Comparative differences in the behavior of TiO2 and SiO2 food additives in food ingredient solutions

Yusoff, Ridhwan; Nguyen, Luong T. H.; Chiew, Paul; Wang, Zheng Ming; Ng, Kee Woei

2018

Yusoff, R., Nguyen, L. T. H., Chiew, P., Wang, Z. M., & Ng, K. W. (2018). Comparative differences in the behavior of TiO2 and SiO2 food additives in food ingredient solutions. Journal of Nanoparticle Research, 20(3), 76-. doi:10.1007/s11051-018-4176-8

https://hdl.handle.net/10356/105439

https://doi.org/10.1007/s11051-018-4176-8

Journal of Nanoparticle Research

Comparative Differences in the Behaviour of TiO_2 and SiO_2 Food Additives in Food Ingredient Solutions

--Manuscript Draft--

Manuscript Number:	NANO-D-17-01799R1
Full Title:	Comparative Differences in the Behaviour of TiO_2 and SiO_2 Food Additives in Food Ingredient Solutions
Article Type:	Original research
Keywords:	Nanoparticles; Titanium dioxide; silicon dioxide; protein; sucrose; corona
Corresponding Author:	Kee Woei Ng Nanyang Technological University SINGAPORE
Corresponding Author Secondary Information:	
Corresponding Author's Institution:	Nanyang Technological University
Corresponding Author's Secondary Institution:	
First Author:	Ridhwan Yusoff
First Author Secondary Information:	
Order of Authors:	Ridhwan Yusoff
	Luong TH Nguyen
	Paul Chiew
	Zheng Ming Wang
	Kee Woei Ng
Order of Authors Secondary Information:	
Funding Information:	
Abstract:	Nanotechnology is widely used in the food industry to improve the colour, taste and texture of food products. However, concerns regarding potential undesirable health effects remain. It is expected that interaction of engineered nanomaterials (ENMs) with food ingredients will influence their behaviour and the resulting corona. Nonetheless, there are limited systematic studies conducted to clarify this understanding. Herein, we investigated the behaviour and corona formation of food grade titanium dioxide (TiO_2) and silicon dioxide (SiO_2) in solutions of model food ingredients bovine serum albumin and sucrose. Measurements using dynamic light scattering (DLS) indicated that both TiO_2 and SiO_2 nanoparticles displayed a decrease in agglomerate sizes in the presence of both food ingredients. Both particles were negatively charged in all the conditions tested. Corona adsorption studies were carried out using multiple complementary methods including Fourier transformed infrared (FTIR) spectroscopy, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-ToF-MS), transmission electron microscopy (TEM), micro bicinchoninic acid (BCA) protein assay and thermogravimetric analysis (TGA). Comparative investigation showed that sucrose could disperse both particles more effectively than bovine serum albumin and that SiO_2 displayed greater adsorption capacity for both bovine serum albumin and sucrose, compared to TiO_2. Taken collectively, this study demonstrated the importance of considering food ingredient effects when mapping the behaviour of ENMs in food products. Such understanding could be significant in the evaluation of biological effects, such as toxicity, of ENMs used in food products.
Response to Reviewers:	Manuscript ID: NANO-D-17-01799 Authors' response to reviewers' comments:

We thank the reviewers for their constructive suggestions and criticisms. We have now addressed all the comments accordingly, and made edits in red, where appropriate. A list of our point-by-point replies is appended herein:

Reviewer #1

1. Reviewer's question: The Introduction should have been written in a more precise way. It seemed to be too elaborate more like a thesis. It should be concise and informative.

Author's answer: We have shortened the introduction by removing parts that are less directly relevant to the study.

2. Reviewer's question: In the methods part, the methods of similar instruments should have been under single heads. The authors may have written separate paragraphs for different materials, but the head should have been one. Also, the authors should have been concise as similar methods are repeated every time a new material is discussed (e.g. pristine SiO2 and SiO2/BSA corona etc.)

Author's answer: We accept the suggestion and have shifted the characterization of pristine nanoparticles to the Supporting Information, Section 1. All methods of similar instruments have been revised and grouped under single heads.

3. Reviewer's question: The quality of images (specially the ToF and FTIR spectra) is not clear. Text inside the figures are not visible.

Author's answer: We have improved the resolution of the FTIR and MALDI-ToF MS spectra images, and enlarged embedded texts for clarity.

4. Reviewer's question: The TEM images are not clear. Lattice images and SAED patterns showing crystallographic orientation are not provided.

Author's answer: We have improved the resolution of the TEM images. In this study, TEM was performed to provide primary information including the morphology and size of the nanoparticles studied, as a verification of the quality of these commercially available materials. Lattice images and selected area electron diffraction (SAED) patterns were not evaluated as these are well reported in the literature*, which we have now included. Additional texts have been added on page 6 to explain this.

*Reference:
1) Yang, Y., et al., Survey of food-grade silica dioxide nanomaterial occurrence, characterization, human gut impacts and fate across its lifecycle. Sci Total Environ, 2016. 565: p. 902-912.
2) Chen, H., et al., The effects of orally administered Ag, TiO2 and SiO2 nanoparticles on gut microbiota composition and colitis induction in mice. NanoImpact, 2017. 8: p. 80-88.
3) Lorenzetti, M., et al., TiO2 (Nano)Particles Extracted from Sugar-Coated Confectionery. Journal of Nanomaterials, 2017. 2017: p. 1-14.

5. Reviewer's question: The authors mentioned XRD in the text but there was no XRD spectrum analysis in the manuscript. When the authors are working with pure phase crystalline material, the crystallographic study must be adequately provided. The authors should at least perform a particle size and strain calculation from the spectrum which has high relation with the surface energy of the particle.

Author's answer: We have moved the XRD spectra figures to Supporting Information, Figure S1. We did not perform a particle size and strain calculation from the XRD spectra as these are well established, commercially available nanoparticles which have been well characterized by others. We conducted XRD to verify the crystalline structures of the particles. Nonetheless, we have now included literature* that provide more in-depth XRD analyses of the same nanoparticles. Additional texts have been added on page 6 to explain this.

*Reference:
1) Faust, J.J., et al., Food grade titanium dioxide disrupts intestinal brush border microvilli in vitro independent of sedimentation. Cell Biol Toxicol, 2014. 30(3): p. 169-88.
2) Athinarayanan, J., et al., Presence of nanosilica (E551) in commercial food products: TNF-mediated oxidative stress and altered cell cycle progression in human lung fibroblast cells. Cell Biol Toxicol, 2014. 30(2): p. 89-100.

6. Reviewer's question: The manuscript also lacked the BET (N2 Adsorption-Desorption isotherm) pore size distribution profile.

Author's answer: We have included the pore size distribution profile in Supporting Information, Section 2.4.

7. Reviewer's question: All the experiments were performed in vitro. It would have been good if the analysis had some in vivo analysis or at least analysis on some blood samples to make the data more realistic towards living systems.

Author's answer: We agree that in vivo studies will be required to achieve more realistic understanding of the interaction between these nanoparticles and living systems. However, the in vivo environment is highly complex and dynamic, making it difficult to draw conclusions that correlate to specific food ingredients in the system. We therefore carried out the current study to establish fundamental understanding of the nanoparticles' behaviour in specific food ingredients, as a basis to move on to more complex models in the future, with the eventual goal of conducting physiologically relevant toxicology studies. The current study has provided a glimpse of how the food ingredient solutions influence nanomaterial behavior. Moving forward, future studies could include evaluating nanomaterial behavior in complex digestae containing both food ingredients and GI tract fluids, and the simulation of GI tract translocation to derive a full picture of nanomaterial behavior across the entire GI tract. Such understanding will no doubt be necessary to determine the toxicological influence of a specific nanomaterial in the GI tract.

8. Reviewer's question: The conclusion should have depicted the impact being carried by the paper in a more mathematical way. Presently, it was more descriptive in nature.

Author's answer: We acknowledge the comment and added more quantitative conclusions as suggested on page 10.

9. Reviewer's question: The ANNOVA results should have been elaborated a bit more. may be a table showing the ANNOVA results should have been adequate.

Author's answer: We have included details of the ANOVA results in Supporting Information, Section 2.1.

Reviewer #2

1. Reviewer's question: Page 2, "Abstract" section, line 9 and page 5, "Fourier Transform Infrared spectroscopy" section, line 2: "fourier" should be replaced by "Fourier".

Author's answer: We have corrected this as the reviewer suggested.

2. Reviewer's question: Page 4, "Methods" section, line 2: "Transmission Electron Microscopy" should be replaced by "Transmission electron microscopy".

Author's answer: We have corrected this as the reviewer suggested.

3. Reviewer's question: Page 5, "Brunauer-Emmett-Teller specific surface area" section, line 4: Which P/P0 values were used in the calculation of specific surface area?

Author's answer: 8 points BET standard programme (P/Po values from 0 to 0.2) was

used to calculate the specific surface area. We have included the information in the Supporting Information, methods section.

4. Reviewer's question: Page 5, "Stability of TiO2 and SiO2 nanoparticles in food ingredient solutions" section, line 6: Instead of "protein and carbohydrates", "protein and carbohydrate" is more appropriate.

Author's answer: We have corrected this as the reviewer suggested.

5. Reviewer's question: Page 5, "Fourier Transform Infrared spectroscopy" section: "Fourier Transform Infrared spectroscopy" should be replaced by "Fourier transform infrared spectroscopy".

Author's answer: We have corrected this as the reviewer suggested.

6. Reviewer's question: Page 6, lines 5, 9, 16 and 23: Please use small letters in the spelling of subsection titles.

Author's answer: We have corrected this as the reviewer suggested.

7. Reviewer's question: Page 7, "Stability of TiO2 and SiO2 nanoparticles in food ingredient solutions" section, line 12: After this sentence, I suggest the authors that the following sentences and references should be given.
"The magnitude of the zeta potential is very important in determining the stability of the colloidal systems. In general, particles having the zeta potential values higher than +30 mV or lower than -30 mV are considered as stable dispersions (Duman and Tunc, 2009; Tunc et al., 2012).".
*Duman O, Tunc S (2009) Electrokinetic and rheological properties of Na-bentonite in some electrolyte solutions, Microporous Mesoporous Mater. 117: 331-338.
*Tunc S, Duman O, Kanci B (2012) Rheological measurements of Na-bentonite and sepiolite particles in the presence of tetradecyltrimethylammonium bromide, sodium tetradecyl sulfonate and Brij 30 surfactants, Colloid Surf. A, 398: 37-47.

Author's answer: We have included these on page 7 in the main text.

8. Reviewer's question: Page 9, "Sucrose corona" section, lines 11 and 12: A few TGA curves of samples mentioned should be presented as supporting material because they can be of interest for the readerships of this journal.

Author's answer: We have included representative TGA curves in Supporting Information, Section 2.8.

