5-2023

Microwave Synthesis of Carbon Dot Nanoparticles

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Microwave Synthesis of Carbon Dot Nanoparticles

A Thesis

Presented to

the Faculty of the Department of Chemistry

East Tennessee State University

In Partial Fulfillment

of the Requirements for the HID Degree

Bachelor of Science in Chemistry

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by

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2022-2023

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Keywords: Carbon Dots, Nanoparticles, Microwave Method

ABSTRACT

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This study aimed to improve the known microwave method to produce carbon dot nanoparticles from ethylenediamine and citric acid. Carbon dots have recently gained much attention as they have diverse applications, such as bioimaging and drug delivery reagents as cancer theranostics. Research was focused on establishing the ideal time for the synthetic reaction to produce carbon dot nanoparticles with the microwave method. After several trials, the 16-minute trial provided the best results based on Fourier transform infrared spectroscopy, ultraviolet-visible spectroscopy, fluorescence spectroscopy, and ultraviolet exposure.

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LIST OF ABBREVIATIONS

CDs	Carbon Dots
NPs	Nanoparticles
FL	Fluorescence
UV	Ultraviolet Light
LED	Light Emitting Diode
QDs	Quantum Dots
CDs NPs	Carbon Dots Nanoparticles
FTIR	Fourier Transform Infrared Spectroscopy
UV-Vis	Ultraviolet-Visible Spectroscopy
FL-S	Fluorescence Spectroscopy
CA	Citric Acid
ED	Ethylenediamine
DI	Deionized
WL	Wavelength
ABS	Absorbance

CHAPTER 1. INTRODUCTION

Lots of people die because of cancer each day. In the United States in 2019, there were 146.2 cancer caused deaths per 100,000 standard population. This was approximately 100 deaths higher per 100,000 people than the following leading cause of death.[1] Carbon Dots (CDs) are widely studied as theranostic nanoparticles for cancer treatment and diagnosis at the same time.

Carbon Dots are nanoparticles (NPs) with sizes less than 10 nanometers[2] with unique properties, such as fluorescence (FL).[3] FL is present when CD solutions are exposed to ultraviolet light (UV) due to the size of their sp^2 orbitals and abundance of oxidized surface defects.[4] As nanoparticles with large surface areas, CDs are modified chemically to be used in many fields including cancer therapy, solar cells, light emitting diode (LED), and bioimaging. For example, CDs are used to replace metal fluorophores in luminescent solar concentrators and photovoltaic cells.[5] With their unique optical properties, CDs are being researched for LED technology. Carbon dots nanoparticles (CDs NPs) could replace current phosphors and semiconductor quantum dots (QDs) in LED technology because of CDs' FL, CDs' low cost, and CDs' low environmental impact.[6]

CDs are also used in bioimaging. Bioimaging is a growing field of medical technology that is noninvasive and gives medical professionals a view of specific biological activity.[7] Unlike fluorescent dyes that are used in bioimaging today, CDs are effective for bioimaging with their low cytotoxicity, them not suffering from narrow excitation bands, and their lack of quick photobleaching.[8] In bioimaging, CDs are used to identify biomarkers on cell membranes, biomarkers in the cytoplasm of cells, and as a reagent to help diagnose the type, size, and position of tumors.[9] Yang et al. reported that microwave synthesized CDs showed an ability to identify cancer cells with overexpressed folate receptors. In their paper, the overexpressed folate

receptors are common in certain types of cancer.[10] Cell membrane protein CD133 has been recognized as a useful biomarker to identify tumor initiating cells. In a study, anti-CD133 monoclonal antibodies were attached to NPs to find tumor initiating cells.[11]

CDs have been researched as a cancer drug delivery agent. Doxorubicin is a drug that has proven viable for eliminating cancer cells.[12] Researchers were able to successfully attach folic acid and Doxorubicin to CDs that were synthesized via microwave method.[9] As more research is being conducted on CDs NPs and cancer, using CDs to fight cancer cells in a clinical setting should be viable in the future. Current clinical chemotherapies to treat cancer are not specific for cancer cells.[13] An effective cancer therapy should only terminate cancer cells. Targeted therapy looks to complete this by targeting cancer cells with a ligand before elimination of cancer cells with a drug.

With these applications mentioned, it is important to study the synthetic techniques to prepare carbon dots. The two approaches for synthesizing CDs are the bottom-up approach and the top-down approach. A bottom-up approach synthesizes CDs by adding carbon molecules together to form CDs, and a top-down approach synthesizes CDs by reducing a carbon source into CDs. Examples of bottom-up techniques for synthesizing CDs include hydrothermal method, solvothermal method, and microwave method. Examples of top-down synthetic techniques for synthesizing CDs include laser ablation, electrochemical, and arc discharge.[14]

The scheme utilized in this research was a bottom-up microwave method. The chemicals were mixed in a beaker, with equal molar amounts, and heated in the microwave. In the microwave method, the reaction relies on interactions between the electromagnetic waves from the microwave and the electric dipoles of the molecules. Multiple carbon sources can be used to synthesize CDs via microwave method including carbon sources found in the kitchen and nature.

