-to-noise (S/N) ratio.

2.2.4. Transmission electron microscopy

TEM samples were prepared by first re-suspending the washed NP-biomolecule corona in DI water. Two drops of the suspension were deposited on the copper grids and left to dry in a desiccator overnight. Images were captured at randomly selected locations on the grids at 160,000 X magnification with an accelerating voltage of 120 kV (TEM, Carl Zeiss Libra 120).

2.2.5. Thermogravimetric analysis

The mass proportion of biomolecules adsorbed onto the NPs was quantified using the thermogravimetric analyzer (TGA, TA Instruments Q500, New Castle). The collected NPs were frozen at -80 °C for 24 h and lyophilized for 24 h before analysis. Weight loss curves of the samples were recorded at a heating rate of 20 °C/min up to 900 °C, in a nitrogen-filled environment.

2.2.6. In vitro sedimentation, diffusion and dosimetry (ISDD)

ISDD was performed using the simulations developed by Hinderliter and coworkers (Hinderliter et al. 2010). The MATLAB program was downloaded from their website and the aggregate density method was used. Aggregate density or effective density of the particle was measured using the method reported by DeLoid et al. (2014). Briefly, 1 mL of 1 mg/mL particle suspension was spun down in packed cell volume tubes (87005: Techno Plastic Products, Switzerland). The volume of the pellet was determined using the easy-read device provided together with the PCV tubes (87010: Techno Plastic Products, Switzerland). This volume was used to calculate the effective density (DeLoid et al. 2014). The effective density was a key parameter for the ISDD simulation program which calculates the particle deposition profile as well as the fraction area-under-curve (fracAUC) which is a measure of the time-weighted deposited dosage of particles.

1 2.2.7. *Statistical analysis*

2 All quantitative data were presented as mean $\pm$ standard deviation (SD). Statistical analyses
3 were carried out using Student's *t*-test or one-way analysis of variance (ANOVA) with Tukey's
4 post-hoc testing. For all comparisons, statistical significance was accepted when *p* values were
5 less than 0.05.

3. Results and discussion

3.1. Physicochemical properties of pristine P25 and E171 NPs

Thorough characterization of P25 and E171 NPs was performed to provide information about their size, morphology, crystal structure as well as specific surface area (Fig. S1, Supporting Information). P25 NPs had spherical to polygonal morphologies with an average particle size of 22 ± 4 nm (Fig. S1A). The particles had a phase composition of 82.6% anatase and 17.4% rutile (Fig. S1B). The nitrogen adsorption-desorption isotherm of P25 NPs is shown in Fig. S1C. The measured specific surface area as determined by the BET method was $54.03 \text{ m}^2/\text{g}$. Unlike P25 NPs, E171 NPs had primary particle size ranging from 40 to 360 nm, with 32% of the particles being less than 100 nm in at least one dimension (Fig. S1D). The particles had spherical to polygonal morphologies, and existed in the anatase phase (Fig. S1E). Fig. S1F shows the nitrogen adsorption-desorption isotherm of E171 NPs. The measured specific surface area of E171 NPs by the BET method was $8.05 \text{ m}^2/\text{g}$ (Yusoff et al. 2018).

3.2. Stability of P25 and E171 NPs in food ingredient solutions

The size of the particles within a food product may change considerably due to a number of physicochemical phenomena such as flocculation, coalescence, and Ostwald ripening (Yada et al. 2014). Thus in order to have fundamental knowledge of the physicochemical changes (e.g. agglomeration state and surface charge variation) of the NPs when they interact with food ingredient solutions, hydrodynamic characterization of the NPs was carried out using DLS. Fig. 1 reports the hydrodynamic sizes of P25 and E171 NPs in the different media used. The mean hydrodynamic sizes of both NPs suspended in water were 127.9 ± 17.9 and 365.7 ± 27.6 nm, respectively, which suggest that the primary particles had agglomerated in an aqueous environment, as previously reported (Weir et al. 2012; Yang et al. 2014). In the presence of BSA,

1 E171 showed a decrease in the size of their agglomerates, while no significant changes in
2 hydrodynamic sizes were observed for P25 (Fig. 1A). A distinct shift of zeta potential values from
3 positive ($+31.6 \pm 1.2$ mV) to negative (-25.6 ± 1.1 mV) suggests adsorption of proteins onto the
4 P25 NP surfaces (Fig. 1B). Studies have shown that NPs exposed to proteins molecules adopted
5 the physicochemical properties of the adsorbed protein shell. For instance, serum protein
6 adsorption onto positively charged gold nanorods and polyethylene-coated magnetite has been
7 shown to switch their positive surface charge to negative (Alkilany et al. 2009; Arsianti et al.
8 2010). Other findings have suggested that surface charge density has a direct effect on the binding
9 of NPs with cells (Bouwmeester et al. 2014). Taken together, protein adsorption affects the surface
10 properties of the particles, which may influence their uptake and biodistribution (Casals and Puentes
11 2012; Lesniak et al. 2012). When P25 NPs were dispersed in sucrose solution, negligible change
12 in the hydrodynamic size and zeta potential values was observed at the low sucrose concentration.
13 However, at the high sucrose concentration, there was a drastic increase in hydrodynamic size,
14 which suggested a heightened state of agglomeration. Such condition led to unstable and highly
15 agglomerated dispersion, with a measured size of 1533.2 ± 188.2 nm, approximately an order of
16 magnitude greater in size compared to those measured in water. In good agreement with this
17 agglomeration, the zeta potential dropped markedly from $+31.6 \pm 1.2$ mV to $+6.54 \pm 0.54$ mV,
18 signaling significant reduction in the electrostatic repulsion force needed to maintain the NPs in
19 their colloidal form. In contrast with P25, parallel experiments with E171 showed greater NP
20 stability in the presence of sucrose. We have previously demonstrated that protein and sucrose
21 coronas on E171 NPs were responsible for the stability of these particles in suspension (Yusoff et
22 al. 2018). The binding of these biomolecules to the NP surfaces help to disperse the NPs in an
23 aqueous environment by providing steric hindrance and electrostatic repulsion. For instance, the