9. Reviewer's question: Pages 10-12, "References" section: This section should be revised carefully. For example;
*In this section, please do not use reference number. Only, references should be listed alphabetically.
*In some references such as references "1", "2", "20", "23", "28", "29", "30", etc., please check the use of small and capital letters during the spelling of journal names.
*According to the format of this journal, journal names should be abbreviated in all references (See references "1", "2", "11", etc.).
*In references "4", "15", "26" and "40", please use subscript for "2" in "TiO2".
*In reference "5", book name should be indicated.
*In references "9", "15", "20", "24", "32", "38", "39" and "41", the titles of articles should be written by small letters.
*In reference "20", "berg CA" and "ACS nano" should be replaced by "Berg CA" and "ACS Nano", respectively.
*In references "26" and "40", "TiO(2)" should be replaced by "TiO2".
*In reference "37", please use subscript for "2" and "3" in the spelling of "Fe2O3".

Author's answer: We have corrected all these as the reviewer suggested.

10. Reviewer's question: Page 13, "Table 1": "BSA:Sucrose (mg/mL)" should be replaced by "BSA:Sucrose" or "BSA (mg/L) : Sucrose (mg/mL)".

Author's answer: We have corrected this as the reviewer suggested.

11. Reviewer's question: Page 17, Fig. 2(a): In Fig. 2(a), the name of x-axis should be indicated. Furthermore, superscript should be used for "-1" in "cm-1".

Author's answer: We have corrected these as the reviewer suggested.

Reviewer #3

1. Reviewer's question: The authors have taken different concentration of matrixes for instance: 1. aqueous solution; 2. Sucrose and 3. BSA; and thereafter comparing the nanoparticles behavior. They should explain the reason behind selecting these concentrations? How they can be compared even their concentration is also different?

Author's answer: The concentrations of food models (protein and sucrose) used for the study were based on market analysis of food products that are known to contain the 2 engineered nanomaterials used in the study. Similarly, the choice of nanoparticle concentrations was based on actual amounts of nanoparticles present in real food products, according to published reports. Additional texts have been added on pages 4-5 to explain this. Comparisons made between the food ingredients and nanoparticles were qualitative and were meant to demonstrate the differing trends of effects rather than absolute differences.

2. Reviewer's question: The TiO2 nanoparticles are having a wide size distribution (40-360 nm), while it is narrow for silica nano particles. Again, size has a crucial role to play on their interaction with surroundings. How author can compare these two in same matrix though their size distribution is totally different. They should rather take monodispersed nano particles for study and make significant conclusion.

Author's answer: Indeed, food grade TiO2 (E171) nanoparticles have a broad size distribution ranging from 40 to 360 nm. However it is important to note that this is an intrinsic property of this food additive, and this is the actual material that is found in real foods today. According to our literature survey*, reports on the behaviour and toxicity of nano-TiO2 in foods are scarce, but nearly all the papers we found used E171 as the model TiO2 particle. Using any other monodispersed TiO2 particles (either synthesized in the lab or commercially available with a homogeneous size distribution) will significantly limit the practical relevance of the study as those particles will be irrelevant to food applications. It will also be difficult to draw parallels with real food products for subsequent risk assessment purposes, since the particles are expected to behave differently in the food matrices due to differences in their pristine properties.

Consequently, scientists have argued that greater effort should be spent on characterizing the properties of actual nanoparticles used in food products (E171) as opposed to non-food additives and reference nanoparticles (e.g. P25), in relation to their environmental fate and toxicity to humans. Therefore, using E171 nanoparticles in the current study is highly relevant to establishing meaningful understanding of TiO2 nanoparticles in foods. As described above, comparisons made between the nanoparticles were qualitative and were meant to demonstrate the differing trends of effects rather than absolute differences.

*Additional references:

1) Weir, A., et al., Titanium dioxide nanoparticles in food and personal care products. Environ Sci Technol, 2012. 46(4): p. 2242-50.
2) Faust, J.J., et al., Food grade titanium dioxide disrupts intestinal brush border microvilli in vitro independent of sedimentation. Cell Biol Toxicol, 2014. 30(3): p. 169-88.
3) Periasamy, V.S., et al., Identification of titanium dioxide nanoparticles in food products: induce intracellular oxidative stress mediated by TNF and CYP1A genes in human lung fibroblast cells. Environ Toxicol Pharmacol, 2015. 39(1): p. 176-86.
4) Song, Z.M., et al., Biological effect of food additive titanium dioxide nanoparticles on intestine: an in vitro study. J Appl Toxicol, 2015.
5) Bettini S et al., Food-grade TiO2 impairs intestinal and systemic immune homeostasis, initiates preneoplastic lesions and promotes aberrant crypt development

in the rat colon. Sci Rep 7:40373, 2017.

3. Reviewer's question: The TEM images should also be provided at large scale (ex. 1 micron) to see the overall effect. A small scale alone is not enough to conclude behavior.

Author's answer: For accurate number based particle size distribution analysis using TEM, we only viewed and imaged the particles at high magnification (scale bars of maximum 200 nm). Most published reports used similar magnifications for their analysis, and the results obtained were comparable. Nonetheless, we have now provided TEM images at larger scale (500 nm) in Supporting Information, Figure S9.

4. Reviewer's question: For DLS analysis, could author provide PDI index with each measurement to see true measurement as a function of matrix effect?

Author's answer: We have included all PDI values from the DLS measurements in Supporting Information, Section 2.2.

Reviewer #4

1. Reviewer's question: The paper entitled "Comparative differences in the behaviour of TiO2 and SiO2 food additives in food ingredient solutions" analyses the behaviour of SiO2 and TiO2 particles upon dispersion in complex matrices like food ingredients. The topic is important, and the experimental work is carried out in a systematic way. However, among the several aspects investigated it is not clear which one is the most critical, as the study reports nice experimental results but does not explain the reasons for the observed behaviour. For instance in the conclusions paragraphs one can read: "…[SiO2 nanoparticles displayed greater corona adsorption capacity for both bovine serum albumin and sucrose, compared to TiO2]" Why? Which one is the most critical parameter? The authors should try to provide an explanation. More detailed comments and suggestions: *Porosity for the particles results to be a critical parameter, but no values for the porosity are given. Not only the surface area (as the author do), but also the porosity might be determined easily from the nitrogen sorption analysis reported in Figure S1, by applying the BJH method.

Author's answer: We have revised the conclusions and other relevant sections to better explain our observations. We have also included the porosity of the nanoparticles as highlighted in the Results section (Page 6) and Supporting Information, Section 2.4.

2. Reviewer's question: Did the authors use any specific precaution for the sample preparation at TEM? The samples shall be carbon rich, usually these samples pose challenges as the carbon-rich shell of materials may undergo degradation under the effect of the electron beam.

Author's answer: Acquisition of the nanoparticle-corona micrographs was done with utmost care using TEM Carls Zeiss Libra 120, with a low accelerating voltage of 120 kV. They were viewed under medium brightness level to prevent localised heating and degradation of samples. Several micrographs were taken at different locations of the grids to ensure representative images were captured, and results were consistent across samples. We are therefore confident that the organic layers observed around the nanoparticles surfaces were representative of the original corona, albeit in a dry state. We have further included images of control samples (pristine nanoparticles) in Supporting Information, Figure S5.

Reviewer #5

1. Reviewer's question: The paper addresses the issue of nanoparticles, TiO2 and SiO2, in food ingredients. The authors make a good effort in trying to use BSA and sucrose to represent protein and carbohydrate in food ingredient solutions. The method is very effective but could be improved. The results are very clear and precise. It is very well written overall. The detection limit of FTIR should be mentioned. As

	mentioned in the paper, below the detection limit so the result did not pick up the sucrose. (Page 9 Sucrose corona) Author's answer: Due to low amounts of sucrose bound to the TiO2 and SiO2 particle surfaces, we were not able to obtain the relevant sucrose peaks and determine the practical detection limit of FTIR for these samples. Since IR radiation needs to transmit through the nanoparticle-corona samples, we believe that particles themselves, being inorganic in nature, would have masked some of the signal. Moreover, the detection limit of FTIR is dependent on the nature of specific samples. Based on this study alone, we are therefore unable to quantify this detection limit for sucrose corona on the particles, and we are also unable to draw such reference from the literature because there are none. 2. Reviewer's question: pH in the experiment should be mentioned as it can be the factor for forming corona. (Page 5 Stability of TiO2 and SiO2 nanoparticles in food ingredients solutions) Author's answer: We have included the information in the main text on page 4. 3. Reviewer's question: Temperature should be varied in the experiment. Food ingredients can be used in different temperature. Also the corona formation occurs differently according the temperature. Author's answer: We agree that the effect of temperature should be investigated as it is one of the parameters that affect corona formation. Food matrix composition was chosen as the variable since the objective of our study was to establish fundamental understanding of the nanoparticles' behaviour in various food ingredient solutions. We will include temperature change into our future study to understand the influence of temperature on corona formation. 4. Reviewer's question: For future recommendation, future experiment could extend for more protein and carbohydrate substances. Author's answer: We have included this valid recommendation in the Conclusion on page 10.
Suggested Reviewers:	Joel N Meyer Duke University joel.meyer@duke.edu Expert in studying nanomaterial behaviour in suspensions
	Bernd Nowack Swiss Federal Laboratories for Materials Science and Technology nowack@empa.ch Expert in nanomaterial behaviour in the environment
Additional Information:	
Question	**Response**
Scientific Justification (Available to Reviewers)	With concerns over the safety of ingesting nanomaterials used as food additives in processed foods, there is pressing need to further our current understanding of how the properties and behaviour of these nanomaterials evolve over their life cycle in the food product, and through the GI tract. Such understanding will be necessary for the purpose of designing more realistic experiments that take into account the state of the nanomaterials in question, especially for the purpose of understanding toxicology influences. However, there is little fundamental information about how commercially available nanoparticle based food additives behave in different food ingredients. In this manuscript, we report our results in establishing the behaviour of, and corona formation on food-grade titanium dioxide (TiO2) and silicon dioxide (SiO2) in solutions of model food ingredients bovine serum albumin and sucrose. Our results systematically demonstrate that nanoparticle composition influences the behaviour of nanoparticles in common food ingredient solutions, in terms of agglomeration, surface charge and corona formation.

