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# Effects of Hydrophobicity and Hydrophilicity on Adsorption of Blood Plasma Protein

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This study was completed by Tori Zimmerman, Morgan Miller and Ruibo Hu, under the guidance of Dr. Nita Sahai.

## Abstract

In recent times, Nanoparticles (NPs) are used in medical practices especially for their contribution to drug delivery. Blood plasma protein binds to titanium dioxide nanoparticles and prevent the delivery of drugs to the intended target. When the protein binds to the NPs, the NPs in turn change the secondary structure of the blood plasma protein. This can affect drug delivery and thrombosis. The different shapes of these NPs influence the binding affinity and adsorption of the blood plasma protein. The research analyzed the NPs for two variables, shape and hydrophobicity. We conducted this experiment to further research effects of hydrophobicity and hydrophilicity of the NPs on the binding properties of the blood plasma protein. Fg had a higher binding affinity than HSA to the NPs. HSA failed to adsorb on any of the nanoparticles. Anatase had a higher binding affinity than the sample of NTs. These results indicate hydrophilicity and hydrophobicity does have an effect on protein adsorption. Further research could give us insight on the differences in secondary structure between NPs of different shapes and different levels of hydrophobicity or hydrophilicity.

**Acknowledgements**

I would like to express my appreciation to Dr. Sahai for allowing us the ability to work in the lab and advising this project. I would also like to thank Morgan Miller and Ruibo Hu for your contributions and efforts on this project. Additionally, I am grateful to Jiadong Chen for providing us with protocols and samples to use for this experiment. Thank you to the Honors faculty advisor, Dr. Bagatto, and readers, Dr. Londraville and Dr. Ramirez, for your support.

# Table of Contents

## Introduction

Nanoparticles are structures that range from 1 nanometer to 100 nanometers in size (Murthy, 2007). These particles are utilized and studied to improve various science fields such as engineering and medical science. Nanotechnology, the study and manipulation of nanoparticles, has been applied to research in medicine to explore drug delivery and medical techniques capable of reducing damage to human organs and tissues.

Titanium dioxide ($TiO_2$, titania) is a chemical compound that can be found in three forms which are anatase, rutile, and brookite. $TiO_2$ is apparent in various applications, anywhere from makeup to candy. It is approved by the FDA to be used as an additive to pharmaceutical products and food (Skocaj et al., 2011). More recently, nano-sized particles of $TiO_2$ have been utilized as nanotechnology has advanced as a reputable field of scientific study.

Moreover, titanium dioxide nanoparticles ($TiO_2$ NPs) are studied due to concerns regarding possible negative impacts of exposure to the human body. This can be contributed to the increased presence of $TiO_2$ NPs in many body care and food products, such as sunscreen. Thus, this research is extremely important in the medical field to prevent negative health outcomes.

Additionally, $TiO_2$ NPs research has shown that $TiO_2$ NPs adsorb blood plasma proteins after the NPs bind to the protein (J. Chen, M.S. Thesis, 2020). This may lead to structural changes in a protein's structure and may lead to changes in regulating thrombosis or the drug carried by a $TiO_2$ NP may not be properly delivered to the targeted tissue. Researching the effects of $TiO_2$ NPs on thrombosis and drug delivery is imperative to benefiting medical practices.

Previous research was conducted by Jiadong Chen in the Sahai group to better understand how protein adsorption can be affected by different shapes and hydrophobicity of $TiO_2$ NPs (J. Chen, M.S. Thesis, 2020). Chen studied three different shapes of $TiO_2$ NPs: nanorods (NRs), nanotubes (NTs), and rutile hexagonal (HNPs) and their interaction with two blood plasma proteins, fibrinogen (Fg) and human serum albumin (HSA). Fg provides a beneficial model because of its importance in formation of clots, while HSA makes a good model because of its abundance in blood plasma in the human body.

Albumins are subject to structural changes when in the presence of particles with hydrophobic surfaces due to strong hydrophobic interactions (J. Chen, M.S. Thesis, 2020). Highly hydrophobic particle surfaces tend to have high protein binding affinity, while highly hydrophilic particle surfaces tend to have low protein binding affinity. Furthermore, changes to protein structure can affect the response of proteins in the blood to drugs and antibodies.

Chen's research showed that binding of all three types of $TiO_2$ NPs to blood plasma protein were different. He noted that the hydrophobicity and different shapes of these NPs did have an effect on the binding affinity and protein adsorption. HNPs were found to be more hydrophobic compared to NTs and NRs.

According to the adsorption isotherms for HSA and Fg, the binding affinity was highest for HNPs, followed by NTs and NRs, respectively (J. Chen, M.S. Thesis, 2020). HSA proved to have a significantly lower binding affinity to all three types of $TiO_2$ NPs than Fg. All types of $TiO_2$ NPs produced similar changes in protein structure for Fg. This indicated that if the surface has low protein binding affinity, the shape and hydrophobicity of the $TiO_2$ NPs will affect the conformation and amount of the adsorbed protein.

HNPs have the highest binding affinity of all three types of NPs because both NRs and NTs contain hydrophilic OH bonds on their surfaces. The presence of these OH bonds of the surface of NTs and NRs lowers the interactions with hydrophobic blood plasma proteins. The larger size of Fg allowed for stronger interactions with the surfaces of the $TiO_2$ NPs, leading to higher binding affinity than HSA.