1 formation of hydrogen bonds between the hydroxyl groups in sucrose and molecules creates
2 stability by overcoming the van der Waals attraction forces between particles (Gawali et al. 2017).
3 Interestingly, in a mixture of food ingredient solutions, no noticeable changes in the hydrodynamic
4 size were observed for P25 NPs. The zeta potential values remained negative, which suggests that
5 BSA molecules were preferentially adsorbed on the NPs surface over the sucrose molecules, thus
6 suppressing agglomeration under such conditions. For E171, different extents of improvement on
7 NP dispersion were observed. Collectively, the results highlight that corona adsorption is strongly
8 dependent on the composition of the media. Evaluation of the changes in agglomeration state and
9 surface charge variation of the NPs in the test media (e.g. food ingredient solutions) is essential as
10 these changes may have a significant impact on the observed toxicological responses.

11 3.3. P25 and E171 NP corona in food ingredient solutions

12 3.3.1. Protein corona

13 We characterized the identity and composition of the NP corona using methods described
14 previously (Giri et al. 2014; Kokkinopoulou et al. 2017; Yusoff et al. 2018). FTIR analysis of P25-
15 BSA samples detected the presence of amide I and II vibrational peaks at 1650 cm^{-1} and 1540 cm^{-1} ,
16 respectively (Fig. 2A), which was otherwise absent on the pristine P25 sample. This suggests
17 protein corona formation on the BSA-treated P25 samples. Notably, the intensity of the protein
18 bands was more remarked in P25 than E171, which is indicative of the differences in protein
19 adsorption capacity between the two particles. Under TEM, a translucent layer of material was
20 observed to cover both NP surfaces following treatment with BSA (Fig. 2B-E), providing visual
21 observation and further supports the protein corona formation. We next quantitatively determined
22 the composition of the adsorbed proteins on the surfaces of the NPs with TGA. Our analyses
23 showed stark differences in the total amounts of adsorbed proteins per unit mass for both particle

types (Fig. 2F), wherein P25 displayed significantly higher protein corona adsorption (approximately 8-10 times) than E171, across the concentrations tested. It was observed that the amount of protein adsorbed onto the surface of P25 NPs decreased as the protein concentration of the model solution was increased. This trend was unexpected and in stark contrast with the protein corona adsorption profile on E171, which was observed to increase concomitantly with increasing protein concentration in the medium. Two possible scenarios could explain the trend observed. The experiment to measure protein adsorption separates the non-adsorbed proteins through centrifugation at 17000 g for 15 min. We used the ISDD model to predict the size range which can deposit completely in such conditions and it was noted that anything below ~100 nm may not be completely deposited within 15 min. Having more BSA would stabilize the NPs and create a distribution with more particles nearer to the primary size of < 100 nm. These particles would also be the most potent in terms of adsorption as they have the greatest surface area to volume ratio. Therefore, in high BSA samples, a greater fraction of the most potent adsorbers could be lost during centrifugation resulting in an underestimated measurement of protein adsorption and showing a false trend. To confirm this, we performed ICPMS (data not shown) on the supernatant after centrifugation to see whether the amount of NPs lost was substantial enough to show a reverse trend. The amounts measured in the supernatant however were very minimal (<1%) for all treatments and therefore this could not explain the trend on its own.

Protein adsorption in the context of our experiments could be of two kinds. Surface adsorption on the nanoparticles or chelation/trapping of protein in the spaces inside particle agglomerates. Increasing the concentration of BSA can stabilize the P25 particles and thereby break up the bigger agglomerates. This can increase the surface area of NPs available for protein adsorption but reduces spaces between nanoparticles which can trap proteins. Since P25 already

1 have a large surface area to volume ratio, it is possible that the impact of new surface creation is
2 minimal while the disruption of inter-particle spaces has an impact large enough to show a negative
3 correlation. If this is true, it is plausible to expect that by increasing the BSA concentration, at
4 some point all large agglomerates would be broken up and therefore the adsorption amount would
5 stabilize. This was likely the case in our study as after a drop in protein adsorption from low BSA
6 to medium BSA condition, there was no further change in protein adsorption from medium BSA
7 to high BSA. In the case of E171, since the particles are much larger (lower surface area to volume
8 ratio), it is possible that the impact of new surface creation was much more significant and
9 therefore a positive correlation was found.