Comparative Differences in the Behaviour of TiO_2 and SiO_2 Food Additives in Food Ingredient Solutions

Ridhwan Yusoff[1], Luong T. H. Nguyen[1], Paul Chiew[2], Zheng Ming Wang[2 *], and Kee Woei Ng[1 *]

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

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

[*]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

Abstract

Nanotechnology is widely used in the food industry to improve the colour, taste and texture of food products. However, concerns regarding potential undesirable health effects remain. It is expected that interaction of engineered nanomaterials (ENMs) with food ingredients will influence their behaviour and the resulting corona. Nonetheless, there are limited systematic studies conducted to clarify this understanding to date. Herein, we investigated the behaviour and corona formation of food grade titanium dioxide (TiO_2) and silicon dioxide (SiO_2) in solutions of model food ingredients including bovine serum albumin (BSA) and sucrose. Measurements using dynamic light scattering (DLS) showed that both TiO_2 and SiO_2 nanoparticles displayed a decrease in agglomerate sizes in the presence of both food ingredients. Both particles were negatively charged in all the conditions tested. Corona adsorption studies were carried out using multiple complementary methods including Fourier transformed infrared (FTIR) spectroscopy, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-ToF-MS), transmission electron microscopy (TEM), micro bicinchoninic acid (BCA) protein assay and thermogravimetric analysis (TGA). Comparative investigation showed that sucrose could disperse both particles more effectively than BSA and that SiO_2 displayed greater adsorption capacity for both BSA and sucrose, compared to TiO_2. Taken collectively, this study demonstrated the importance of considering food ingredient effects when mapping the behaviour of ENMs in food products. Such understanding could be significant in the evaluation of biological effects, such as toxicity, of ENMs used in food products.

Keywords

nanoparticles, titanium dioxide, silicon dioxide, protein, sucrose, corona

Introduction

Over the last few decades, nanotechnology has extended its reach into numerous fields ranging from engineering, healthcare, personal care to food applications (Chaudhry et al. 2008; Wiechers and Musee 2010). In the food industry, bulk food grade titanium dioxide (TiO_2) and silicon dioxide (SiO_2) have been extensively used as food additives to enhance the color, taste, and texture of processed foods. These are additives approved by the European Food Safety Authority (EFSA), and coded as E171 and E551, respectively (Chaudhry et al. 2008). TiO_2 is used as a white pigment to impart brightness to certain foodstuffs such as confectionery and dairy products (Weir et al. 2012), while SiO_2 is used as an anticaking agent to maintain flow properties in powder products (Dekkers et al. 2011). Several studies have reported that a considerable fraction of E171 was found to contain nano-sized particles of dimensions less than 100 nm, while all of the primary particles of E551 were nano-sized (Chen et al. 2013; Dekkers et al. 2011; Weir et al. 2012). As a large number of food products containing these permitted food additives can be found in the market, extensive public exposure to these nanomaterials through ingestion is anticipated (Cushen et al. 2012; Vance et al. 2015).

There are ample reports in the literature that document the potential deleterious effects of nanoparticles on the environment and public health. Reports on the ability of nanoparticles penetrating into cells, tissues and organs, because of their ultrafine dimensions, are aplenty (Karlsson et al. 2009; Wu et al. 2011). We have also shown that these particles, through different mechanisms, could interfere with endogenous cell signaling machinery to elicit a variety of responses that are consistent with exposure to toxins (Setyawati et al. 2013a; Setyawati et al. 2013b; Zhao et al. 2013). Specific to nanomaterials used in food products, a recent study has suggested that chronic exposure to food grade TiO_2 nanoparticles could promote colon micro-inflammation and contribute to the development of colorectal cancer in rats (Bettini et al. 2017; Urrutia-Ortega et al. 2016), while previous studies have shown that food grade SiO_2 nanoparticles could cause metabolic stress in intracellular environments (Athinarayanan et al. 2015; Athinarayanan et al. 2014). However, safety assessments are often hampered by the lack of information concerning the interactions of these orally ingested nanoparticles at realistic concentrations (Bellmann et al. 2015; Rossi et al. 2014; Wang et al. 2013; Yada et al. 2014). Nanoparticles are highly reactive and their physicochemical properties depend on the surrounding matrix as well as perturbations of the environment (Smolkova et al. 2015). Upon introduction into complex matrices such as foods or body fluids, these nanoparticles will interact with an assortment of biomolecules including food ingredients such as proteins, sugars, and lipids, which potentially form a decorative layer around the particles commonly known as the corona (Lynch and Dawson 2008; Monopoli et al. 2012). This corona imparts a unique identity to the nanoparticles by altering the agglomerated size, surface properties, and interfacial composition, which can influence their subsequent biological interactions. As a result of these modifications, the corona has been shown to affect the uptake and distribution of nanoparticles (Casals and Puntes 2012; Lesniak et al. 2012).

Several reports have argued the beneficial and hazardous biological effects of a nanoparticle corona to cells. While this may help to shield the nanoparticle or prevent leaching of mobile entities such as ions from the nanoparticles (Casals and Puntes 2012; Garvas et al. 2015; Tedja et al. 2012), the adsorption of proteins may also increase toxicity

by interfering with normal cell signaling pathways from both outside and inside a cell (Johnston et al. 2012; Zanganeh et al. 2016). Taken together these studies showed that characterization of the corona is important for understanding how exposure to nanoparticles affects the biological responses of cells and organisms.

In the context of food, this issue is complex because the nanoparticles are immersed in a dynamic environment across the life cycle of the product – food ingredients during production of the food; the final food matrix; and different sections of the gastrointestinal tract that comes with evolving mechanical, chemical and biological environment. In this study, we therefore aimed to start by carrying out systematic experiments to establish fundamental understanding of the behaviour patterns of E171 TiO_2 and E551 SiO_2 particles in model food ingredients including a protein (bovine serum albumin) and a carbohydrate (sucrose). Key properties such as agglomerated particle size, surface charge, identity and quantity of corona biomolecule composite were determined. The results presented plug the current knowledge gap of E171 and E551 interaction with food ingredients and form the basis to move on to complex food systems and gastrointestinal tract environment, with the eventual goal of establishing the health implications of ingesting these nanomaterials.

Materials and Methods

Materials

Food grade titanium dioxide (TiO_2; E171) and silicon dioxide (SiO_2; E551) were obtained from commercial suppliers. 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.

Methods

Stability of TiO_2 and SiO_2 nanoparticles in food ingredient solutions

Dynamic light scattering (DLS, Zetasizer Nano-ZS, Malvern Instruments, UK) was used to evaluate the state of agglomeration of nanoparticles in the food ingredient solutions. TiO_2 and SiO_2 nanoparticles were suspended in DI water at a concentration of 2 mg/mL and were used as stock solutions for further dispersion in food ingredient solutions. An appropriate amount of each nanoparticle stock solution was added to each of the food ingredient solution to obtain a final nanoparticle concentration of 1 µg/mL and 1 mg/mL for TiO_2 and SiO_2 nanoparticles, respectively. BSA and sucrose, which are the model protein and carbohydrate respectively, were dissolved in DI water at a concentration of 200 mg/mL and further diluted to obtain various concentrations (Table 1). The concentrations tested were based on market analysis of food products that are known to contain the engineered nanomaterials. The colloidal suspensions were vortexed for 15 s and sonicated in a water bath for 30 min at 50 Hz. Each sample was tested in triplicates and the mean hydrodynamic sizes and zeta potential values were reported. Control samples were prepared in the absence of food ingredients and similarly analyzed. All solutions were prepared at pH 6.8 ± 0.1 and measurements were carried out at 25 ± 0.1 ^{o}C. Preliminary tests showed that the final nanoparticle concentration was adequate to avoid multiple scattering of the DLS measurements. In addition, it is

important to note that the nanoparticle concentrations tested fall within the range reportedly used in food products (Dekkers et al. 2011; Weir et al. 2012).

TiO_2 and SiO_2 nanoparticle corona in food ingredient solutions

TiO_2 and SiO_2 nanoparticles with a final concentration of 1 mg/mL were dispersed in different concentrations of food ingredient solutions (Table 1) and incubated at 37 ^oC for 24 h. The samples were collected by centrifugation at 2680 and 17000 g for SiO_2 and TiO_2 nanoparticles, respectively, for 15 min and washed thrice with DI water to remove unbound and loosely bound biomolecules. An array of methods was employed to analyze the corona qualitatively and quantitatively.

Fourier transform infrared spectroscopy. The identities of the nanoparticle-biomolecule corona were determined using Fourier transform infrared spectroscopy (FTIR, Perkin Elmer Spectrum GX, USA). Prior to analysis, the collected food ingredient solution treated nanoparticles were frozen at -80 ^oC for 24 h and freeze-dried for 24 h. The samples were mixed well with potassium bromide (KBr) powder and compressed to form pellets before measurement was carried out. Transmission spectra were recorded from 4000 cm^{-1} to 400 cm^{-1} at a resolution of 4 cm^{-1} over 64 scans.

Transmission electron microscopy. Samples were prepared by first re-suspending the washed nanoparticle-biomolecule corona in DI water. Two drops of the suspension were deposited on the carbon-coated copper grids (Ted Pella, Inc., USA) and left to dry in a desiccator before analysis. Images were captured using TEM (Carl Zeiss Libra 120) with an accelerating voltage of 120 kV, at randomly selected locations on the grids at 160,000 X magnification.

Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry. Analysis of proteins adsorbed on the nanoparticles was performed using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-ToF-MS, Axima Performance, Shimadzu Biotech, UK) equipped with a 337 nm laser and operated in linear mode. Briefly, after adding 100 µL of DI water to the washed samples, 0.7 µL of sample and 0.7 µL of sinapic acid matrix solution (Sigma-Aldrich, USA) were deposited onto a stainless-steel plate. The samples were left to dry at room temperature before analysis. Laser power was set to 120 J and the spectra collected were the sum of 150 profiles.

Micro bicinchoninic acid. The protein attached on the nanoparticles was quantified using micro bicinchoninic acid assay (BCA, Thermo Fisher Scientific, USA) according to the manufacturer's instructions. Briefly, after removing the unbound and loosely bound proteins from the nanoparticles, the samples were mixed with 100 µL of the working solution of BCA reagent and incubated at 37 ^oC for 2 h. The samples were centrifuged, and the collected supernatants were transferred to a 96-well plate for measurement. Absorbance at the wavelength of 562 nm was recorded using microplate reader (Infinite 200, Tecan Inc., Switzerland). The amount of adsorbed proteins was calculated by using a calibration curve obtained from the BSA standard provided in the kit.

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

Statistical analysis

All quantitative data were presented as means ± standard deviation (SD). Statistical analyses were carried out using one-way analysis of variance (ANOVA) with Tukey's post-hoc testing. For all comparisons, differences were considered significant when *p* values were less than 0.05. A detailed analysis of the ANOVA results is included in Supporting Information, Section 2.1.