All three types of NPs adsorbed on HSA and produced different changes in their secondary structure. HSA had stronger hydrophobic interactions with HNPs because they contain more hydrophobic surfaces than NTs and NRs (J. Chen, M.S. Thesis, 2020). Thus, interactions between HNPs and HSA created more changes in structure than interactions of HSA with NTs or NRs, due to their highly hydrophilic surfaces.

Chen's research showed that blood plasma protein had the highest binding affinity for hexagonal nanoparticles (HNPs) and the lowest binding affinity for the nanorods (NRs), with the nanotubes (NTs) falling in between. However, the effects of NP shape and hydrophobicity/hydrophilicity were convoluted because the hexagonal shaped rutile HNPs were hydrophobic compared to the elongated NTs and NRs which were more hydrophilic. Therefore, the goal of the present study was to separate out the effects of surface hydrophobicity/hydrophilicity from shape effects. Results can lead to improvements in the process of drugs effectively targeting specific tissues and organs in the body when NPs are in use. Additionally, results could be imperative to benefiting medical practices by formulating NPs that prevent thrombosis.

## Materials and Methods

All experiments for this research were conducted using protocols from Chen's study (J. Chen, M.S. Thesis, 2020). The chemical compounds used for this experiment were all obtained

from Sigma™ (St. Louis, MO, USA) unless stated otherwise. The water utilized for this study was ultrapure water from Thermo Scientific (18.2 MΩ·cm) (Barnstead™ GenPure™ xCAD Plus, Rockford, IN, USA). Sodium hydroxide was obtained from Fisher Scientific (Waltham, MA). Ceramic boats were purchased from Nova-Tech International (Kingwood, TX). 450 Digital Sonifier was obtained from Branson (Brookfield, CT).

The $TiO_2$ NTs and NRs utilized for this experiment were products prepared by Jiadong Chen. The synthesis of $TiO_2$ NPs was conducted starting with rutile HNPs to form nanotubes (NTs) and nanorods (NRs) using the hydrothermal method noted in the research article by Kasuga et al (2009). 20g of solid NaOH was dissolved in 40mL of ultrapure water in order to obtain a concentration of NaOH at ~12M. Later on, a more precise concentration of the NaOH solution was found after titration using oxalic acid (1N) and an indicator, phenolphthalein. Ultrapure water was then added to the NaOH solution to dilute it to a concentration of 10M. 30mL of NaOH solution and 3g of rutile $TiO_2$ powder were both deposited into a hydrothermal autoclave reactor. The autoclave reactor was properly sealed and positioned inside the oven which heated the sample at 120 °C for 20 hours. The mixture was collected from the autoclave and separated equally into four 50mL centrifuge tubes. The tubes were centrifuged at 4000 RCF for 8 minutes and ultrapure water was used to wash the samples after each cycle. This protocol was repeated until the supernatant of the sample met a conductivity under $70\mu S \cdot cm^{-1}$ (VWR® Bench/Portable Conductivity Meter, Radnor, PA, USA). ~40mL of 0.1 HCL was then added to the powder sample and the mixture was sonicated for about 3 minutes. The mixture was then centrifuged under the same conditions as the prior centrifugation. Ultrapure water was used to wash the mixture until the supernatant of the sample achieved a conductivity under $10\mu S \cdot cm^{-1}$.

The sample was then lyophilized (FreeZone 4.5 Liter Console Freeze Dry System, Labconco, Kansas City, MO, USA) and stored under standard conditions.

Characterization of the $TiO_2$ NPs was completed using various techniques including transmission electron microscopy (TEM), Fourier transform infrared (FT-IR), thermogravimetric analysis (TGA), and dynamic light scattering to obtain zeta potentials.

TEM: Characterization by TEM was conducted on the HNPs, NTs, and NRs to determine size and shape. 70% ethanol was used to suspend the nanoparticles and the suspension was sonicated for 3 minutes to achieve a concentration of 1 $mg \cdot mL^{-1}$. 10µL of the suspension was pipetted onto a formvar carbon-coated copper grid (300 mesh, Ted Pella, Redding, CA, USA) and after 3 minutes the sample was blotted with filter paper. The grid was left to air dry overnight and viewed the following day with TEM (JSM- 1230, JEOL, Peabody, MA, USA). ImageJ was set up to estimate the dimensions of the NP of the various TEM images.

FT-IR: Characterization by FT-IR was conducted for all three types of NPs. The range of 600 to 4000$cm^{-1}$ was used to measure the infrared spectra and each sample was scanned 10 times using FT-IR technology (Excalibur FTS 3000, PerkinElmer, Shelton, CT, USA).

TGA: The TGA analysis involved heating each of the samples starting at room temperature increasing the temperature at a rate of 20 $°C \cdot min^{-1}$ until reaching 600°C (TGA550, TA Instruments-Waters LLC, New Castle, DE, USA).