10 When protein adsorption capacity was normalized to the surface area of the particles, the
11 differences became less conspicuous (Fig. 2G). This was expected as adsorption is a surface
12 dependent phenomenon. Nonetheless such an analysis reinforces that these two TiO₂ types are not
13 mass-equivalent of each other especially in terms of their adsorptive capacity, due to the inherent
14 differences in their specific surface area, particle size, and possibly lattice structure. In our study,
15 P25 possessed a large specific surface area of 54.03 m²/g as a result of its small particle size, while
16 E171 had a much smaller specific surface area of 8.05 m²/g owing to its broad particle size
17 distribution. Smaller particles have larger specific surface area and consequently, increased
18 available adsorption sites to interact with surrounding biomolecules. Earlier studies have
19 demonstrated that smaller gold NPs could bind more proteins compared to the larger ones, and the
20 adsorption was proportional to the surface area of the NPs (Dobrovolskaia et al. 2009; Schaffler et
21 al. 2013). Collectively, these studies concluded that the size-dependent interaction between the
22 particles and biomolecules correlated specifically with available NP surface area.

Apart from size and surface area, the surface charge is another parameter that affects the formation and composition of the corona. The influence and effect of surface charge of latex NPs have been analyzed by several groups (Aggarwal et al. 2009; Cedervall et al. 2007; Lundqvist et al. 2008). For instance, Lundqvist et al. (2008) demonstrated that the total bound protein for positively charged NPs (amine-modified polystyrene) increased steadily upon increasing the amount of serum. In contrast, this was not observed for negatively charged NPs (carboxyl-modified polystyrene). The total bound protein amount increased initially upon increasing the amount of plasma added to the NPs. The amount decreased and only increased again at much higher plasma amounts. These observations may be explained that most of the serum proteins are negatively charged under neutral pH conditions. In good correlation with this, the authors showed that the composition of protein coronas was different for negatively and positively charged NPs. In our study, both particles displayed distinctly different surface polarities, whereby P25 showed a highly positive surface charge density of +31.6 mV, while E171 possessed a moderately negative surface charge density of -19.4 mV, in water. BSA, an ubiquitous biomolecule, has an effective zeta potential of -20.3 mV at physiological pH (~7) (Li et al. 2016). As such, BSA could be directly adsorbed onto the P25 surfaces by electrostatic interaction (Ji et al. 2010). Nevertheless, the fact that proteins could also be adsorbed onto negatively charged particles such as E171 suggests the role of non-electrostatic interactions as well (Allouni et al. 2015). A study conducted by McCracken and coworkers (McCracken et al. 2013) showed that P25 TiO₂ treated with simulated intestinal digestive solution led to a strongly negatively charged surface, which attracted a corona of bile salts and proteins. These particles were found to be internalized by intestinal epithelial cells, C2BBel, in vitro. However, if the more relevant E171 had been used in the study, the surface charge could turn out to be less negative and less proteins could have been attracted onto these

particles. Interaction with the C2BBe1 cells could have been different as a result. In another study, Botelho and coworkers (Botelho et al. 2014) showed that P25 TiO₂ (as well as another TiO₂ nanopowder of size below 25 nm) have no effect on the metabolic activity of AGS (gastric epithelial cancer) cells due to the adsorption of FBS proteins on the surface of the nanoparticles which changes the biological behavior of the native nanoparticles. If the more relevant E171 particles had been used, there would be lower protein adsorption than on the P25 TiO₂. This could have led to E171 exhibiting a greater and more representative effect on the viability of AGS cells owing to lesser protective protein corona.

Surface chemistry also has a great influence on the interaction of the particles with the surrounding milieu. Previous studies had reported that cationic dyes (methylene blue) adsorbed more readily to E171 than P25 NPs (Yang et al. 2014), which can be attributed to the presence of phosphate groups on the surface of E171 but not P25 NPs (Dudefoi et al. 2017). These obvious differences in surface chemistry imply different potential for interaction with biomolecules and other components in the food matrix.

3.3.2. Sucrose corona

The FTIR spectra of P25 and E171 NPs incubated in sucrose solution did not present any sucrose associated vibrational peaks over the concentrations tested (Fig. 3A). Nonetheless, under TEM, a distinct, thin layer of material was observed to cover both NP surfaces following exposure to sucrose (Fig. 3B, C), which was absent on untreated NPs. The adsorbed layer, which could only be sucrose, completely coated the single particles as well as larger agglomerates. We believe that the relevant vibrational spectroscopic profiles of sucrose were masked by the presence of the TiO₂ NPs, and were therefore not recorded in the FTIR spectra presented. Conventional FTIR has between 2 to 30 μm resolution, which is likely insufficient for analyzing nanolayers (Khanal et al.