Results and Discussion

Characterization of pristine TiO_2 and SiO_2 nanoparticles

Thorough characterization of TiO_2 and SiO_2 nanoparticles was conducted to provide information about their size, morphology, crystal structure as well as specific surface area (Figure S1, Supporting Information). TEM analysis revealed that E171 TiO_2 nanoparticles had spherical to polygonal morphologies (Figure S1a). The primary particle size ranges from 40 to 360 nm, with 32% of the particles being less than 100 nm in at least one dimension (Figure S2, Supporting Information). TiO_2 nanoparticles displayed diffraction peaks at 2θ = 25.4°, 36.9°, 37.9°, 38.7° and 48.1°, which can be indexed to (101), (103), (004), (112) and (200) planes, respectively (Figure S1b). The diffraction peak positions are consistent with the diffraction pattern of anatase TiO_2 (Reyes-Coronado et al. 2008). The nitrogen adsorption-desorption isotherm of TiO_2 nanoparticles is shown in Figure S1c. The measured specific surface area of TiO_2 nanoparticles by the BET method was 8.05 m^2/g, and the adsorption and desorption average pore radius were 93.380 and 90.262 Å, respectively. The pore size distribution profile is presented in Supporting Information, Section 2.4. Unlike TiO_2 nanoparticles, E551 SiO_2 nanoparticles were irregularly shaped, with primary particle sizes ranging from 20 to 50 nm (Figure S1d). Compared to the TiO_2 nanoparticles, SiO_2 nanoparticles tended to present as large, agglomerated clusters in the TEM images captured. The XRD data depicted in Figure S1e revealed that the SiO_2 nanoparticles were amorphous in nature, with a characteristic broad peak centered at 22°. Figure S1f shows the nitrogen adsorption-desorption isotherm of SiO_2 nanoparticles. The measured specific surface area of SiO_2 nanoparticles as determined by the BET method was 140.29 m^2/g. The adsorption and desorption average pore radius were 141.442 and 138.354 Å, respectively. The pore size distribution profile is presented in Supporting Information, Section 2.4. Crystallographic orientation and surface energy analyses of these particles are well documented in the literature (Athinarayanan et al. 2014; Chen et al. 2017; Faust et al. 2014; Lorenzetti et al. 2017; Yang et al. 2016) and therefore not repeated here. Collectively, our particle characterization data obtained herein were consistent with those reported elsewhere (Dekkers et al. 2011; Weir et al. 2012).

Stability of TiO_2 and SiO_2 nanoparticles in food ingredient solutions

To have fundamental overview of the physical properties of the nanoparticles when they interact with food ingredient solutions, hydrodynamic size characterization of the nanoparticles was carried out using DLS. Figure 1 reports the hydrodynamic size and zeta potential of TiO_2 and SiO_2 nanoparticles in the various media used. The polydispersity index (PDI) of all measurements is included as Table S1, Supporting Information. The mean hydrodynamic sizes of these nanoparticles in water were 338.2 ± 41.9 and 366.4 ± 20.5 nm, respectively, which suggest that the primary particles had agglomerated in an aqueous environment, as expected. In the presence of BSA, both TiO_2 and SiO_2 nanoparticles showed a decrease in the size of their agglomerates, with a measured size of 222 ± 4.93 and 346.2 ± 61.4 nm, respectively. Rapid binding of proteins to the nanoparticle surfaces forms a protein corona (Lynch and Dawson 2008), which helps to disperse the nanoparticles in an aqueous environment by providing steric hindrance and electrostatic repulsion (Wells et al. 2012). Zeta potential measurement was also performed to understand changes in the surface charge of the nanoparticles, which correlate directly to binding of biomolecules onto the particle surfaces (Casals et al. 2010). Both nanoparticles registered negative surface charges in water, with zeta potential values of -19.4 ± 0.81 and -35.1 ± 0.12 mV, respectively. The magnitude of the zeta potential is important in determining the stability of colloidal systems. In general, particles having absolute zeta potential values higher than 30 mV are considered as stable dispersions due to the strong charge-charge repulsion between particles (Duman and Tunç 2009; Tunç et al. 2012). Upon dispersion in BSA, zeta potential for both particles dropped, more so for TiO_2 than SiO_2 nanoparticles, which suggested that more stable nanoparticle dispersions were acquired. Interestingly, when TiO_2 and SiO_2 nanoparticles were introduced into sucrose solutions, both particles, particularly SiO_2, displayed a remarkable reduction in hydrodynamic size accompanied by decreasing zeta potential that is correlated to increasing sucrose concentration. It has been demonstrated that polysaccharides can help to passivate surfaces of iron oxide nanoparticles and render them colloidally stable (Gawali et al. 2017). The formation of hydrogen bonds between the hydroxyl groups in sucrose and water molecules creates stability by overcoming the van der Waals attraction forces between particles. Increasing hydroxyl groups on the surfaces of nanoparticles, due to sucrose adhesion, also resulted in increasing negative surface charges, which consequently contributed to the colloidal stabilization. In a mixture of food ingredient solutions, different extents of improved nanoparticle dispersion were observed for TiO_2 and SiO_2 nanoparticles. Specifically, in a mixture of low BSA and high sucrose concentration solution, both particles exhibited a significant reduction in their agglomerated sizes. This dispersion phenomenon suggests that corona adsorption was strongly affected by the composition of the media. The influence of ionic strength and pH of media on the stability of TiO_2 and SiO_2 nanoparticles were also explored (Figure S3 and S4, Supporting Information). Both particles agglomerated at high ionic strength and low pH conditions due to electrostatic screening effects, which were indicated by the marked drops in magnitudes of zeta potential values.

TiO_2 and SiO_2 nanoparticle corona in food ingredient solutions

Protein corona

It is well accepted that the hard corona plays a pivotal role in determining a biological system's physiological response to a nanomaterial, compared to the soft corona (Lynch et al. 2007). Therefore, in this study, we attempted to characterize the hard corona formed in the model food ingredient solutions. In cases where BSA was included in the media, the presence of a protein corona on the nanoparticles was confirmed using three different techniques namely FTIR, MALDI-ToF-MS and TEM. The FTIR spectra of TiO_2 nanoparticles incubated in BSA recorded the presence of amide I and II vibrational peaks at 1650 cm^{-1} and 1540 cm^{-1}, respectively (Figure 2a). Amide group vibrations of the backbone received the most attention in protein IR spectroscopy because they are native to all proteins and are widely used in protein secondary structure studies (Song et al. 2012). Figure 2b shows a MALDI-ToF-MS spectrum of TiO_2 nanoparticles after exposure to BSA. The collected spectrum showed singly charged BSA ions at a mass to charge (m/z) ratio of 66,472 Da (Park and Blick 2013), clearly demonstrating the presence of a protein corona on the TiO_2 nanoparticles surface. MALDI-ToF-MS has proven to be a useful technique in detecting proteins adsorbed onto nanoparticle surfaces due to its simplicity in sample preparation and ability to perform rapid screenings (Chiang et al. 2011). Moreover, this technique permits the analysis of the corona in-situ, thus averting any unintended denaturation and fragmentation that may occur during sampling. To provide additional qualitative support for better understanding of the nanoparticle-protein corona, we present TEM images of the pristine particles (Figure S5, Supporting Information) compared to particles that had been exposed to BSA. At high magnification, the bare TiO_2 nanoparticles presented clear boundaries without any surface coating or layer. On the other hand, the BSA-treated TiO_2 nanoparticles had a translucent layer of material on the surfaces (Figure 2c). Unsurprisingly, thickness of the corona layer correlated directly to the BSA concentration in the media (Figure 2d). Similar to TiO_2, the FTIR spectra of BSA-treated SiO_2 nanoparticles contained amide I and II vibrational peaks (Figure 3a). The acquired MALDI-ToF-MS spectrum showed a sharp peak centered at 66,376 Da, which corresponded to singly charged BSA ions (Figure 3b). However, there was unexpected absence of a corona covering the SiO_2 nanoparticles surfaces, based on TEM examination (Figure 3c). SiO_2 nanoparticles are commonly reported to be porous (Canham 2014), which could have resulted in absorption of biomolecules into the particles rather than adsorption onto the surfaces. Visualization techniques such as TEM are therefore not suitable for characterizing the corona on such nanoparticles.

Beyond the qualitative assessments presented, we sought to strengthen protein corona characterization by adopting two dissimilar quantitative methods to measure BSA adsorption, i.e. micro BCA assay and TGA. The weight loss curves of BSA-adsorbed particles as measured from TGA are shown in Figure S6, Supporting Information. The results of both techniques were consistent, indicating that the amounts of adsorbed proteins on TiO_2 nanoparticles increased with increasing BSA concentration, with a maximum value of about 2% of total mass registered when BSA concentration was 20 mg/mL (Figure 2e). A similar trend was also observed for SiO_2 nanoparticles (Figure 3d). Not surprisingly, SiO_2 nanoparticles displayed higher adsorption capacity than TiO_2 nanoparticles, up to about 4% of total mass when BSA concentration was 4 mg/mL, which can be ascribed to its larger specific surface area

and porosity, thus providing increased available adsorption sites for interaction (Dobrovolskaia et al. 2009; Xiong et al. 2013). Apart from particle size, shape, surface charge and surface chemistry also affect the formation and composition of the corona around nanoparticles (Kharazian et al. 2016).

Sucrose corona

The FTIR spectra of TiO_2 nanoparticles incubated in sucrose solution did not present any sucrose associated vibrational peaks over the concentrations tested (Figure 4a). Nonetheless, under TEM, a distinct, thin layer of material was observed to cover the TiO_2 nanoparticle surfaces following exposure to sucrose (Figure 4c, d), which was absent on untreated nanoparticles. The adsorbed layer, which could only be sucrose, completely coated the single particles as well as larger agglomerates. It is worth noting that the sucrose corona layer was thinner on the TiO_2 nanoparticles incubated in media with a lower concentration of sucrose. The FTIR spectra of sucrose-treated SiO_2 nanoparticles did not contain sucrose vibrational peaks (Figure 5a). We believe that the amount of sucrose corona fell below the limit of detection of FTIR, thus the relevant vibrational peaks did not show up in both nanoparticle types. The sucrose corona could also have been masked by the inorganic nanoparticles. Similar to BSA treated samples, the absence of a coating covering the SiO_2 nanoparticles surface, based on TEM analysis, was apparent (Figure 5c). TGA results (Figure 4b and 5b) showed that the amount of adsorbed sucrose increased with increasing sucrose concentration for both particles. The weight loss curves of sucrose-adsorbed particles as measured from TGA are shown in Figure S7, Supporting Information. Notably, in a highly concentrated sucrose medium (100 mg/mL), SiO_2 nanoparticles displayed up to 20 times higher adsorption capacity than TiO_2 nanoparticles, indicating greater binding affinity of sucrose molecules to SiO_2 than TiO_2 nanoparticles at such a concentration.