The microwave method for CDs is considered a green synthesis process.[15] The largest pro of microwave synthesis is the short time of synthesis. Time can be saved, and more reactions can be completed when synthesizing CDs with microwave method. Problems with using a microwave for synthesis include the CDs having a large size distribution and isolating CDs after the reaction. The microwave method to synthesize CDs is simple, environmentally conscious, and time saving.[16] Microwave method should be considered a viable technique to synthesize CDs. In Yang et al.'s research, they obtained a quantum yield of 25% on their microwave synthesized CDs.[10] Another group, Zhai et al., synthesized CDs microwave method with citric acid (CA) and various amines. Their best trial was with a reaction between CA and ethylenediamine (ED). They recommended a reaction time that was long enough for complete carbonization.[17]

Plan for this research was to complete microwave syntheses of CDs to discover the ideal time for heating the reaction. The procedure used was guided by the literature.[18] Reaction was conducted with a microwave, purification was conducted by dialysis, lyophilization was conducted using a freeze dryer, and characterization was conducted with spectroscopy. The independent variable for the research was the time of the reaction in the microwave. The goal of this research was to find the best time for synthesizing CDs with functional groups, absorbance, and FL. This research utilized Fourier transform infrared spectroscopy (FTIR) to confirm the functional groups of carboxylic acid and amine. Ultraviolet-visible spectroscopy (UV-Vis) was utilized to confirm they were CDs and to quantitate their absorbance. Fluorescence spectroscopy (FL-S) was utilized to confirm and quantitate the FL of the CDs.

Characterization is an important component of this research because CDs do not have a specific organic structure or chemical formula. CDs are comprised of a central core of sp^2 and sp^3 carbon atoms that form lattices with functional groups attached to the surface.

Characterization techniques for CDs include microscopy, spectrometry, spectroscopy, and diffraction.[19] Spectroscopic characterization for CDs for this research included FTIR, UV-Vis, and FL-S. UV was used to confirm products' FL. Characterization solves the challenge of identifying CDs.

CHAPTER 2. EXPERIMENTAL

Materials and Instrumentals

Microwave, dialysis tubing (3,500 MWCO), Shimadzu IR-Prestige FTIR spectrometer, Shimadzu Pharma-Spec UV-1700 UV-Vis spectrometer, and Jobin Yvon Inc. FluoroMax-3 FL spectrometer. The chemical reagents used were deionized (DI) water, dry ice, citric acid, and ethylenediamine.

Methods

In a 400 mL beaker, 5.000 g of solid citric acid (0.0260 mol) was dissolved in 50.0 mL of deionized water. And then, 1.740 mL of ethylenediamine (0.0260 mol) was added into the solution.

The solution was stirred and placed in the microwave with 625 watts. The watch glass was placed on top of the beaker. The solution concentrations and conditions were kept the same for the trials except for the time of the reactions. The reaction was run for 4 minutes, 8 minutes, 12 minutes, 16 minutes, 20 minutes, and 24 minutes (T-4, T-8, T-12, T-16, T-20 and T-24). The product was stored in the fridge overnight covered in parafilm. On the second day, 30 mL of DI water was added to dissolve the product again.

The product solution was placed in water bath for dialysis for 24 hours. Once dialysis was done, the resulting liquid was transferred into a vial and stored in the refrigerator.

The lyophilization was completed with a freeze-drying machine. The purified product solutions were transferred into the fast-freeze flasks and stored in the freezer for 40 minutes. After 40 minutes, the frozen samples were attached to the freeze dryer for vacuum drying overnight.

The final color and yield of product obtained depended on the time of the reaction. The solid product was redissolved in DI water to prepare 0.01 mg/mL solutions for spectroscopy. The UV-Vis and FL-S were carried out for quantitative absorbance and quantitative fluorescence data respectively. The solid product was examined with FTIR for functional groups.