2016). TGA results (Fig. 3D) showed that the amount of adsorbed sucrose increased with increasing sucrose concentration for both particles, which was contrary to the protein adsorption trend observed on P25 (Fig. 2F). Similar to the protein corona analysis, the sucrose corona on P25 at all sucrose concentrations tested was significantly higher than that of E171. Once again, when the numbers were normalized to surface area, both particles became comparable (Fig. 3E).

3.3.3. Complex coronas

The FTIR spectra of P25 and E171 NPs incubated in mixtures of BSA and sucrose at varying concentrations recorded the presence of amide I and II vibrational peaks, with no sucrose peaks detected (Fig. 4A). TEM micrographs showed a coating on the NP surfaces following exposure to the same mixtures (Fig. 4B-E). TGA results revealed that a low BSA and high sucrose mixture produced a higher amount of adsorbed proteins and sucrose molecules combined for P25 NPs, while the reverse is true for E171 NPs (Fig. 4F). When the numbers were normalized to surface area, both particles became comparable (Fig. 4G). Although the mechanisms of interaction and differences in such mechanisms between NP types are unclear, these findings suggest that in a solution of proteins and sugars, proteins could be the major contributor of a corona on both P25 and E171 NP surfaces.

3.4. Effective dosage of P25 and E171 NPs

In the context of toxicology assessments, dosage is a key parameter. Classification of a substance as toxic or safe is in essence a measure of the dosage needed to induce significant adverse effects. While simple to grasp on the surface, to pin down a single number as the dosage is often a much more complicated task. Most *in vitro* studies use concentration as the parameter to define dosage. However, in the simplest of *in vitro* tests using 2D monolayer cell cultures, assigning concentration as the key parameter has severe limitations, which we addressed herein.

1 The test NP is added as a suspension onto a monolayer of cells which lay at the bottom of a culture
2 vessel. The onset of exposure is marked at the moment the suspension is pipetted into the vessel.
3 However, the NP load comes into contact with the cells increasingly over time as the particles
4 sediment onto the bottom of the vessel where the monolayer of cells finally interacts with them. It
5 is therefore the **number of particles** which deposit over time that is the effective dosage able to
6 cause any adverse, or otherwise, effect (Sohal et al. 2018). That is the first reason why assuming
7 concentration to represent dosage is erroneous. Secondly, concentration is a volumetric
8 measurement or a 3D parameter. Once deposited onto the monolayer of cells, the particles cannot
9 be measured in terms of volumetric concentration. Instead, a more appropriate measure of their
10 abundance would be their surface concentration, i.e. the **number of particles** per unit area of the
11 culture surface.

12 In addition, effective dosage based on the explanation above is a function of the particles'
13 ability to sediment and diffuse in the culture plate. These in turn are dependent on the physical
14 properties of the particle in both primary as well as aggregated state. The properties include
15 primary particle size, hydrodynamic size, particle density and aggregate's effective density
16 (DeLoid et al. 2014). P25 and E171 have contrasting values for most of the above listed parameters
17 which translates into a contrasting sedimentation profile between them. The calculated effective
18 density of P25 and E171 in water are $1.236 \pm 0.005 \text{ g/cm}^3$ and $1.820 \pm 0.183 \text{ g/cm}^3$ ($n = 2$),
19 respectively, which means that E171 deposits much faster than P25, as shown in Fig. 5. In a typical
20 24 h incubation period, less than 30% of P25 NPs are expected to deposit. In the same time frame,
21 almost 70% of E171 are expected to deposit. By integrating the fraction deposited over time curve,
22 the "Time Weighted Average" factor is derived. This factor has units of frac.hr and is a measure
23 of the time-weighted dosage of the particles. The TWA factor is more than two-fold higher for

E171 (8.602) compared to P25 (4.044), implying that any tests performed using P25 to mimic E171 would underestimate the dosage and its toxicity by more than two times. This is further evidence against generalizing P25 to model food-grade E171 particles.

Taken together, our results demonstrate both qualitatively and quantitatively that the known physicochemical property differences between P25 and E171 NPs bring about significantly different functional outcomes which could in turn result in contrasting effects in toxicology studies.

4. Conclusion

With the increasing use of commercial nanomaterials in many consumer products including food applications, it is imperative to identify potential impacts of nanomaterials on human health and the environment. The distribution of nanomaterials in any specific environment is governed by modes of release, physicochemical properties, and their consequent interactions with other entities in that environment. Complete risk assessment requires comprehensive physical-chemical characterization of the nanomaterials including chemical composition, size, morphology, surface area, crystallinity, and other properties. In this study, we assessed the behavior patterns of reference P25 TiO₂ NPs and food-grade E171 TiO₂ NPs in model food biomolecules BSA (protein) and sucrose (carbohydrate). We found that P25 NPs agglomerated more significantly in high sucrose concentrations and presented positive surface charges in sucrose, in contrast to E171. P25 NPs attracted significantly more BSA and sucrose corona formation per unit mass of material. P25 NPs also sedimented much more slowly in water compared to E171. These results demonstrated that differences in the inherent properties between P25 and E171 NPs contributed to the observed differences in particle behavior in the different food ingredient components, and also the corona

characteristics studied. Therefore, we conclude that although they are homogenous and thus used as a reference material in many toxicity studies, P25 TiO₂ NPs should not be used as a substitute for E171 in toxicity tests for the purpose of food-related risk assessment.