Complex coronas

The FTIR spectra of TiO_2 nanoparticles incubated in both BSA and sucrose at varying concentrations recorded the presence of amide I and II vibrational peaks, with no sucrose peaks detected (Figure 6a). These results are concordant with our observation in Figure 4a, which showed the absence of sucrose vibrational peaks in the samples tested. TEM micrographs showed a coating on the TiO_2 nanoparticle surfaces following exposure to mixtures of BSA and sucrose in solution (Figure 6c, d). Likewise, FTIR analysis of SiO_2 nanoparticles incubated in both BSA and sucrose recorded the presence of amide I and II vibrational peaks with no sucrose peaks detected (Figure 7a). TEM examination revealed the absence of a corona layer surrounding the SiO_2 nanoparticle surfaces (Figure 7c, d), which was consistent with our earlier observations of SiO_2 samples in solutions of single food ingredients. The weight loss curves of complex coronas-adsorbed particles as measured from TGA are shown in Figure S8, Supporting Information. The TGA results (Figure 6b and 7b) suggest that the nanoparticles exhibited differences in their preferential adsorption of biomolecules in a solution of food ingredient mixtures, although the mechanisms of interaction and differences in such mechanisms between nanoparticle types are unclear. In addition, it is worth noting that the amount of corona on SiO_2 nanoparticles treated in a low BSA and high sucrose concentration medium was significantly higher than in either of the individual components. This suggests that the combination of

ingredients may promote synergistic formation of bio-coronas, further demonstrating the importance of understanding corona formation in the context of the relevant environment of interest.

Conclusion

In summary, our results demonstrated that BSA and sucrose were able to stabilize both TiO_2 and SiO_2 nanoparticles in suspension. Sucrose was more effective than BSA in stabilizing the suspensions, being able to reduce hydrodynamic sizes of the particles by up to 60%. The shift towards smaller hydrodynamic sizes was accompanied by more negative zeta potential values. The rapid binding of biomolecules to the nanoparticles surface resulted in the formation of a corona. This surface coating prevented the nanoparticles from agglomeration by providing steric hindrance and electrostatic repulsion. Complementary methods were used to comprehensively characterize the nanoparticle-coronas complexes. Our data showed that E551 SiO_2 nanoparticles were capable of adsorbing twice more bovine serum albumin and up to twenty times more sucrose, at the concentrations tested, compared to E171 TiO_2, owing to its larger specific surface area and measured porosity. The understanding generated from this study will be useful in formulating experimental designs to evaluate the influence of engineered nanomaterials, such as toxicity, on biological systems. Future experiments could also extend for other protein and carbohydrate substances such as whey proteins and glucose, respectively.

Acknowledgements

The authors would like to acknowledge Dr Ch'ng Ai Lee, Helen Phang and Dr Wu Yuansheng from AVA for their technical assistance and critical assessment of this work. The authors would also like to acknowledge the Facility for Analysis, Characterisation, Testing & Simulation (FACTS) at NTU for technical assistance in TEM and XRD analyses.

References

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

Athinarayanan J, Periasamy VS, Alsaif MA, Al-Warthan AA, Alshatwi AA (2014) Presence of nanosilica (E551) in commercial food products: TNF-mediated oxidative stress and altered cell cycle progression in human lung fibroblast cells. Cell Biol Toxicol 30:89-100 doi:10.1007/s10565-014-9271-8

Bellmann S et al. (2015) Mammalian gastrointestinal tract parameters modulating the integrity, surface properties, and absorption of food-relevant nanomaterials. Wiley Interdiscip Rev Nanomed Nanobiotechnol 7:609-622 doi:10.1002/wnan.1333

Bettini S et al. (2017) Food-grade TiO_2 impairs intestinal and systemic immune homeostasis, initiates preneoplastic lesions and promotes aberrant crypt development in the rat colon. Sci Rep 7:40373 doi:10.1038/srep40373

Canham L (2014) Porous silicon and functional foods. Handbook of Porous Silicon:985-997 doi:10.1007/978-3-319-05744-6_101

Casals E, Pfaller T, Duschl A, Oostingh GJ, Puntes V (2010) Time evolution of the nanoparticle protein corona. ACS Nano 4:3623-3632 doi:10.1021/nn901372t

Casals E, Puntes V (2012) Inorganic nanoparticle biomolecular corona: formation, evolution and biological impact. Nanomedicine 7:1917-1930 doi:10.2217/NNM.12.169

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

Chen H et al. (2017) The effects of orally administered Ag, TiO_2 and SiO_2 nanoparticles on gut microbiota composition and colitis induction in mice. NanoImpact 8:80-88 doi:10.1016/j.impact.2017.07.005

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

Chiang CK, Chen WT, Chang HT (2011) Nanoparticle-based mass spectrometry for the analysis of biomolecules. Chem Soc Rev 40:1269-1281 doi:10.1039/c0cs00050g

Cushen M, Kerry J, Morris M, Cruz-Romero M, Cummins E (2012) Nanotechnologies in the food industry – Recent developments, risks and regulation. Trends Food Sci Technol 24:30-46 doi:10.1016/j.tifs.2011.10.006

Dekkers S et al. (2011) Presence and risks of nanosilica in food products. Nanotoxicology 5:393-405 doi:10.3109/17435390.2010.519836

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

Duman O, Tunç S (2009) Electrokinetic and rheological properties of Na-bentonite in some electrolyte solutions. Microporous and Mesoporous Materials 117:331-338 doi:10.1016/j.micromeso.2008.07.007

Faust JJ, Doudrick K, Yang Y, Westerhoff P, Capco DG (2014) Food grade titanium dioxide disrupts intestinal brush border microvilli in vitro independent of sedimentation. Cell Biol Toxicol 30:169-188 doi:10.1007/s10565-014-9278-1

Garvas M, Testen A, Umek P, Gloter A, Koklic T, Strancar J (2015) Protein corona prevents TiO_2 phototoxicity. PLoS One 10:e0129577 doi:10.1371/journal.pone.0129577

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

Johnston H, Brown D, Kermanizadeh A, Gubbins E, Stone V (2012) Investigating the relationship between nanomaterial hazard and physicochemical properties: Informing the exploitation of nanomaterials within therapeutic and diagnostic applications. J Control Release 164:307-313 doi:10.1016/j.jconrel.2012.08.018

Karlsson HL, Gustafsson J, Cronholm P, Moller L (2009) Size-dependent toxicity of metal oxide particles-a comparison between nano- and micrometer size. Toxicol Lett 188:112-118 doi:10.1016/j.toxlet.2009.03.014

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

Lesniak A, Fenaroli F, Monopoli MP, Berg CA, Dawson KA, Salvati A (2012) Effects of the presence or absence of a protein corona on silica nanoparticle uptake and impact on cells. ACS Nano 6:5845-5857

Lorenzetti M, Drame A, Šturm S, Novak S (2017) TiO_2 (nano)particles extracted from sugar-coated confectionery. J Nanomater 2017:1-14 doi:10.1155/2017/6298307

Lynch I, Cedervall T, Lundqvist M, Cabaleiro-Lago C, Linse S, Dawson KA (2007) The nanoparticle-protein complex as a biological entity; a complex fluids and surface science challenge for the 21st century. Adv Colloid Interface Sci 134-135:167-174 doi:10.1016/j.cis.2007.04.021

Lynch I, Dawson KA (2008) Protein-nanoparticle interactions. Nano Today 3:40-47 doi:10.1016/s1748-0132(08)70014-8

Monopoli MP, Åberg C, Salvati A, Dawson KA (2012) Biomolecular coronas provide the biological identity of nanosized materials. Nat Nanotechnol 7:779-786 doi:10.1038/nnano.2012.207

Park J, Blick RH (2013) A silicon nanomembrane detector for matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) of large proteins. Sensors (Basel) 13:13708-13716 doi:10.3390/s131013708

Reyes-Coronado D, Rodriguez-Gattorno G, Espinosa-Pesqueira ME, Cab C, de Coss R, Oskam G (2008) Phase-pure TiO_2 nanoparticles: anatase, brookite and rutile. Nanotechnology 19:145605 doi:10.1088/0957-4484/19/14/145605

Rossi M, Cubadda F, Dini L, Terranova ML, Aureli F, Sorbo A, Passeri D (2014) Scientific basis of nanotechnology, implications for the food sector and future trends. Trends Food Sci Technol 40:127-148 doi:10.1016/j.tifs.2014.09.004

Setyawati MI et al. (2013a) Cytotoxic and genotoxic characterization of titanium dioxide, gadolinium oxide, and poly(lactic-co-glycolic acid) nanoparticles in human fibroblasts. J Biomed Mater Res A 101:633-640 doi:10.1002/jbm.a.34363

Setyawati MI et al. (2013b) Titanium dioxide nanomaterials cause endothelial cell leakiness by disrupting the homophilic interaction of VE-cadherin. Nat Commun 4:1673 doi:10.1038/ncomms2655

Smolkova B, El Yamani N, Collins AR, Gutleb AC, Dusinska M (2015) Nanoparticles in food. Epigenetic changes induced by nanomaterials and possible impact on health. Food Chem Toxicol 77:64-73 doi:10.1016/j.fct.2014.12.015

Song L, Yang K, Jiang W, Du P, Xing B (2012) Adsorption of bovine serum albumin on nano and bulk oxide particles in deionized water. Colloids Surf B Biointerfaces 94:341-346 doi:10.1016/j.colsurfb.2012.02.011

Tedja R, Lim M, Amal R, Marquis C (2012) Effects of serum adsorption on cellular uptake profile and consequent impact of titanium dioxide nanoparticles on human lung cell lines. ACS Nano 6:4083–4093

Tunç S, Duman O, Kancı B (2012) Rheological measurements of Na-bentonite and sepiolite particles in the presence of tetradecyltrimethylammonium bromide, sodium tetradecyl sulfonate and Brij 30 surfactants. Colloids Surf A Physicochem Eng Asp 398:37-47 doi:10.1016/j.colsurfa.2012.02.006

Urrutia-Ortega IM et al. (2016) Food-grade titanium dioxide exposure exacerbates tumor formation in colitis associated cancer model. Food Chem Toxicol 93:20-31 doi:10.1016/j.fct.2016.04.014

Vance ME, Kuiken T, Vejerano EP, McGinnis SP, Hochella MF, Jr., Rejeski D, Hull MS (2015) Nanotechnology in the real world: Redeveloping the nanomaterial consumer products inventory. Beilstein J Nanotechnol 6:1769-1780 doi:10.3762/bjnano.6.181

Wang H, Du L-J, Song Z-M, Chen X-X (2013) Progress in the characterization and safety evaluation of engineered inorganic nanomaterials in food. Nanomedicine 8:2007-2025 doi:10.2217/NNM.13.176

Weir A, Westerhoff P, Fabricius L, Hristovski K, von Goetz N (2012) Titanium dioxide nanoparticles in food and personal care products. Environ Sci Technol 46:2242-2250 doi:10.1021/es204168d

Wells MA, Abid A, Kennedy IM, Barakat AI (2012) Serum proteins prevent aggregation of Fe_2O_3 and ZnO nanoparticles. Nanotoxicology 6:837-846 doi:10.3109/17435390.2011.625131

Wiechers JW, Musee N (2010) Engineered inorganic nanoparticles and cosmetics: Facts, issues, knowledge gaps and challenges. J Biomed Nanotechnol 6:408-431 doi:10.1166/jbn.2010.1143

Wu J, Wang C, Sun J, Xue Y (2011) Neurotoxicity of silica nanoparticles: Brain localization and dopaminergic neurons damage pathways. ACS Nano 5:4476–4489