CHAPTER 3. RESULTS AND DISCUSSION

The reaction was between citric acid and ethylenediamine in a microwave. The time of reaction served for the independent variable. *Tables 1a and 1b* summarize the results. The equation used for product yield was the mass of final product divided by the mass of citric acid used, five grams, times 100.[18]

Table 1a: Physical Results

Reaction Time	**4 Minutes** (T-4)	**8 Minutes** (T-8)	**12 Minutes** (T-12)	(T-16 (#1)) **16 Minutes** (T-16 (#2))	
State of Matter After Reaction	Normal Liquid	Viscous Liquid	Viscous Liquid and Solid with Air Pockets	Solid with Air Pockets	
Color After Reaction	Yellow	Green-Yellow	Red-Brown with Green-Yellow	Red-Brown with some Green-Yellow	
Final Product Mass (g)	0.293	0.448	0.673	0.945	0.635
State of Final Product	Solid	Solid	Solid	Solid	Solid
Yield (Wt.%)	5.86	8.96	13.5	18.9	12.7

Table 1b: Physical Results

Reaction Time	**20 Minutes** (T-20)	(T-24 (#1)) **24 Minutes** (T-24 (#2))	
State of Matter After Reaction	Solid with Air Pockets	Solid with Air Pockets	
Color After Reaction	Red-Brown	Red-Brown	
Final Product Mass (g)	0.788	N.A.	1.114
State of Final Product	Solid	Liquid	Solid
Yield (Wt.%)	15.8	N.A.	22.3

The longer reactions yielded the color that was desired, a reddish-brown indicated by literature.[18] This color was not present in the T-4 and T-8 post-reaction samples. There was no reported yield for T-24 (#1) in *Table 1b*. T-24 (#1)'s final product was a liquid because too much reaction product solution was freeze dried at once. This solid clump melted during transportation of the sample and dissolved all the carbon dot product making a liquid final product for T-24 (#1). A similar clump was seen with T-24 (#2)'s product after lyophilization. Though the frozen clump of T-24 (#2)'s was removed, away from the rest of its product.

Based on the results, the reaction between citric acid and ethylenediamine needed to be run for more time than 4 or 8 minutes to produce the red-brown color mentioned. That color may indicate the functional groups necessary for future drug and targeting agents' attachment to the carbon dots, the carboxylic acid and the amine groups.[13] Those two groups were proven with the FTIR spectrums. The FTIR results were interpreted based on an FTIR table.[20]

Table 2: FTIR Results

Product	Absorption (cm^{-1})	Functional Group	T-4	T-8	T-12	T-16 (#1)	T-16 (#2)	T-20	T-24 (#1)	T-24 (#2)
O–H Stretching	3,700 - 3,200	–OH or Water	No	No	No	Yes	No	No	No	No
O–H Stretching	3,600 - 2,500 (Weak or Medium)	Carboxylic Acid	Yes (Strong)	Yes (Strong)	Yes (Weak)	Yes	Yes (Very Weak)	Yes	Yes	Yes
N–H Stretching	3,300	$–NH_2$ or –NH	No	No	Yes	Yes	Yes	Yes	No	Yes
C=O Stretching	Around 1,687	Carboxylic Acid	Yes (Weak)	Yes (Weak)	Yes	Yes	Yes	Yes	1,705	Yes
N–H Bending	Around 1,650	Amine	No	No	Yes	Yes	Yes	Yes	Yes	Yes
N–O Stretching	1,550	Nitro Compound	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes

The O–H stretch at 3,600 cm^{-1} was seen on T-16 (#1) only. The O–H peak may indicate T-16 (#1) was not dry enough for FTIR. The typical O–H stretch of the carboxylic acid was seen between 3,600 cm^{-1} and 2,500 cm^{-1} for all the products. N–H stretching was seen at 3,300 cm^{-1} for all spectrums other than T-4, T-8, and T-24 (#1), or they were covered by carboxylic acid

peak. There were strong peaks seen around 1,687 cm^{-1}, representing the C=O stretch of a carboxylic acid. There were peaks for amine seen around 1,650 cm^{-1} in each product except T-4 and T-8. The peak at 1,550 cm^{-1} showed a nitro group. Therefore, the samples T-12, T-16, and T-20 were the ones to have both –COOH and $–NH_2$ groups in their FTIR spectra.

UV-Vis spectroscopy was used to quantify the absorbance of the products. In a separate study, UV-Vis spectroscopy was conducted on CDs NPs, and the carbon dots in that study absorbed the most at a wavelength of 341 nanometers.[21] This suggests that carbon dots were present in the solutions for spectroscopy here because each UV-Vis spectrum exhibited its highest absorbance at a wavelength close to an x-axis value of 341 nanometers. Two tests were run for each product indicated by Series 1 and Series 2. A table summarizing the UV-Vis results follows on the next page.