Acknowledgements

The authors would like to acknowledge Helen Phang from the Agri-Food & Veterinary Authority of Singapore (AVA) for her technical assistance and critical assessment of this work. The authors would also like to acknowledge the Facility for Analysis, Characterization, Testing and Simulation (FACTS) at NTU for technical assistance in TEM and XRD analyses. This study was supported by the Agri-Food & Veterinary Authority of Singapore (M4061593.070 to NKW).

Conflict of interest

The authors declare that they have no conflicts of interest.

Appendix A. Supplementary Data

Supplementary information (SI) contains additional method and result sections. Supplementary data to this article can be found online at:

References

- Aggarwal P, Hall JB, McLeland CB, Dobrovolskaia MA, McNeil SE (2009) Nanoparticle interaction with plasma proteins as it relates to particle biodistribution, biocompatibility and therapeutic efficacy *Adv Drug Deliv Rev* 61:428-437 doi:10.1016/j.addr.2009.03.009
- Alkilany AM, Nalaria PK, Hexel CR, Shaw TJ, Murphy CJ, Wyatt MD (2009) Cellular uptake and cytotoxicity of gold nanorods: molecular origin of cytotoxicity and surface effects *Small* 5:701-708 doi:10.1002/sml.200801546

1 Allouni ZE, Gjerdet NR, Cimpan MR, Hol PJ (2015) The effect of blood protein adsorption on
2 cellular uptake of anatase TiO₂ nanoparticles Int J Nanomed 10:687-695
3 doi:10.2147/IJN.S72726

4 Arsianti M, Lim M, Marquis CP, Amal R (2010) Polyethylenimine Based Magnetic Iron-Oxide
5 Vector: The Effect of Vector Component Assembly on Cellular Entry Mechanism,
6 Intracellular Localization, and Cellular Viability Biomacromolecules 11:2521-2531

7 Athinarayanan J, Alshatwi AA, Periasamy VS, Al-Warthan AA (2015) Identification of nanoscale
8 ingredients in commercial food products and their induction of mitochondrially mediated
9 cytotoxic effects on human mesenchymal stem cells J Food Sci 80:N459-464
10 doi:10.1111/1750-3841.12760

11 Bettini S et al. (2017) Food-grade TiO₂ impairs intestinal and systemic immune homeostasis,
12 initiates preneoplastic lesions and promotes aberrant crypt development in the rat colon Sci
13 Rep 7:40373 doi:10.1038/srep40373

14 Botelho MC, Costa C, Silva S, Costa S, Dhawan A, Oliveira PA, Teixeira JP (2014) Effects of
15 titanium dioxide nanoparticles in human gastric epithelial cells in vitro Biomed
16 Pharmacother 68:59-64 doi:10.1016/j.biopha.2013.08.006

17 Bouwmeester H, Brandhoff P, Marvin HJP, Weigel S, Peters RJB (2014) State of the safety
18 assessment and current use of nanomaterials in food and food production Trends in Food
19 Science & Technology 40:200-210 doi:10.1016/j.tifs.2014.08.009

20 Brun E et al. (2014) Titanium dioxide nanoparticle impact and translocation through ex vivo, in
21 vivo and in vitro gut epithelia Particle and Fibre Toxicology 11:1-16 doi:10.1186/1743-
22 8977-11-13

23 Casals E, Puentes VF (2012) Inorganic nanoparticle biomolecular corona: formation, evolution and
24 biological impact Nanomedicine 7:1917-1930

25 Cedervall T et al. (2007) Understanding the nanoparticle–protein corona using methods to quantify
26 exchange rates and affinities of proteins for nanoparticles Proc Natl Acad Sci U S A
27 104:2050-2055

28 Chaudhry Q et al. (2008) Applications and implications of nanotechnologies for the food sector
29 Food Addit Contam Part A Chem Anal Control Expo Risk Assess 25:241-258
30 doi:10.1080/02652030701744538

31 Chen XX et al. (2013) Characterization and preliminary toxicity assay of nano-titanium dioxide
32 additive in sugar-coated chewing gum Small 9:1765-1774 doi:10.1002/smll.201201506

33 DeLoid G et al. (2014) Estimating the effective density of engineered nanomaterials for in vitro
34 dosimetry Nat Commun 5:3514 doi:10.1038/ncomms4514

1 DeLoid GM et al. (2017) An integrated methodology for assessing the impact of food matrix and
2 gastrointestinal effects on the biokinetics and cellular toxicity of ingested engineered
3 nanomaterials Part Fibre Toxicol 14:40 doi:10.1186/s12989-017-0221-5

4 Dobrovolskaia MA et al. (2009) Interaction of colloidal gold nanoparticles with human blood:
5 effects on particle size and analysis of plasma protein binding profiles Nanomedicine
6 5:106-117 doi:10.1016/j.nano.2008.08.001

7 Dufefoi W, Terrisse H, Popa AF, Gautron E, Humbert B, Ropers MH (2018) Evaluation of the
8 content of TiO₂ nanoparticles in the coatings of chewing gums Food Addit Contam Part A
9 Chem Anal Control Expo Risk Assess 35:211-221 doi:10.1080/19440049.2017.1384576