Xiong S, George S, Yu H, Damoiseaux R, France B, Ng KW, Loo JS (2013) Size influences the cytotoxicity of poly (lactic-co-glycolic acid) (PLGA) and titanium dioxide (TiO_2) nanoparticles. Arch Toxicol 87:1075-1086 doi:10.1007/s00204-012-0938-8

Yada RY et al. (2014) Engineered nanoscale food ingredients: Evaluation of current knowledge on material characteristics relevant to uptake from the gastrointestinal tract. Comprehensive Reviews in Food Science and Food Safety 13:730-744 doi:10.1111/1541-4337.12076

Yang Y, Faust JJ, Schoepf J, Hristovski K, Capco DG, Herckes P, Westerhoff P (2016) Survey of food-grade silica dioxide nanomaterial occurrence, characterization, human gut impacts and fate across its lifecycle. Sci Total Environ 565:902-912 doi:10.1016/j.scitotenv.2016.01.165

Zanganeh S, Spitler R, Erfanzadeh M, Alkilany AM, Mahmoudi M (2016) Protein corona: Opportunities and challenges. Int J Biochem Cell Biol 75:143-147 doi:10.1016/j.biocel.2016.01.005

Zhao Y, Howe JL, Yu Z, Leong DT, Chu JJ, Loo JS, Ng KW (2013) Exposure to titanium dioxide nanoparticles induces autophagy in primary human keratinocytes. Small 9:387-392 doi:10.1002/smll.201201363

Table Caption

Table 1: Summary of the various concentrations of food ingredient solutions

Nanoparticle/ Food ingredient	BSA (mg/mL)	Sucrose (mg/mL)	BSA (mg/mL) : Sucrose (mg/mL)
TiO_2	1, 10 and 20	1, 50 and 100	1:100, 10:50 and 20:1
SiO_2	1, 2 and 4		1:100, 2:50 and 4:1

Figure Captions

Fig. 1 *Hydrodynamic size and zeta potential analyses.* DLS analysis of (a) TiO_2 and (b) SiO_2 nanoparticles in food ingredient solutions. Data represent mean ± standard deviation (n = 3). $^{*}p < 0.05$ vs. control; $^{\#}p < 0.05$ vs. control (one-way ANOVA with Tukey's post-hoc testing)

Fig. 2 *Analyses of protein corona on TiO_2 nanoparticles.* (a) FTIR spectra of pristine TiO_2, BSA and TiO_2-BSA samples over varying BSA concentrations. Protein peaks are assigned as annotated. (b) MALDI-ToF MS spectrum of TiO_2-BSA sample. Representative TEM micrographs of (c) TiO_2 treated in low BSA concentration and (d) TiO_2 treated in high BSA concentration. Scale bars: 20 nm. Protein corona was identified as a translucent layer surrounding the dark particles. (e) Quantitative analyses of protein corona using BCA and TGA. Data represent mean ± standard deviation (n = 3). $*p < 0.05$ (one-way ANOVA with Tukey's post-hoc testing)

Fig. 3 *Analyses of protein corona on SiO_2 nanoparticles.* (a) FTIR spectra of pristine SiO_2, BSA and SiO_2-BSA samples over varying BSA concentrations. Protein peaks are assigned as annotated. (b) MALDI-ToF MS spectrum of SiO_2-BSA sample. Representative TEM micrograph of (c) SiO_2 treated in high BSA concentration. Scale bar: 20 nm. No protein corona layer was observed. (d) Quantitative analyses of protein corona using BCA and TGA. Data represent mean ± standard deviation (n = 3). $*p < 0.05$ (one-way ANOVA with Tukey's post-hoc testing)

Fig. 4 *Analyses of sucrose corona on TiO_2 nanoparticles.* (a) FTIR spectra of pristine TiO_2, sucrose and TiO_2-sucrose samples over varying sucrose concentrations. No sucrose peaks from the TiO_2-sucrose samples were detected by FTIR. (b) Quantitative analysis of sucrose corona using TGA. Data represent mean ± standard deviation (n = 3). $*p < 0.05$ (one-way ANOVA with Tukey's post-hoc testing). Representative TEM micrographs of (c) TiO_2 treated in low sucrose concentration and (d) TiO_2 treated in high sucrose concentration. Scale bars: 20 nm. Sucrose corona was identified as a translucent layer surrounding the dark particles

Fig. 5 *Analyses of sucrose corona on SiO_2 nanoparticles.* (a) FTIR spectra of pristine SiO_2, sucrose and SiO_2-sucrose samples over varying sucrose concentrations. No sucrose peaks from the SiO_2-sucrose samples were detected by FTIR. (b) Quantitative analysis of sucrose corona using TGA. Data represent mean ± standard deviation (n = 3). $*p < 0.05$ (one-way ANOVA with Tukey's post-hoc testing). Representative TEM micrograph of (c) SiO_2 treated in high sucrose concentration. Scale bar: 20 nm. No sucrose corona layer was observed

Fig. 6 *Analyses of complex coronas on TiO_2 nanoparticles.* (a) FTIR spectra of TiO_2 over varying BSA and sucrose concentrations. (b) Quantitative analysis of corona composite using TGA. Data represent mean ± standard deviation (n = 3). $*p < 0.05$ (one-way ANOVA with Tukey's post-hoc testing). Representative TEM micrographs of (c) TiO_2 treated in low BSA and high sucrose concentration solution and (d) TiO_2 treated in high BSA and low sucrose concentration solution. Scale bars: 20 nm. Corona composite was identified as a translucent layer surrounding the dark particles

Fig. 7 *Analyses of complex coronas on SiO_2 nanoparticles.* (a) FTIR spectra of SiO_2 over varying BSA and sucrose concentrations. (b) Quantitative analysis of corona composite using TGA. Data represent mean ± standard deviation (n = 3). *$p < 0.05$ (one-way ANOVA with Tukey's post-hoc testing). Representative TEM micrographs of (c) SiO_2 treated in low BSA and high sucrose concentration solution and (d) SiO_2 treated in high BSA and low sucrose concentration solution. Scale bars: 20 nm. No corona layer was observed