Dynamic Light Scattering: ζ-potential and isoelectric points of each type of NP were determined for characterization. Surface charge on the surfaces of the NPs were estimated by the isoelectric points. Titrations of 0.05 $mg \cdot mL^{-1}$ of $TiO_2$ NPs with 10 mM NaCl were completed to

attain different pHs ranging from 3 to 11. ζ-potential of the $TiO_2$ NPs was assessed at 25 °C and 2 accumulations were obtained (Nano ZS ZEN3600, Malvern, Westborough, MA). Three measurements were taken at each pH value for every sample.

Adsorption Isotherms: $TiO_2$ NPs were utilized to obtain adsorption isotherms of HSA and Fg. Each NP sample was prepared with a concentration of $10mg{\cdot}mL^{-1}$ in buffer (10mM HEPES and 150mM NaCl, pH 7.4) and tip-sonicated for 20 seconds to avoid aggregation. The blood plasma proteins used for this experiment were HSA (≥96%, ~66.4 kDa) and Fg (≥75%, ~340 kDa). Solutions of protein ($10mg{\cdot}mL^{-1}$) were prepared with buffer and sonicated for a few minutes until the protein was completely dissolved. Concentrations of protein ranging from 0 to $3mg{\cdot}mL^{-1}$ were prepared in PCR tubes using protein solution with an overall volume of 100µL. Each tube had 100µL of the NP solution added until a concentration of $10mg{\cdot}mL^{-1}$ was met. The tubes were placed in trays and incubated for 4 hours at a temperature of 37°C (I24 shaker, New Brunswick Scientific, Edison, NJ, USA). To properly determine if protein was adsorbed, PCR tubes containing controls consisting of 200µL protein solution were also incubated along with the experimental samples. The initial concentration (Ci) identified the protein that remained in the control system's supernatant after centrifugation was completed. After the incubation period was completed, the PCR tubes containing the controls and NP samples were centrifuged and the supernatant was utilized to find the concentration of the protein that remained. This concentration is designated as the equilibrium concentration in solution (Ce). Centrifugation of the PCR tubes was conducted for about 8 minutes at 20,000 rpm (Galaxy Mini Centrifuge, VWR). Beer-Lambert Law was utilized for the concentrations of protein to be determined at 280nm by UV-vis absorbance (Synergy H1 Hybrid Multi-Mode Reader, BioTeck, Winooski, VT, USA). Using 150 µL of the protein solution (0-5 $mg{\cdot}mL^{-1}$) in 96-wellplates, calibration

curves of concentration and absorbance were constructed. The concentration of protein adsorbed onto the $TiO_2$ NPs was defined as qe and determined by calculating the difference between the initial concentration in the control and the protein concentration of the supernatant of each sample of NPs, thus formulated as Ci – Ce. The adsorption isotherms of HSA and Fg adsorbed onto the NTs, NRs, and HNPs were analyzed using the Langmuir adsorption model. The adsorption isotherms and model fits were obtained by Ruibo Hu to solve for $K_{ads}$, the binding affinity for adsorbed particles to surfaces, and $S_t$, the total maximum adsorbed concentration at the surface.

## Results and Discussion

The shape and size of the NPs were characterized by TEM (Figure 1). The commercial Anatase NPs are approximately 25-30 nm in size and sub-hexagonal to cuboidal in shape. The NTs are elongated and hollow as inferred from the lower electron density bulk and higher electron density edges of the NPs. The NTs are ~100 nm long. They were formed by heating the commercial rutile HNPs in an autoclave at 120°C. NTs have a length of 133.2 ± 47 nm (J. Chen, M.S. Thesis, 2020). For the NT cross section, the inner diameters are 4.2 ± 0.9 nm, while the outer diameters are 10.2 ± 1.6 nm. The specific surface area of the NTs is 137 $m^{2} \cdot g^{-1}$, which is much larger than that of the HNPs. This is consistent with the NTs maintaining a hollow structure, compared to the HNPs exhibiting a solid structure. The production of NRs was attempted by hydrothermal treatment at 210°C, but after taking TEM images the NPs still portrayed hollow NTs.

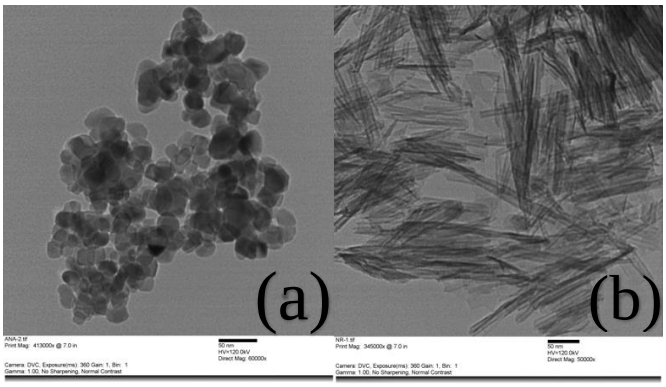


**Figure 1.** TEM images of (a) Anatase and (b) NTs.

The ζ-potential of all three types of NPs was measured as a function of pH (Figure 2). ζ-potential gives insight to the surface charge of the NPs, which is important for the interactions between the NP surfaces and the proteins. The pH at which the ζ-potential is zero is known as the isoelectric point. The isoelectric point of NTs is 2.4, NRs is 3.2, and HNPs is 3.5. The isoelectric points of HSA and Fg, respectively, are 4.7 and 5.5 (J. Chen, M.S. Thesis, 2020). Thus, all the NPs and both proteins are negatively charged at the experimental pH, hence , will repel each other electrostatically. Any adsorption will, therefore, be driven primarily by hydrophobic/hydrophilic interactions.