10 Dufefoi W, Terrisse H, Richard-Plouet M, Gautron E, Popa F, Humbert B, Ropers MH (2017)
11 Criteria to define a more relevant reference sample of titanium dioxide in the context of
12 food: a multiscale approach Food Addit Contam, Part A 34:653-665
13 doi:10.1080/19440049.2017.1284346

14 Foroozandeh P, Aziz AA (2015) Merging worlds of nanomaterials and biological environment:
15 factors governing protein corona formation on nanoparticles and its biological
16 consequences Nanoscale Res Lett 10:221 doi:10.1186/s11671-015-0922-3

17 Gawali SL, Barick BK, Barick KC, Hassan PA (2017) Effect of sugar alcohol on colloidal
18 stabilization of magnetic nanoparticles for hyperthermia and drug delivery applications
19 Journal of Alloys and Compounds 725:800-806 doi:10.1016/j.jallcom.2017.07.206

20 Giri K et al. (2014) Understanding protein-nanoparticle interaction: a new gateway to disease
21 therapeutics Bioconjug Chem 25:1078-1090 doi:10.1021/bc500084f

22 Hinderliter PM, Minard KR, Orr G, Chrisler WB, Thrall BD, Pounds JG, Teeguarden JG (2010)
23 ISDD: A computational model of particle sedimentation, diffusion and target cell
24 dosimetry for in vitro toxicity studies Part Fibre Toxicol 7:36 doi:10.1186/1743-8977-7-
25 36

26 Ji Z et al. (2010) Dispersion and stability optimization of TiO₂ nanoparticles in cell culture media
27 Environ Sci Technol 44:7309-7314

28 Jovanovic B (2015) Critical review of public health regulations of titanium dioxide, a human food
29 additive Integr Environ Assess Manage 11:10-20 doi:10.1002/ieam.1571

30 Khanal D et al. (2016) Biospectroscopy of Nanodiamond-Induced Alterations in Conformation of
31 Intra- and Extracellular Proteins: A Nanoscale IR Study Anal Chem 88:7530-7538
32 doi:10.1021/acs.analchem.6b00665

33 Kharazian B, Hadipour NL, Ejtehadi MR (2016) Understanding the nanoparticle-protein corona
34 complexes using computational and experimental methods Int J Biochem Cell Biol 75:162-
35 174 doi:10.1016/j.biocel.2016.02.008

1 Kokkinopoulou M, Simon J, Landfester K, Mailander V, Lieberwirth I (2017) Visualization of the
2 protein corona: towards a biomolecular understanding of nanoparticle-cell-interactions
3 *Nanoscale* 9:8858-8870 doi:10.1039/c7nr02977b

4 Lesniak A, Fenaroli F, Monopoli MP, A°berg C, Dawson KA, Salvati A (2012) Effects of the
5 Presence or Absence of a Protein Corona on Silica Nanoparticle Uptake and Impact on
6 Cells *ACS Nano* 6:5845-5857

7 Li R, Wu Z, Wangb Y, Ding L, Wang Y (2016) Role of pH-induced structural change in protein
8 aggregation in foam fractionation of bovine serum albumin *Biotechnol Rep* 9:46-52
9 doi:10.1016/j.btre.2016.01.002

10 Lundqvist M, Stigler J, Elia G, Lynch I, Cedervall T, Dawson KA (2008) Nanoparticle size and
11 surface properties determine the protein corona with possible implications for biological
12 impacts *Proc Natl Acad Sci U S A* 105:14265–14270

13 Mattarozzi M, Suman M, Cascio C, Calestani D, Weigel S, Undas A, Peters R (2017) Analytical
14 approaches for the characterization and quantification of nanoparticles in food and
15 beverages *Anal Bioanal Chem* 409:63-80 doi:10.1007/s00216-016-9946-5

16 McClements DJ, DeLoid G, Pyrgiotakis G, Shatkin JA, Xiao H, Demokritou P (2016) The Role
17 of the Food Matrix and Gastrointestinal Tract in the assessment of biological properties of
18 ingested engineered nanomaterials (iENMs): State of the science and knowledge gaps
19 *NanoImpact* 3-4:47-57 doi:10.1016/j.impact.2016.10.002

20 McCracken C, Dutta PK, Waldman WJ (2016) Critical assessment of toxicological effects of
21 ingested nanoparticles *Environmental Science: Nano* 3:256-282 doi:10.1039/c5en00242g

22 McCracken C, Zane A, Knight DA, Dutta PK, Waldman WJ (2013) Minimal intestinal epithelial
23 cell toxicity in response to short- and long-term food-relevant inorganic nanoparticle
24 exposure *Chem Res Toxicol* 26:1514-1525 doi:10.1021/tx400231u

25 Nel AE et al. (2009) Understanding biophysicochemical interactions at the nano-bio interface *Nat*
26 *Mater* 8:543-557 doi:10.1038/nmat2442