Figure 1

Click here to download Figure figure1.tif

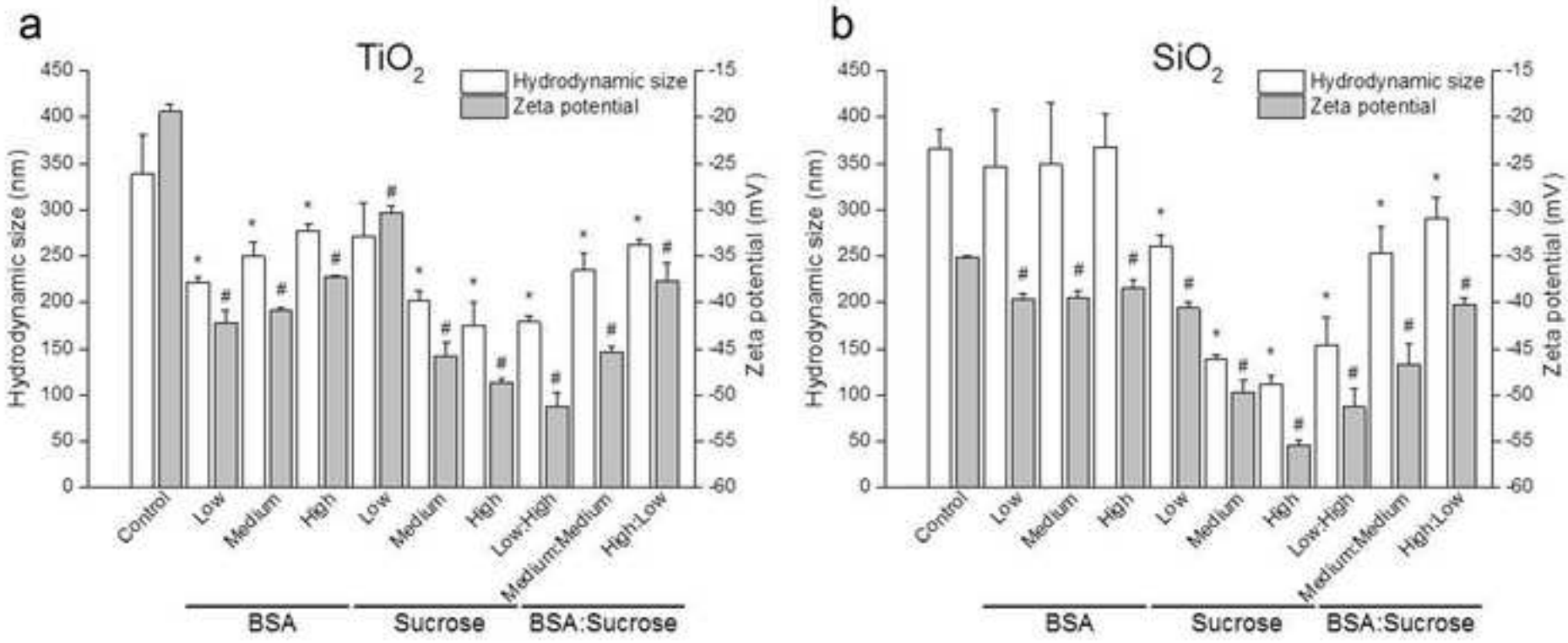


Figure 2

Click here to download Figure figure2.tif

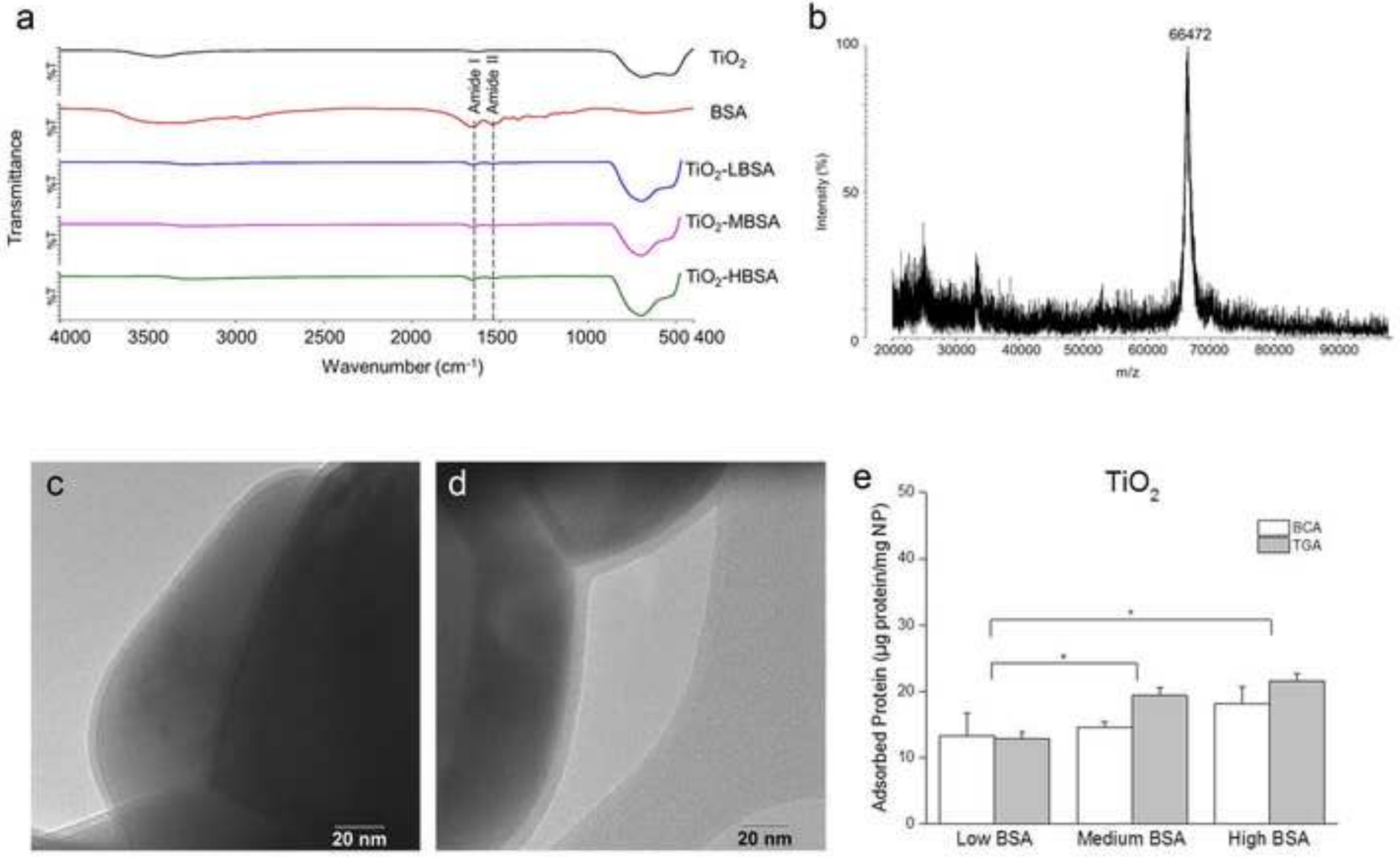


Figure 3

Click here to download Figure figure3.tif

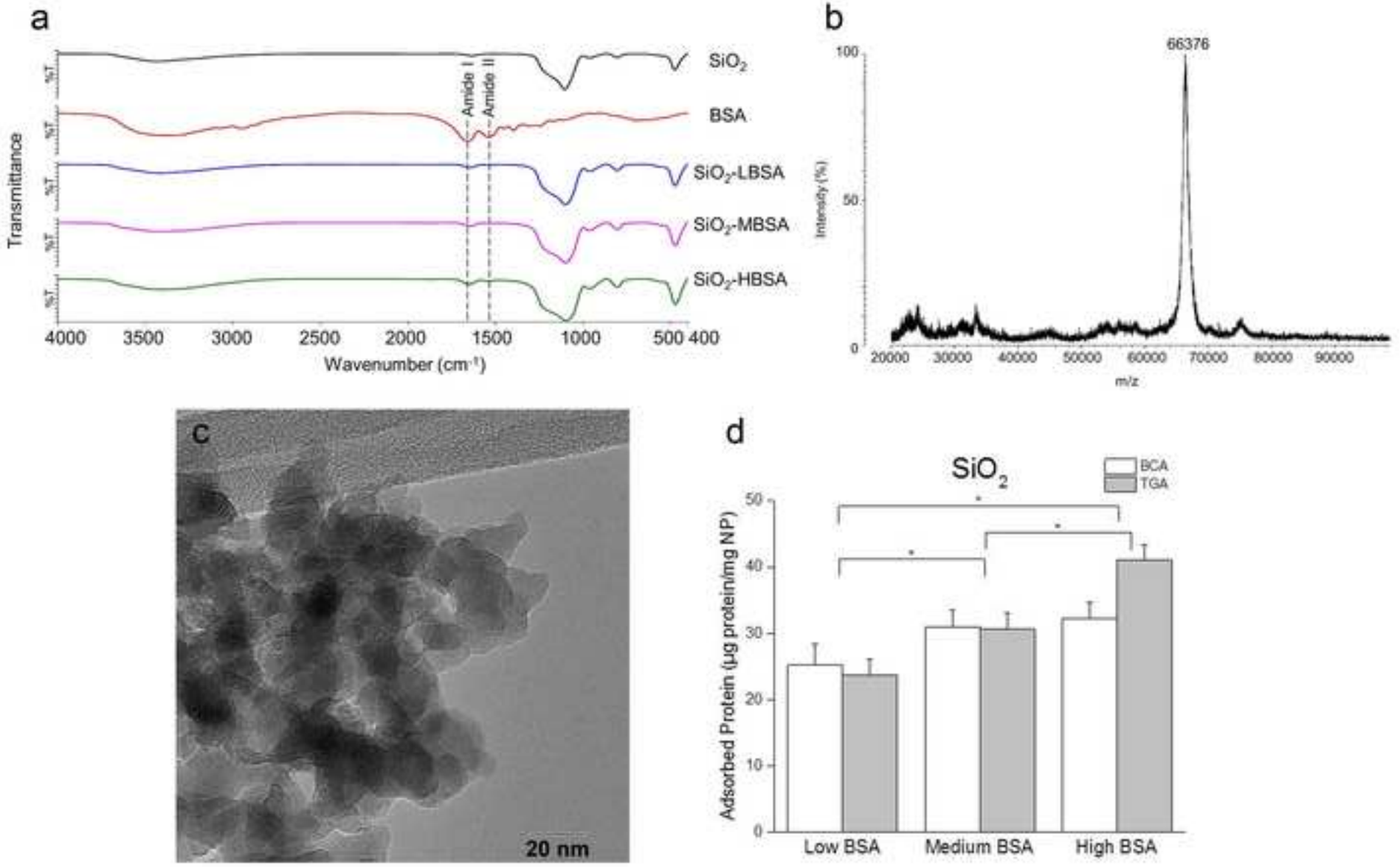


Figure 4

Click here to download Figure figure4.tif