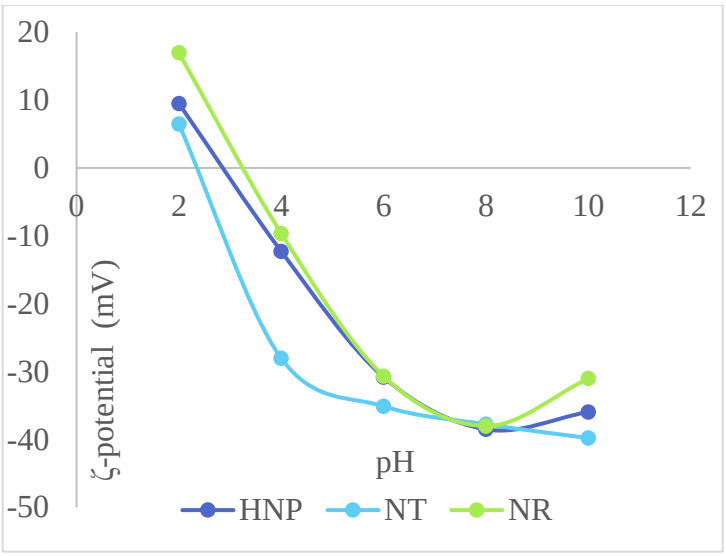


**Figure 2.** The ζ-potentials of all three types of NPs at pHs ranging from 2 to 6.

The relative hydrophobicity/hydrophilicity of surfaces was characterized by FT-IR and TGA. HSA and Fg both possess a lower binding affinity to hydrophilic surfaces when compared to hydrophobic surfaces (J. Chen, M.S. Thesis, 2020). The FT-IR spectra of NTs and NRs both have a broad peak at about 3100cm$^{-1}$ and a sharp peak at about 1600cm$^{-1}$, which indicates the presence of water and -OH bonds on their surfaces (Figure 3). The FT-IR of HNPs shows peaks of much smaller amplitude at both 3100cm$^{-1}$ and 1600cm$^{-1}$, which accurately suggests that very little water or -OH surface groups are associated with HNPs (Figure 3). Therefore, NTs and NRs are shown to be more hydrophilic whereas HNPs are more hydrophobic.