27 Peters RJ et al. (2014) Characterization of titanium dioxide nanoparticles in food products:
28 analytical methods to define nanoparticles *J Agric Food Chem* 62:6285-6293
29 doi:10.1021/jf5011885

30 Relier C et al. (2017) Study of TiO₂ P25 nanoparticles genotoxicity on lung, blood, and liver cells
31 in lung overload and non-overload conditions after repeated respiratory exposure in rats
32 *Toxicol Sci* 156:527-537 doi:10.1093/toxsci/kfx006

33 Schaffler M et al. (2013) Serum protein identification and quantification of the corona of 5, 15 and
34 80 nm gold nanoparticles *Nanotechnology* 24:265103 doi:10.1088/0957-
35 4484/24/26/265103

- 1 Setyawati MI et al. (2013) Titanium dioxide nanomaterials cause endothelial cell leakiness by
2 disrupting the homophilic interaction of VE-cadherin Nat Commun 4:1673
3 doi:10.1038/ncomms2655
- 4 Shi H, Magaye R, Castranova V, Zhao J (2013) Titanium dioxide nanoparticles: a review of current
5 toxicological data Part Fibre Toxicol 10:1-33 doi:10.1186/1743-8977-10-15
- 6 Smolkova B, El Yamani N, Collins AR, Gutleb AC, Dusinska M (2015) Nanoparticles in food.
7 Epigenetic changes induced by nanomaterials and possible impact on health Food Chem
8 Toxicol 77:64-73 doi:10.1016/j.fct.2014.12.015
- 9 Sohal IS, O'Fallon KS, Gaines P, Demokritou P, Bello D (2018) Ingested engineered
10 nanomaterials: state of science in nanotoxicity testing and future research needs Part Fibre
11 Toxicol 15:29 doi:10.1186/s12989-018-0265-1
- 12 Weir A, Westerhoff P, Fabricius L, Hristovski K, von Goetz N (2012) Titanium dioxide
13 nanoparticles in food and personal care products Environ Sci Technol 46:2242-2250
14 doi:10.1021/es204168d
- 15 Yada RY et al. (2014) Engineered Nanoscale Food Ingredients: Evaluation of Current Knowledge
16 on Material Characteristics Relevant to Uptake from the Gastrointestinal Tract
17 Comprehensive Reviews in Food Science and Food Safety 13:730-744 doi:10.1111/1541-
18 4337.12076
- 19 Yang Y, Doudrick K, Bi X, Hristovski K, Herckes P, Westerhoff P, Kaegi R (2014)
20 Characterization of food-grade titanium dioxide: the presence of nanosized particles
21 Environ Sci Technol 48:6391-6400 doi:10.1021/es500436x
- 22 Yusoff R, Nguyen LTH, Chiew P, Wang ZM, Ng KW (2018) Comparative differences in the
23 behavior of TiO₂ and SiO₂ food additives in food ingredient solutions J Nanopart Res 20
24 doi:10.1007/s11051-018-4176-8
- 25 Zhao Y, Howe JL, Yu Z, Leong DT, Chu JJ, Loo JS, Ng KW (2013) Exposure to titanium dioxide
26 nanoparticles induces autophagy in primary human keratinocytes Small 9:387-392
27 doi:10.1002/sml.201201363

1 **Table**

2

3 Table 1. Summary of the concentrations of food ingredient solutions

NP/ Food ingredient	BSA (mg/mL)	Sucrose (mg/mL)	BSA (mg/mL) : Sucrose (mg/mL)
P25	1 and 20	1 and 100	1:100 and 20:1
E171			

4

5

Figure Captions

Fig. 1. DLS measurements of (A) hydrodynamic size and (B) zeta potential of P25 and E171 NPs in food ingredient solutions. Data represent mean $\pm$ standard deviation ($n = 3$). $*p < 0.05$ vs. control for P25; $^{\#}p < 0.05$ vs. control for E171 (one-way ANOVA with Tukey's post-hoc testing).

Fig. 2. *Analyses of protein corona on P25 and E171 NPs.* (A) FTIR spectra of P25 and E171-BSA samples over varying BSA concentrations. Protein peaks are assigned as annotated. Representative TEM micrographs of (B) P25 treated in low BSA concentration, (C) P25 treated in high BSA concentration, (D) E171 treated in low BSA concentration, and (E) E171 treated in high BSA concentration. Scale bars: 20 nm. Protein corona was identified as a translucent layer surrounding the dark particles. TGA measurements of (F) mass of protein adsorbed per mass of particle and (G) mass of protein adsorbed per surface area of particle. Data represent mean $\pm$ standard deviation ($n = 3$); $*p < 0.05$ based on Student's t -test.

Fig. 3. *Analyses of sucrose corona on P25 and E171 NPs.* (A) FTIR spectra of pristine P25 and E171-sucrose samples over varying sucrose concentrations. No sucrose peaks from the P25 and E171-sucrose samples were detected by FTIR. Representative TEM micrographs of (B) P25 and (C) E171 treated in high sucrose concentration. Scale bars: 20 nm. Sucrose corona was identified as a translucent layer surrounding the dark particles. TGA measurements of (D) mass of sucrose adsorbed per mass of particle and (E) mass of sucrose adsorbed per surface area of particle. Data represent mean $\pm$ standard deviation ($n = 3$); $*p < 0.05$ based on Student's t -test.