Table 3: UV-Vis Results

Product	T-4	T-8	T-12	T-16 (#1)	T-16 (#2)	T-20	T-24 (#1)	T-24 (#2)
Series 1 x-axis Apex WL (nm)	344.5	340.5	346.5	343.5	343.5	346.5	341.5	346.0
Series 1 y-axis Apex ABS	0.122	0.154	0.071	0.662	0.640	0.078	0.005	0.044
Series 2 x-axis Apex WL (nm)	347.5	342.5	348.0	342.5	343.5	348.5	341.5	345.0
Series 2 y-axis Apex ABS	0.123	0.154	0.072	0.657	0.640	0.079	0.005	0.044
Average x-axis Apex WL (nm)	346.0	341.5	347.3	343.0	343.5	347.5	341.5	345.5
Average y-axis Apex ABS	0.123	0.154	0.072	0.660	0.640	0.079	0.005	0.044

All the UV-Vis spectra show the typical peak at around 350 nanometers, which is the n to pi star transition from carbonyl bonds.[21] The intensity of peak increases from 0.123 to around 0.660 for the trial of T-16 (#1) and to around 0.640 for the trial of T-16 (#2). Then it is decreased to lower intensity after reaction time is over 20 minutes. According to the UV-Vis data, the 16-minute trial gave the highest absorbance among all the products. The key takeaway from UV-Vis data is that the samples consistently showed their highest absorption values at a wavelength close to an x-axis value of 341 nanometers, representing the presence of carbon dot nanoparticles with similar UV-Vis results to Lin et al.[21]

Fluorescence spectroscopy was conducted on each sample, using ten different excitation wavelengths. In a separate study that conducted FL-S on CDs NPs, the 360-nanometer excitation plot peaked at 450 nanometers on the x-axis.[22] Similarly, every FL-S plot for the 360-nanometer excitations seen with the results for the current microwave study here apexed at an x-axis value of 450 nanometers. This shows likeness between literature FL-S results for carbon dots and the FL-S results of this study that follow.

Table 4: FL-S Results

Product	T-4	T-8	T-12	T-16 (#1)	T-16 (#2)	T-20	T-24 (#1)	T-24 (#2)
Highest Fluorescence Excitation WL (nm)	360	360	360	360	360	360	360	360
360 nm x-axis Apex WL (nm)	450	450	450	450	450	450	450	450
360 nm y-axis Apex FL	292,126	263,040	343,544	1,359,492	1,284,385	224,948	8,086	133,214

All spectrums' fluorescence values peaked using an excitation wavelength of 360 nanometers. The excitation wavelengths ranged from 300 nm to 390 nm with increments of ten nanometers. The changing values of the excitation wavelength had a strong effect on the fluorescence value of the samples. When an excitation wavelength of 300 nanometers was used, the fluorescence value was low. When an excitation wavelength of 360 nanometers was used, the sample showed its highest fluorescence. All the samples showed fluorescence. For the samples, which emit highest fluorescence at 450 nanometers wavelength, among them, T-16 (#1) yielded the highest intensity. It appeared 16 minutes is the most promising time for a high fluorescence. To conclude, the highest quantitative fluorescence value for the carbon dots were exhibited when

contacted by a light with a wavelength of 360 nanometers and at an emission wavelength of 450 nanometers.

Images 1a-1h: Product Solutions Exposed to UV-Light, Top Row from Left to Right: T-4, T-8, T-12, and T-16 (#1) Bottom Row from Left to Right: T-16 (#2), T-20, T-24 (#1), and T-24 (#2)

In the above images, fluorescence was seen when 0.01 milligram per milliliter solutions of CD NPs were exposed to UV-light. Despite the dilute concentration, the product solutions fluoresced blue when exposed to ultraviolet light. This agrees with the results of FL-S that peaked at an x-axis value of 450 nanometers in that the product solutions fluoresced in the blue

portion of the visible light spectrum quantitatively and qualitatively. This correlates the FL-S results with the UV-light results. The samples T-16 (#1) and T-16 (#2) showed the highest fluorescence quantitatively and qualitatively.

CHAPTER 4. CONCLUSION AND FUTURE WORK

In the research, the microwave method with different times were used to synthesize carbon dots. The eight trials were characterized by FTIR, UV-Vis, and Fluorometry. According to the results, it is believed that the reaction time should run around 16 minutes.

Starting from four-minute trial, all the samples contained the two desired functional groups, carboxylic acid and amine group, according to FTIR. Products absorbing the most around 341 nanometers with UV-Vis hinted that carbon dots were present. Products fluorescing quantitatively and qualitatively confirmed that carbon dots were present. The 16-minute trial showed the highest fluorescence quantitatively and qualitatively.

There are options for future work with the microwave reaction. The power level of the microwave could be adjusted, and a hydrothermal vessel could be used instead of a beaker. Combined with purification, the best yield% will be studied. The method will be used to give big quantities of carbon dots for further applications in cancer theranostic research.