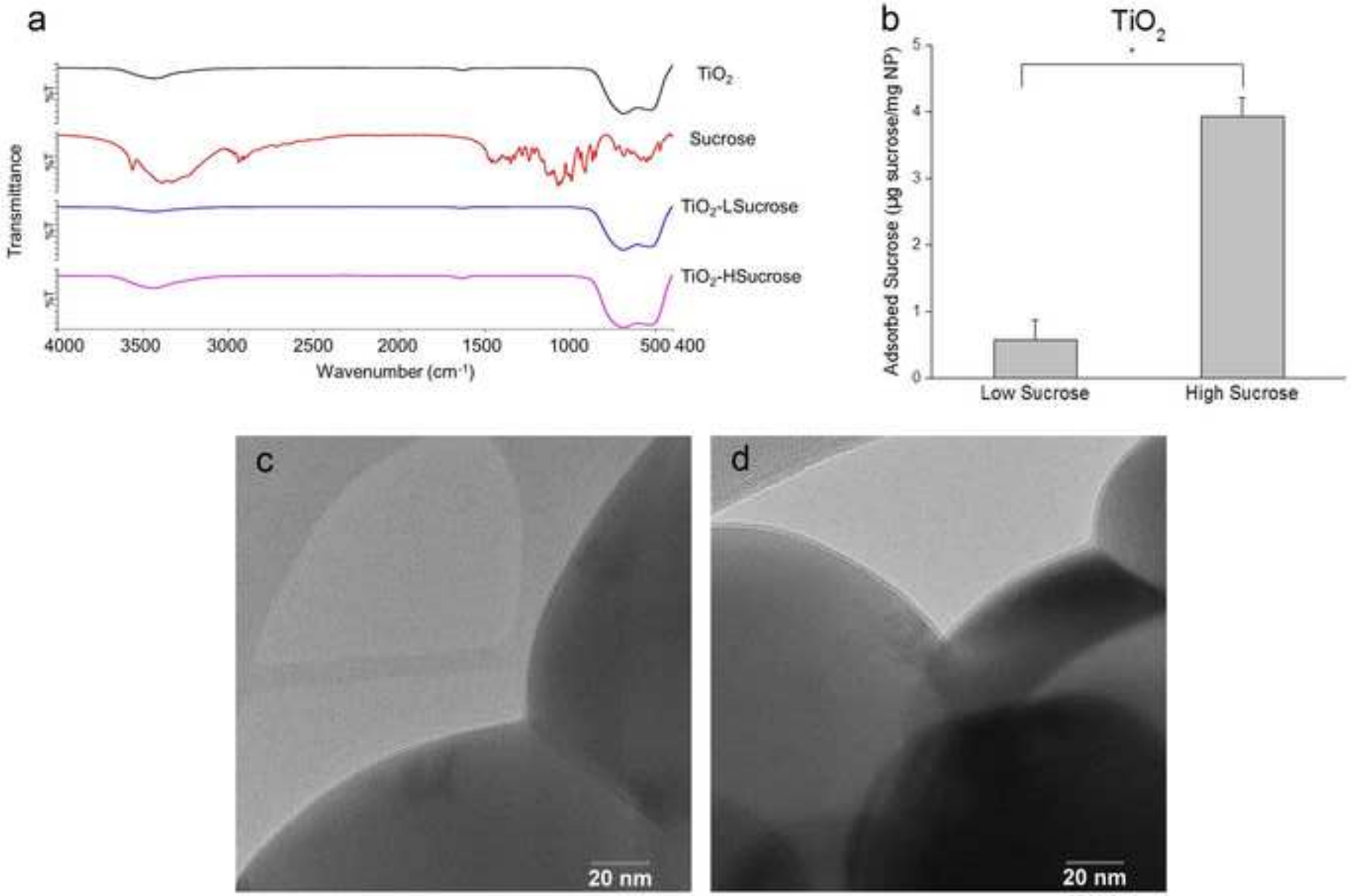


Figure 5

Click here to download Figure figure5.tif

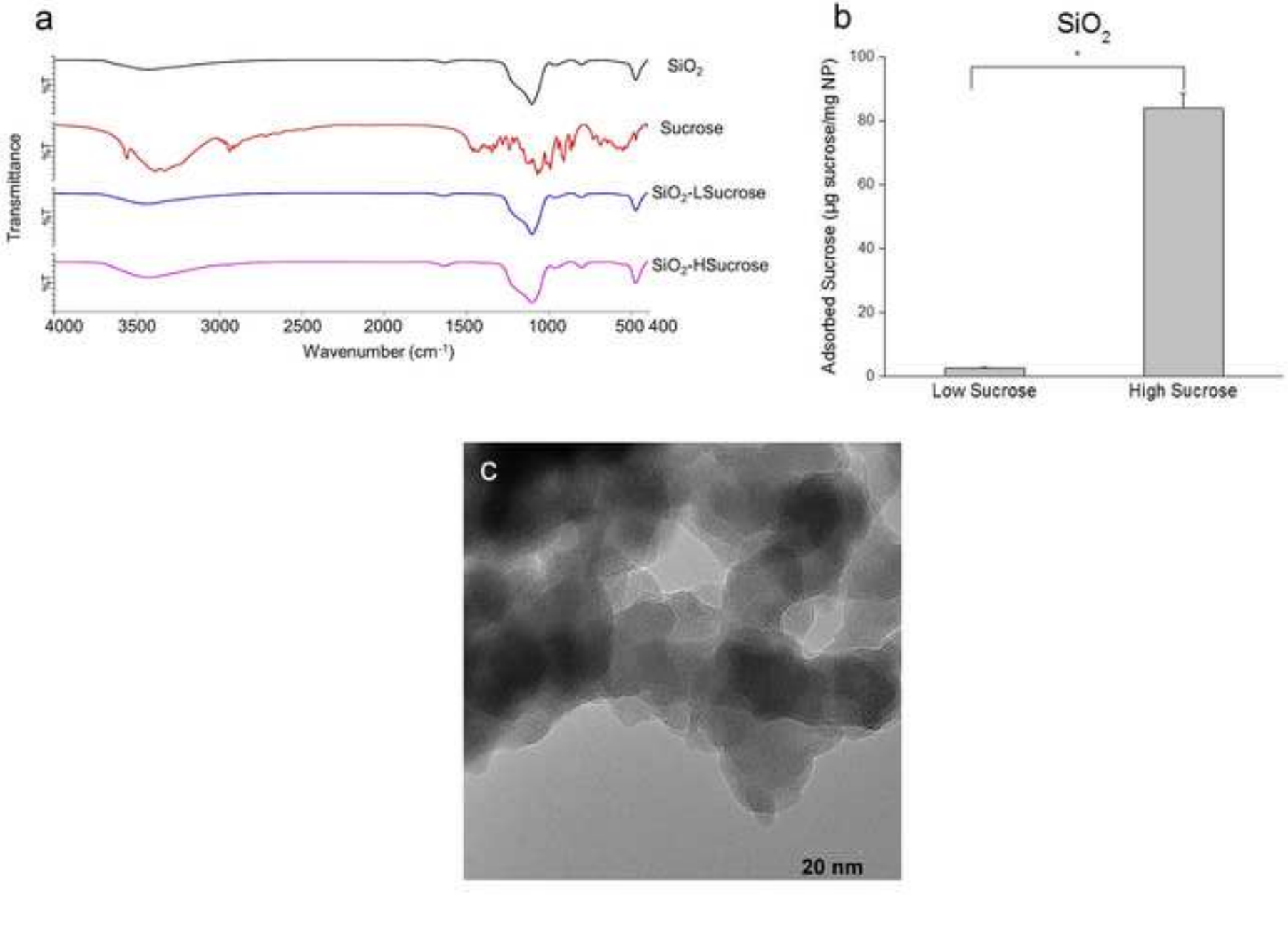


Figure 6

Click here to download Figure figure6.tif

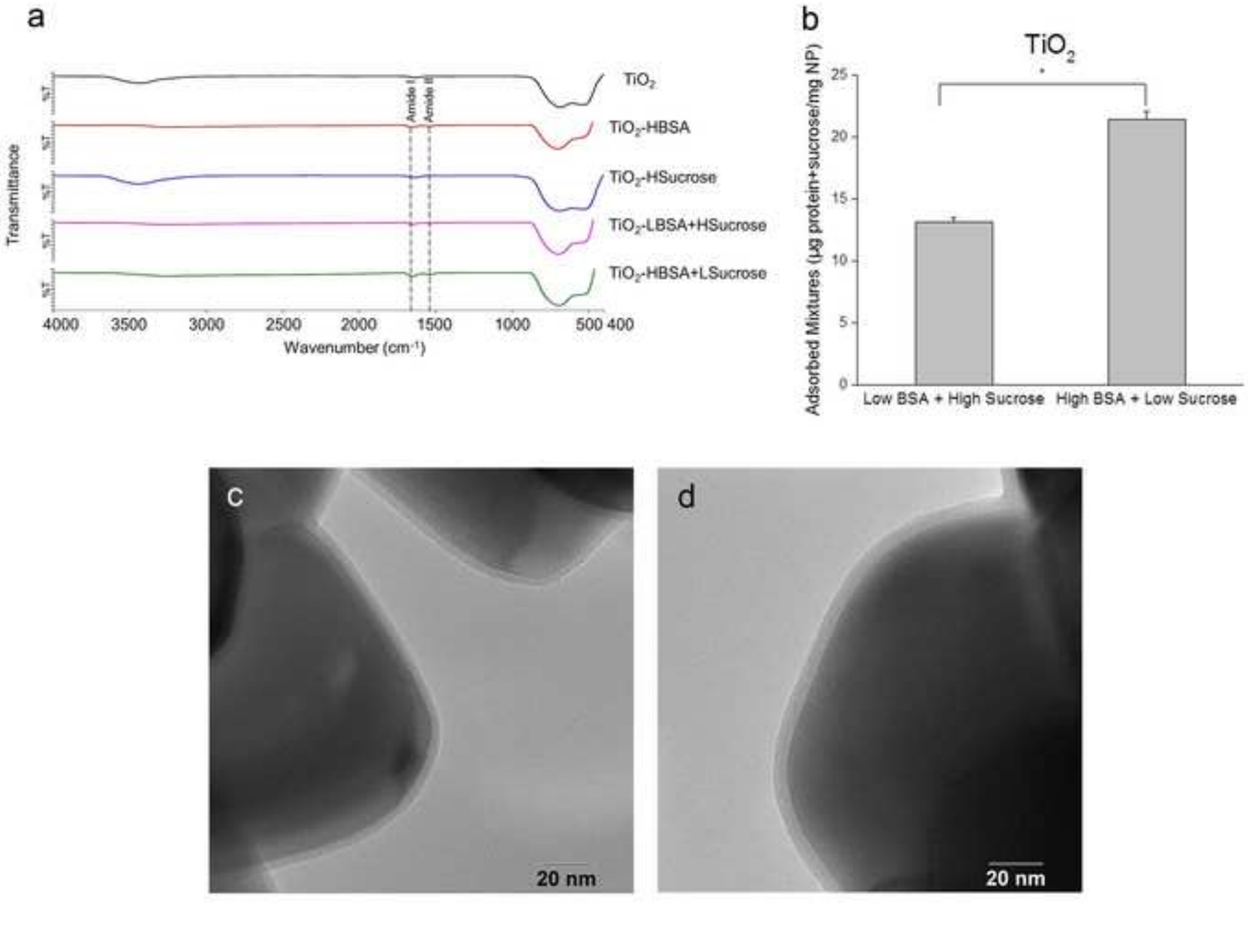
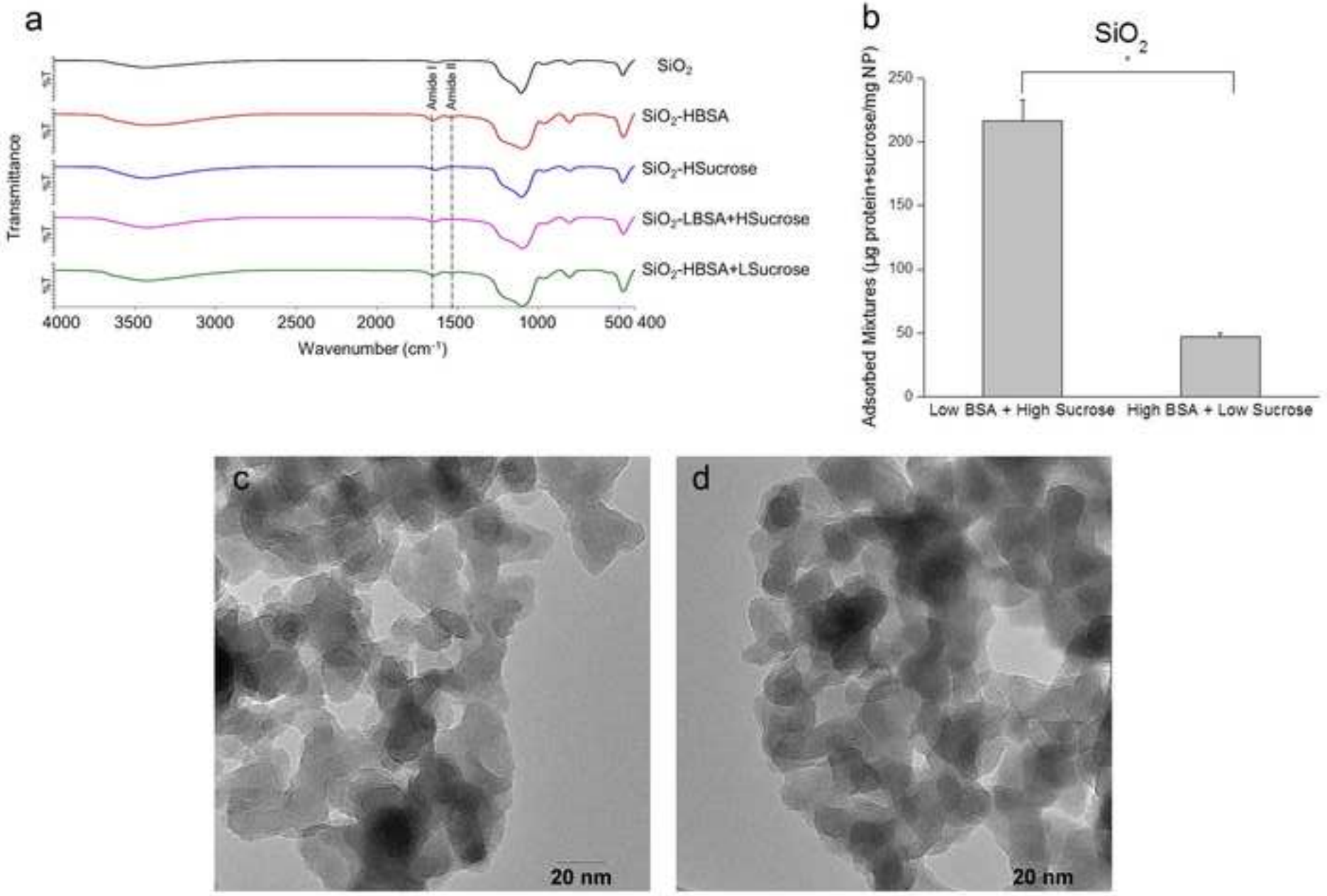


Figure 7

Click here to download Figure figure7.tif



Supplementary material (audio/video files etc)