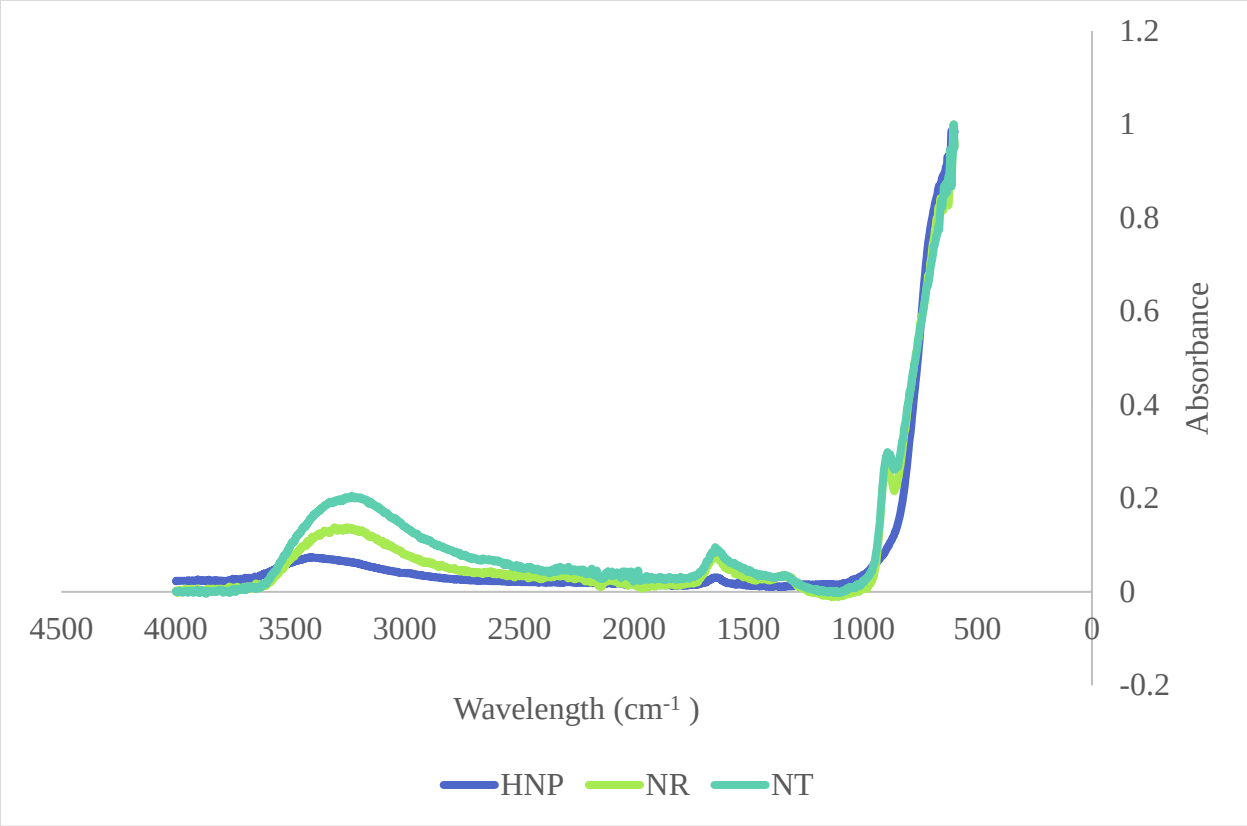


**Figure 3.** Normalized FT-IR spectra of the three types of $TiO_2$ NPs.

The FT-IR spectrum of anatase was very similar to that of rutile (Figure 4). This was an unexpected result because anatase, which is prepared commercially by sol gel synthesis method should have had a greater amount of water and surface -OH species than rutile.

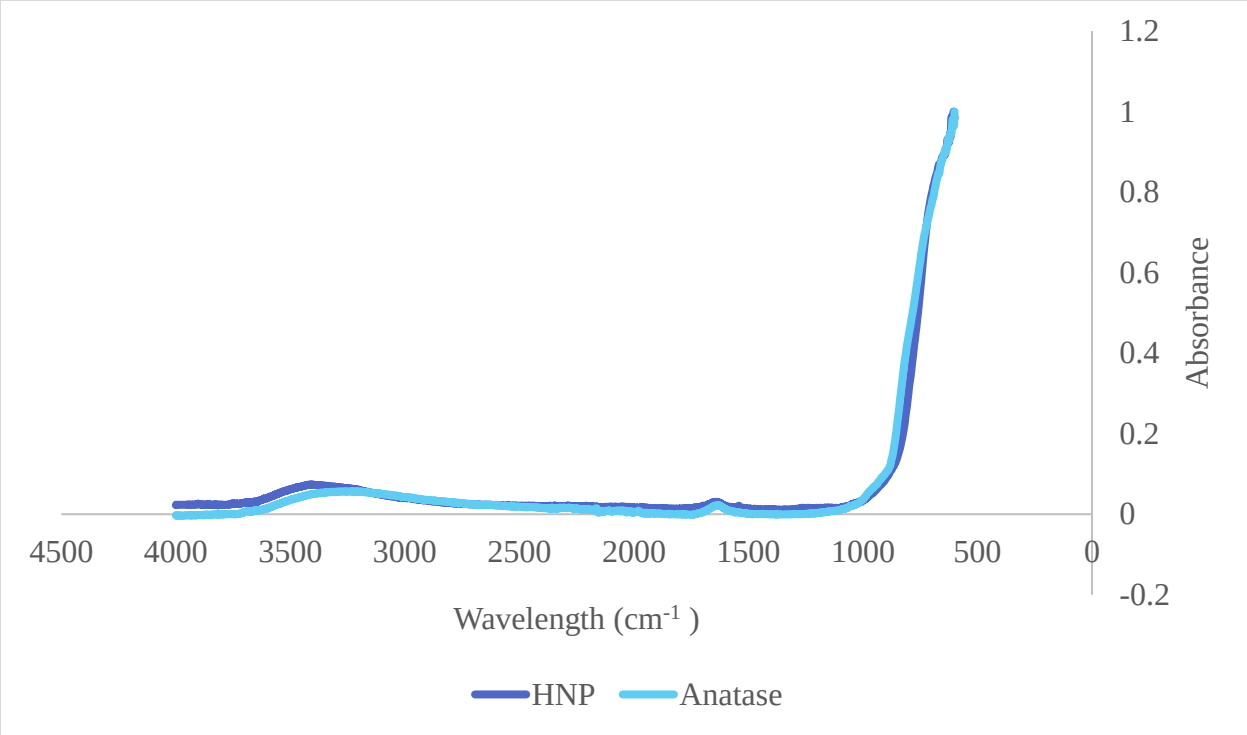


**Figure 4.** FT-IR spectra shows normalized graphs of commercial rutile (HNPs) and commercial Anatase NPs.

The TGA of HNPs, NTs, and NRs was also performed to assess the presence of water or surface -OH bonds for NTs and NRs (Figure 5). Weight loss for HNPs is 0.6%, 12% for NTs, and 13% for NRs (Table 1). The greater weight loss for the NTs and NRs portrays the release of more molecules of water from their surfaces compared to HNPs, indicating their greater hydrophilicity. The hydrophobicity of the HNPs is verified by the almost 0% weight loss.