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APPENDICES

Appendix A: FTIR Spectra

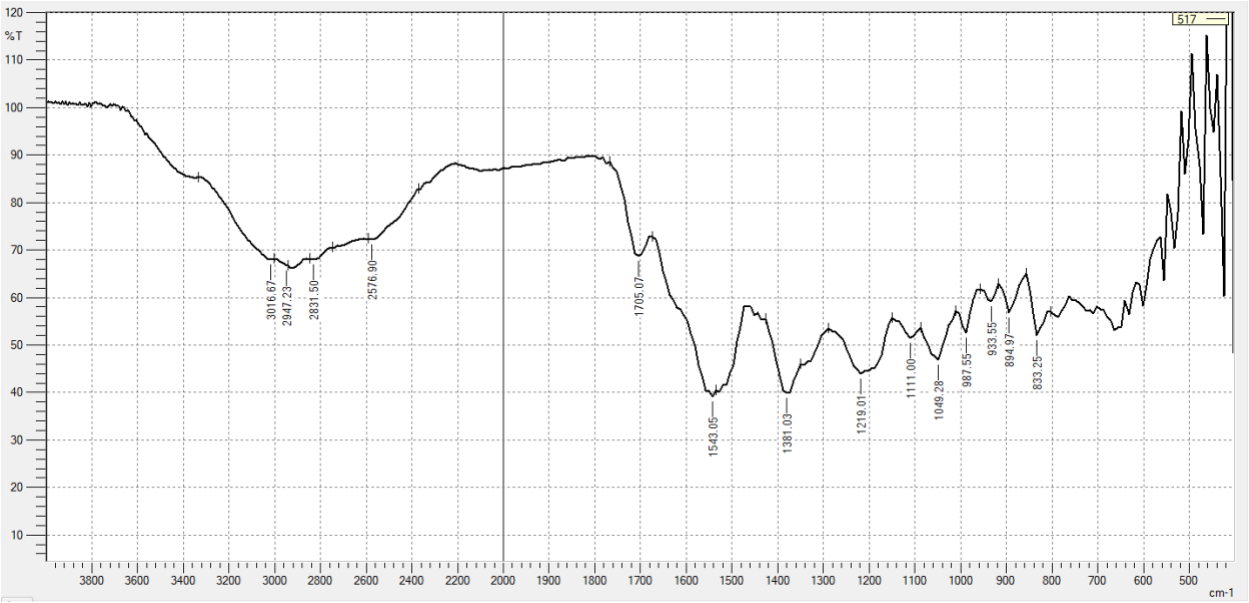

Image 2a: FTIR Spectrum of T-4