Fig. 4. *Analyses of complex coronas on P25 and E171 NPs.* (A) FTIR spectra of P25 and E171 samples over varying BSA and sucrose concentrations. Protein peaks are assigned as annotated. Representative TEM micrographs of (B) P25 treated in low BSA and high sucrose concentration

1 solution, (C) P25 treated in high BSA and low sucrose concentration solution, (D) E171 treated in
2 low BSA and high sucrose concentration solution, and (E) E171 treated in high BSA and low
3 sucrose concentration solution. Scale bars: 20 nm. Corona composite was identified as a
4 translucent layer surrounding the dark particles. TGA measurements of (F) mass of protein and
5 sucrose adsorbed per mass of particle and (G) mass of protein and sucrose adsorbed per surface
6 area of particle. Data represent mean $\pm$ standard deviation ($n = 3$); $*p < 0.05$ based on Student's t -
7 test.

8 **Fig. 5.** ISDD simulation for P25 and E171 NPs stating the time weighted average (TWA) factor
9 for the two particles for media height of 0.263 cm which is consistent with media volume of 0.25
10 mL, 0.5 mL and 2.5 mL for 48, 96 and 6 well cell culture plates, respectively.

11

Deposition profile of P25 and E171 NPs

**Biomolecular Interaction and Kinematics Differences between P25 and E171 TiO₂
Nanoparticles**

Supporting Information

Ridhwan Yusoff¹, Mustafa Hussain Kathawala¹, Luong T. H. Nguyen¹, Magdiel Ingrid Setyawati¹, Paul Chiew², Yuansheng Wu², Ch'ng Ai Lee², Zheng Ming Wang^{2*}, and Kee Woei Ng^{1,3,4*}

¹School of Materials Science and Engineering, Nanyang Technological University, 50 Nanyang Avenue, Singapore 639798

²Laboratories Group, Agri-Food & Veterinary Authority of Singapore, 10 Perahu Road, Singapore 718837

³Environmental Chemistry and Materials Centre, Nanyang Environment & Water Research Institute, 1 Cleantech Loop, CleanTech One, Singapore 637141

⁴Skin Research Institute of Singapore, 8A Biomedical Grove, Singapore 138648

*Correspondence:

Zheng Ming Wang, Laboratories Group, Agri-Food & Veterinary Authority of Singapore, 10 Perahu Road, Singapore 718837. E-mail : WANG_Zheng_Ming@ava.gov.sg

Kee Woei Ng, School of Materials Science and Engineering, Nanyang Technological University, 50 Nanyang Avenue, Singapore 639798. E-mail: kwng@ntu.edu.sg

Methods

Characterization of pristine P25 and E171 NPs

Transmission Electron Microscopy. The particle size and morphology of P25 and E171 NPs were analyzed using Transmission electron microscopy (TEM, Carl Zeiss Libra 120) with an accelerating voltage of 120 kV. Samples were prepared by dispersing a small quantity of NPs in ethanol by 30 min of sonication. Two drops of the suspension were placed on a 300-mesh carbon-coated copper grids (Ted Pella, Inc., USA) and allowed to dry in a desiccator overnight. The grids were placed on a filter paper to remove excess liquid. Particle size, shape, and size distribution were characterized using images of each sample, acquired at five random locations on the grids to ensure representative images were captured. The average particle size was determined as an arithmetic mean of the measured diameters of approximately 100 particles using Java image tool software (ImageJ).

X-ray diffraction. The crystal structures of P25 and E171 NPs were determined using x-ray diffraction (XRD, Shimadzu) operated at 40 kV and 30 mA with a Cu-K α radiation source ($\lambda = 1.5406 \text{ \AA}$). Samples were placed on the sample holder and irradiated at 2θ values ranging from $10\text{-}80^\circ$ with a step size of 0.02° and a scan rate of $4^\circ/\text{min}$. Experiments were done at room temperature and diffraction patterns were analyzed with the Match! Software (Crystal Impact, Germany) to assign diffraction peaks to known crystalline structures integrated in the software.

Brunauer-Emmett-Teller specific surface area. The specific surface area of P25 and E171 NPs accessible to nitrogen molecules were measured using the Brunauer-Emmett-Teller (BET, Micromeritics surface area analyzer TriStar II 3020) method. Samples were degassed at 200°C for 4 h under vacuum prior to analysis. Nitrogen adsorption-desorption isotherms were measured

at 78 K in flowing nitrogen. 8 points BET standard programme (P/P_0 values from 0 to 0.2) was used to compute the specific surface area.

Supplementary Data

Fig. S1. (A) Representative TEM micrograph of P25 particles. Scale bar: 20 nm. (B) XRD spectrum of P25. (C) BET nitrogen adsorption-desorption isotherms of P25. (D) Representative TEM micrograph of E171 particles. Scale bar: 200 nm. (E) XRD spectrum of E171. (F) BET nitrogen adsorption-desorption isotherms of E171.