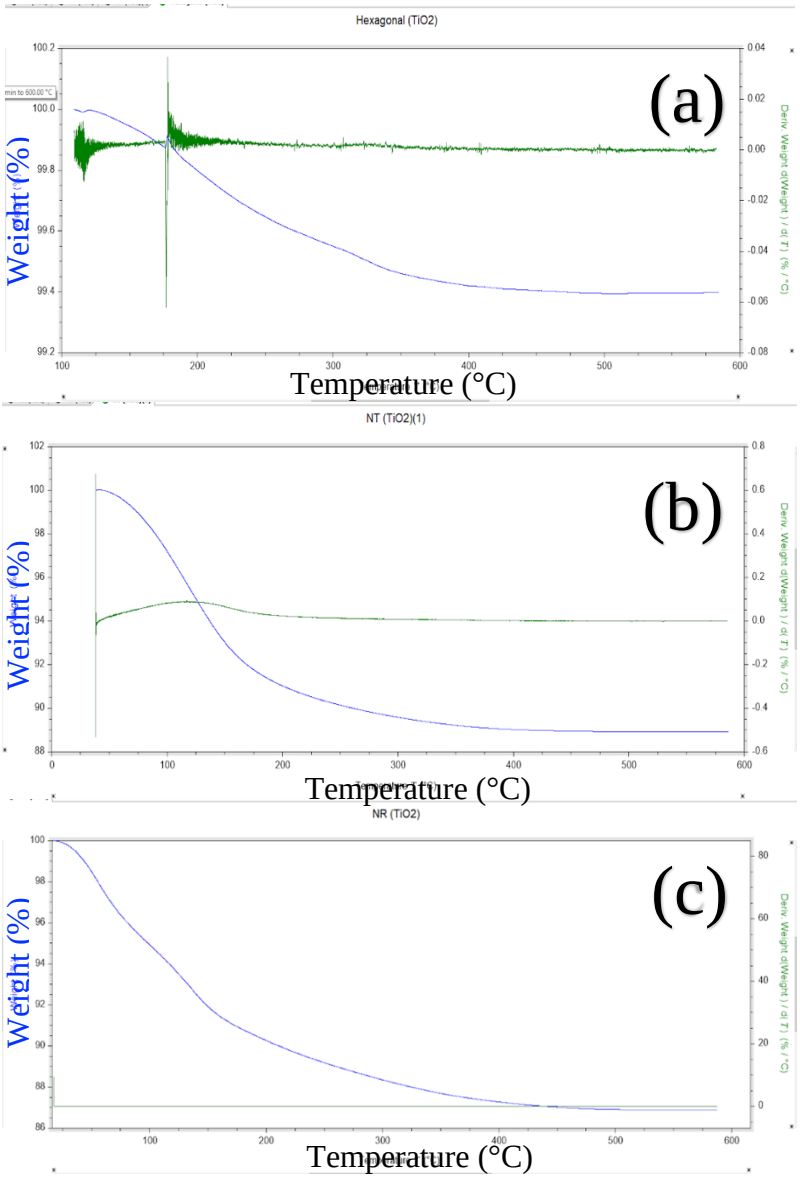


**Figure 5.** TGA analysis of (a) HNPs, (b) NTs, and (c) NRs.

**Table 1.** Weight loss % for each type of NP.

| TGA Analysis Graph | Weight Loss % |
|---|---|
| NR | 13 |
| NT | 12 |
| HNP | 0.6 |

We wanted to conduct this experiment to identify the differences between the three shapes of NPs, with regards to protein binding affinity and surface interaction. However, complications arose and protein adsorption on HNPs and NRs could not be conducted. We attempted to make the HNPs more hydrophilic through many trials, but this failed to occur. Therefore, we continued with NTs and NRs. However, we ran out of NRs and had to produce more to proceed with the experiment. We followed the same hydrothermal method as mentioned in Chen's study (J. Chen, M.S. Thesis, 2020). Nevertheless, after obtaining TEM images it was apparent that the NRs were still hollow and consistent with the structure of NTs. Thus, we continued this study utilizing NTs and Anatase.

Adsorption isotherms of Fg and HSA were obtained for Anatase and NTs, which have different shapes, and are both deemed to be hydrophilic based on their TGA profiles (Figure 6). All four graphs show an initial increase in the amount of protein adsorbed. However, HSA adsorption on both NPs decreases, while Fg adsorption plateaus for both. Therefore, Fg was successfully adsorbed by the NTs and Anatase NPs, while HSA was not adsorbed by either NP sample. This could be due to error with reproducing Chen's protocol as he obtained results showing protein adsorption for all NP samples (J. Chen, M.S. Thesis, 2020). This difference in results of the present study and Chen's study may be because HSA adsorption is too weak.