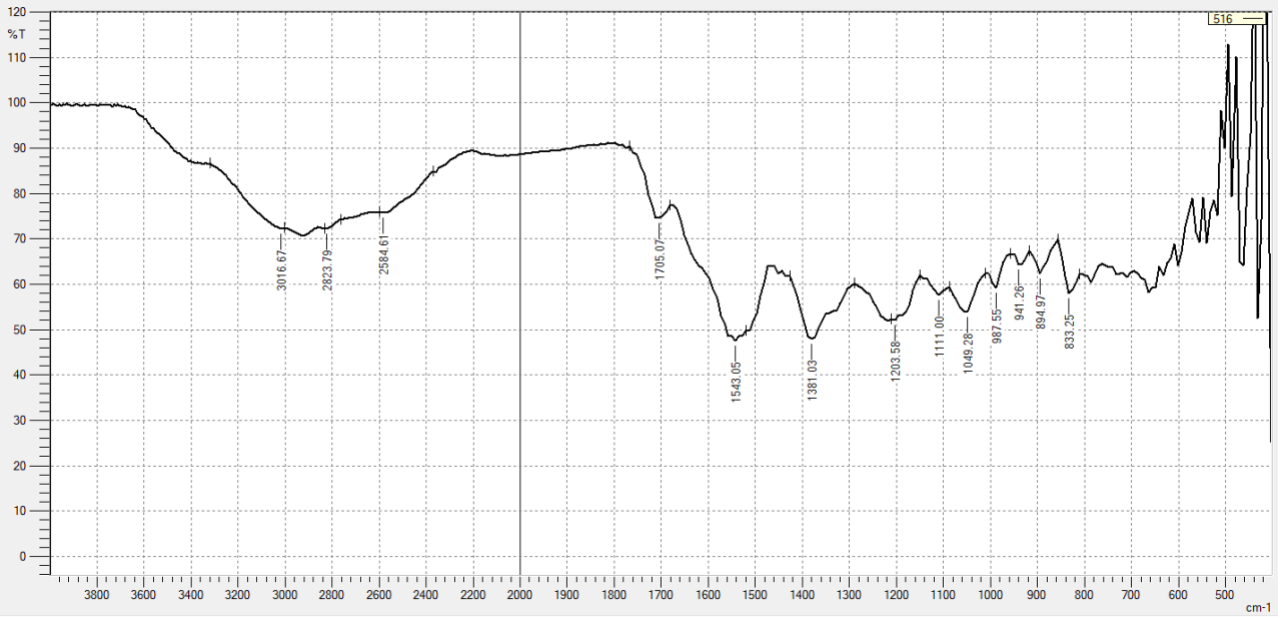

Image 2b: FTIR Spectrum of T-8

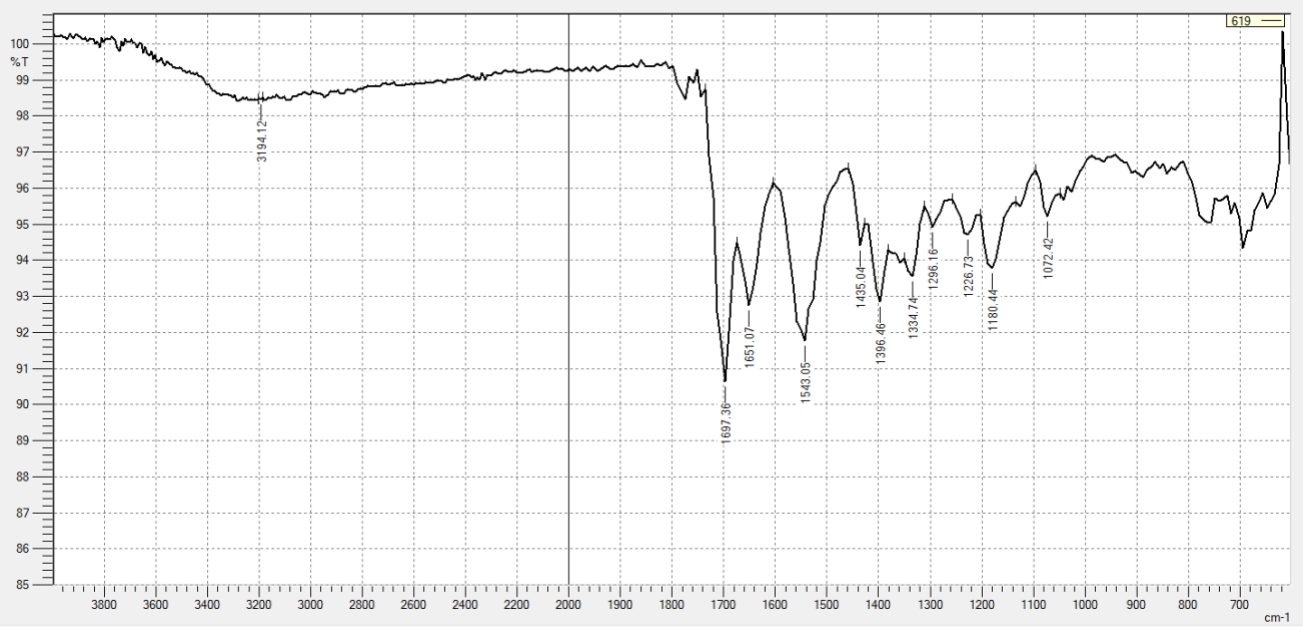

Image 2c: FTIR Spectrum of T-12

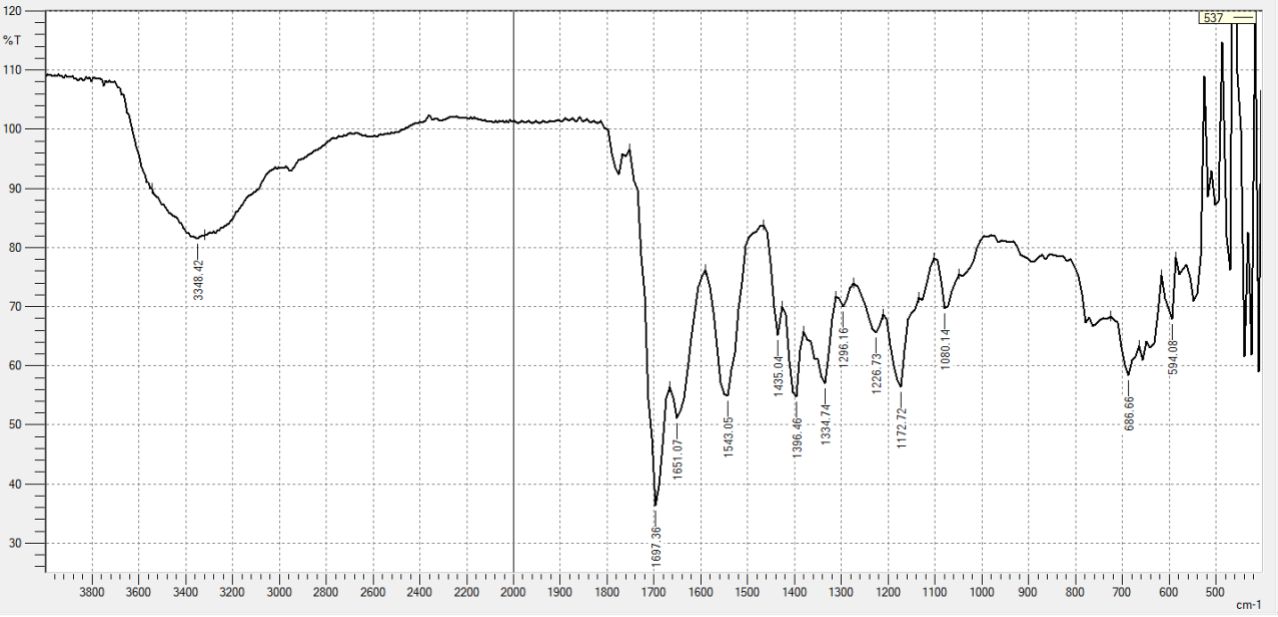

Image 2d: FTIR Spectrum of T-16 (#1)

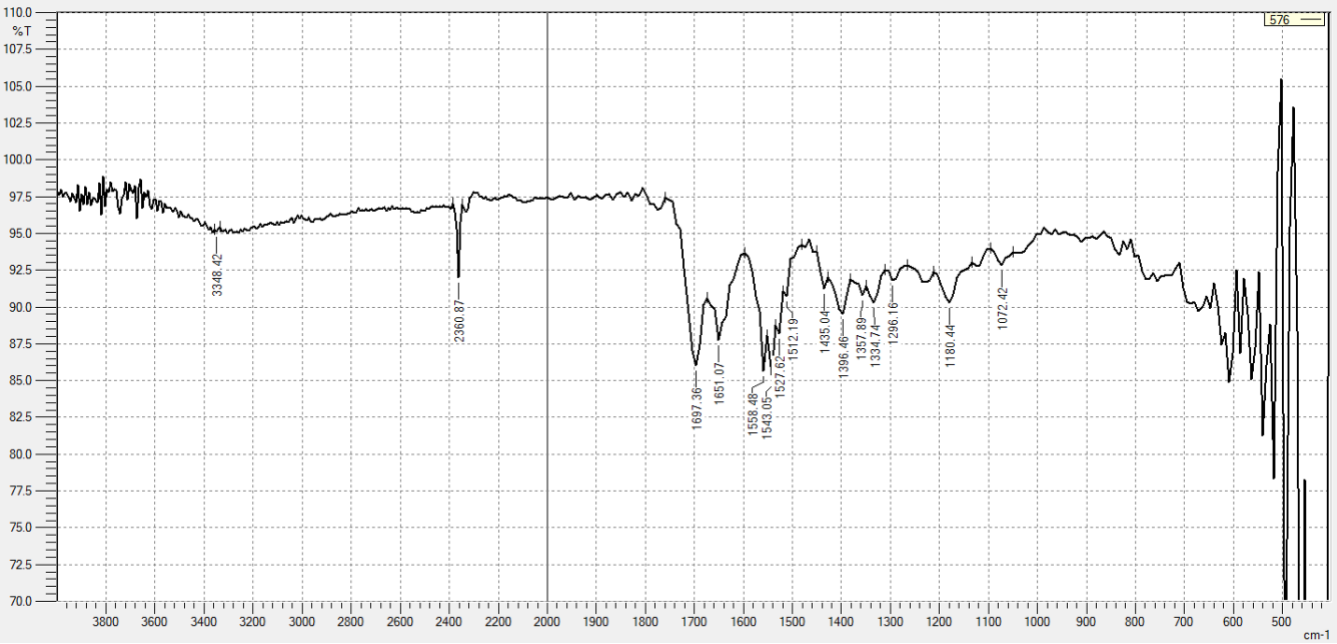

Image 2e: FTIR Spectrum of T-16 (#2)