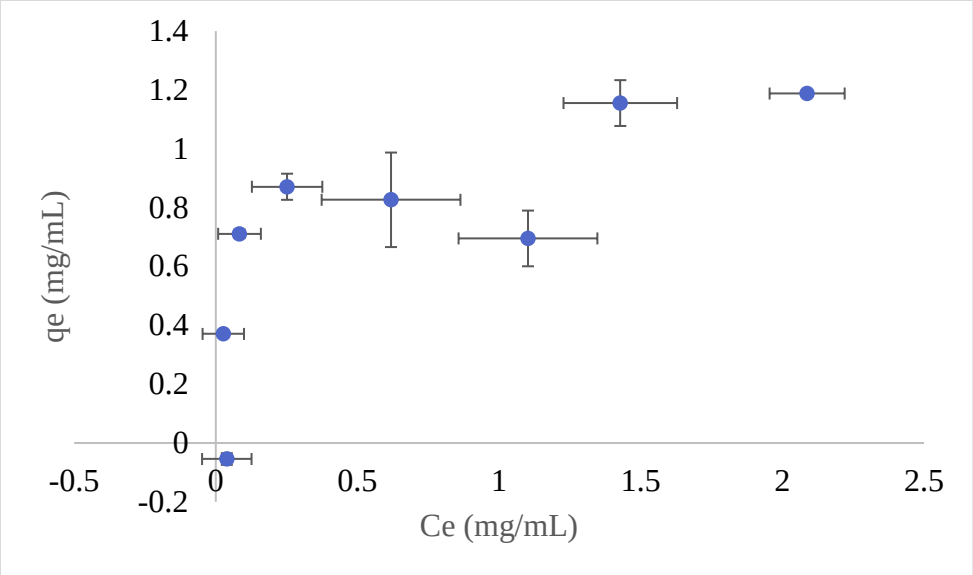


**Figure 6a.** Adsorption isotherm for Fg on Anatase NPs. Error bars show the standard deviation of measurements on three replicates of each sample.

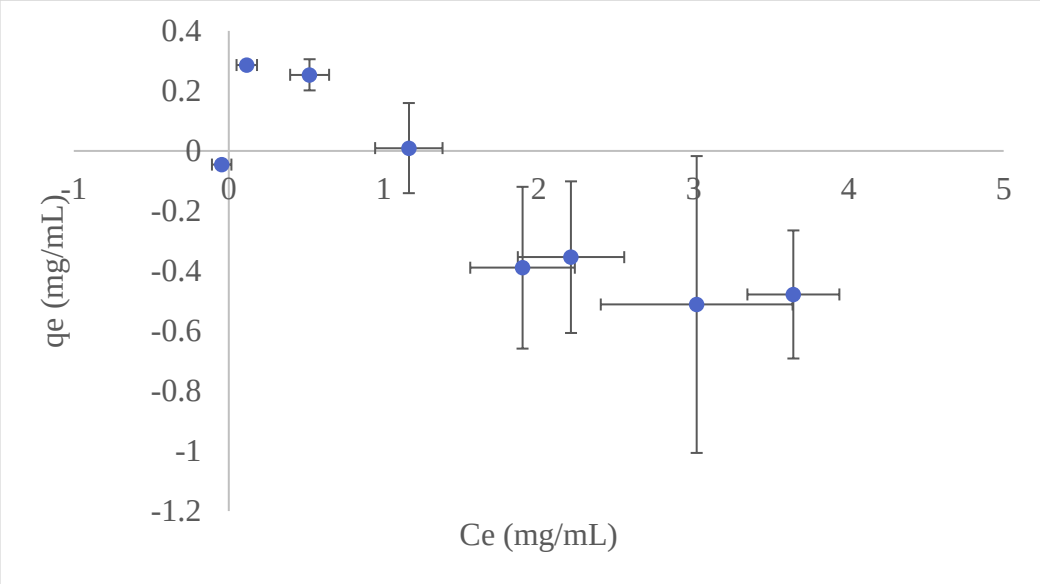


**Figure 6b.** Adsorption isotherm for HSA on Anatase NPs. Error bars show the standard deviation of measurements on three replicates of each sample.

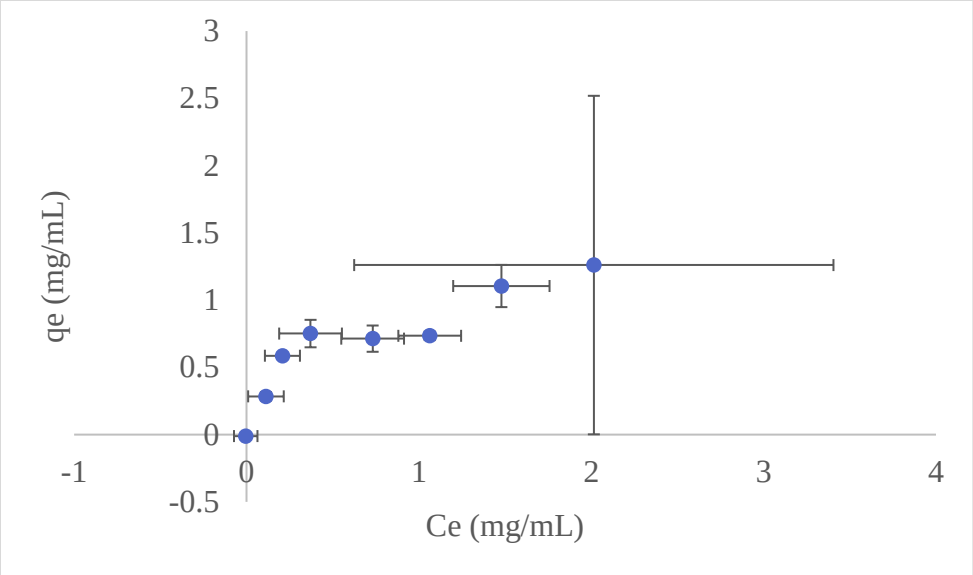


**Figure 6c.** Adsorption isotherm for Fg on NTs. Error bars show the standard deviation of measurements on three replicates of each sample.

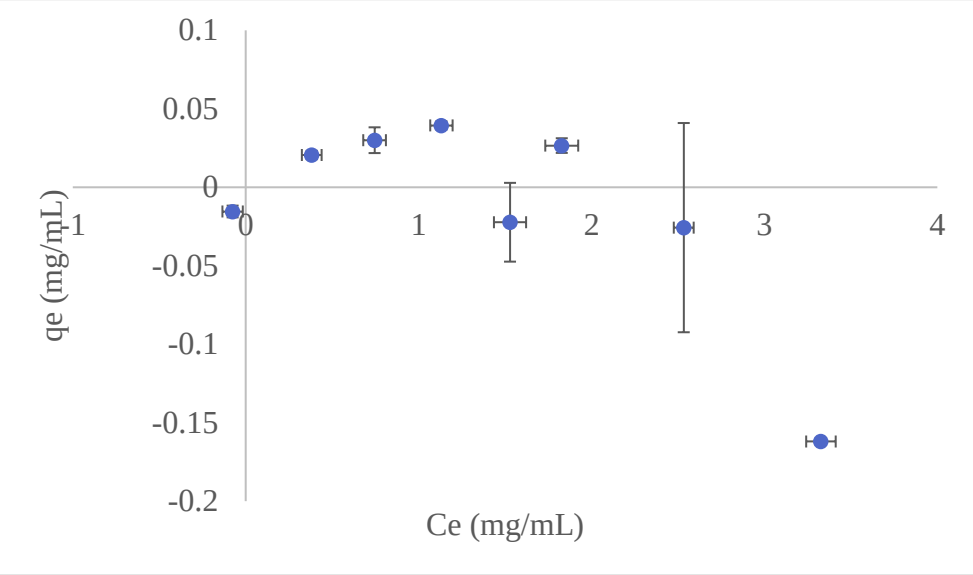


**Figure 6d.** Adsorption isotherm for HSA on NTs. Error bars show the standard deviation of measurements on three replicates of each sample.

The Langmuir model was utilized to find $K_{ads}$ and $S_t$ values from the adsorption isotherm data (Table 2). The model could not be used on the samples containing HSA because protein failed to adsorb onto the samples. Thus, $K_{ads}$ and $S_t$ values could only be found for the Fg adsorption on anatase and NTs. The binding affinity was higher for Anatase NPs than NTs consistent with the

observation that, at lower Ce values, the initial increase in amount of adsorbed protein (qe) is greater for anatase than for NTs. This can be attributed to difference in shape and perhaps in crystal structure. The maximum adsorbed concentrations are similar for both NTs and Anatase.

**Table 2.** Total surface concentration and binding affinity for Anatase and NTs.

| Protein | Type of NPs | $S_t$ ($\mu mol \cdot m^{-2}$) | $K_{ads}$ ($L \cdot mmol^{-1}$) |
|---|---|---|---|
| Fibrinogen | Anatase | 1.17068602 | 11.7013699 |
| | NT | 1.30310138 | 3.07082833 |

## Conclusion

This experiment was conducted to continue Chen's research on the effects of hydrophilicity and hydrophobicity on adsorption of blood plasma protein on $TiO_2$ NPs (J. Chen, M.S. Thesis, 2020). Adsorption of blood plasma protein affects the use of $TiO_2$ NPs for medical use when thrombosis or poor drug delivery can occur. Shape and hydrophobicity/hydrophilicity of titanium dioxide nanoparticles could alter the effects of a drug if $TiO_2$ is present in the drug delivery vehicle.

We examined HSA and Fg adsorption on Anatase NPs and NTs, which are both hydrophilic but have different shapes. HSA failed to adsorb on either anatase or NTs. Fg adsorbed onto both, consistent with previous results from the Sahai lab (J. Chen, M.S. Thesis, 2020). Fg had a higher binding affinity on anatase than on NTs. Thus, shape seems to have affected Fg adsorption. Further research in the Sahai group will focus on the effects of NP shape and hydrophobicity/hydrophilicity on protein structure where protein structure will be

determined by circular dichroism. This would give insight to effects of NPs on potential thrombogenicity of the NPs.

## References

Chen, J., Ustriyana, P., Banerjee, A., & Sahai, N. (2020). *Shape and Hydrophobicity Effects of Titanium Dioxide Nanoparticles on Blood Plasma Protein Adsorption*. (Unpublished master's thesis). The University of Akron, Akron, Ohio.

Deng, Z. J., Mortimer, G., Schiller, T., Musumeci, A., Martin, D., & Minchin, R. F. (2009). Differential plasma protein binding to metal oxide nanoparticles. *Nanotechnology*, *20*(45), 455101.

Kasuga, T., Hiramatsu, M., Hoson, A., Sekino, T., & Niihara, K. (1999). Titania nanotubes prepared by chemical processing. *Advanced materials*, *11*(15), 1307-1311.

Lundqvist, M., Sethson, I., & Jonsson, B. H. (2004). Protein adsorption onto silica nanoparticles: conformational changes depend on the particles' curvature and the protein stability. *Langmuir*, *20*(24), 10639-10647.

Murthy, S. K. (2007). Nanoparticles in modern medicine: state of the art and future challenges. *International journal of nanomedicine*, *2*(2), 129-141.

Skocaj, M., Filipic, M., Petkovic, J., & Novak, S. (2011). Titanium dioxide in our everyday life; is it safe?. *Radiology and oncology*, *45*(4), 227-247.