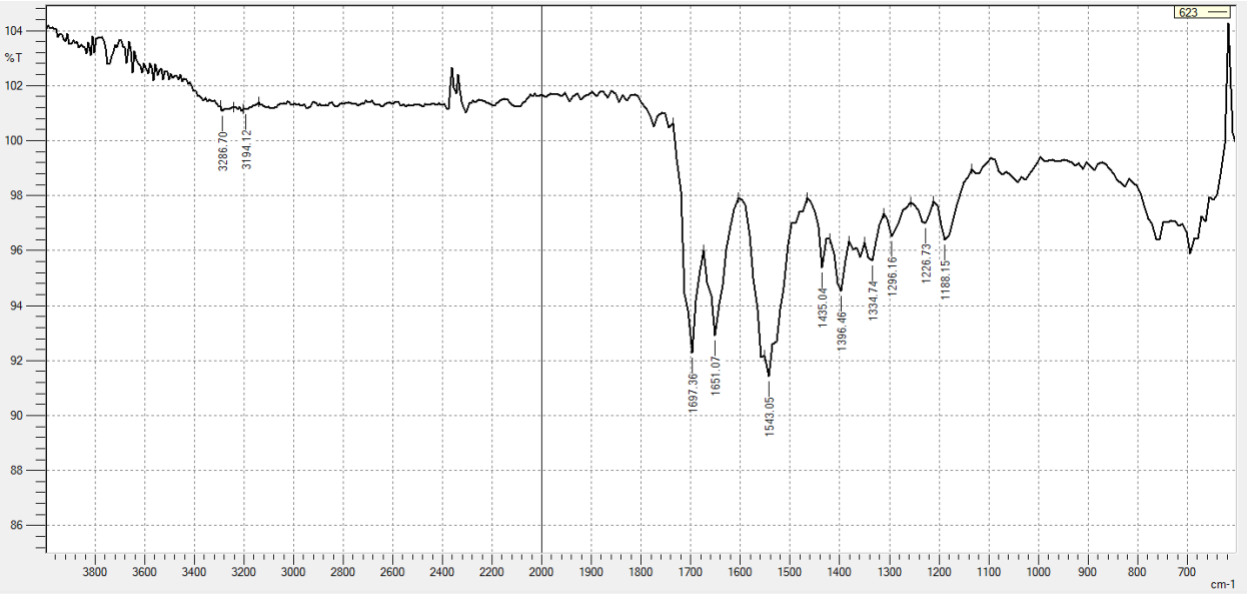

Image 2f: FTIR Spectrum of T-20

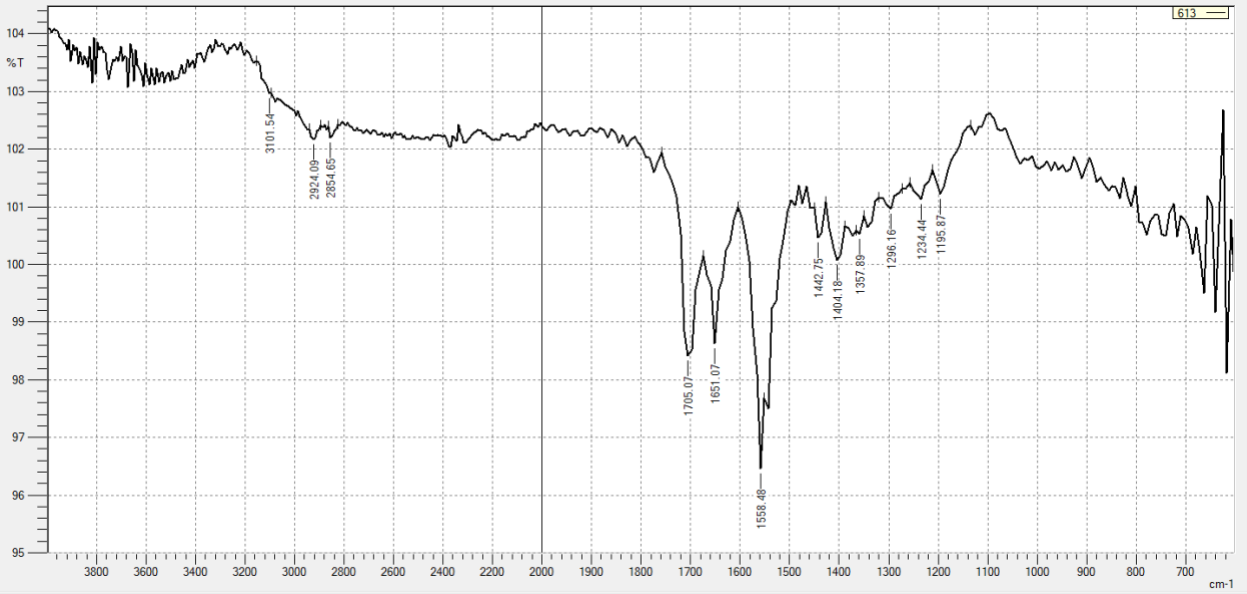

Image 2g: FTIR Spectrum of T-24 (#1)

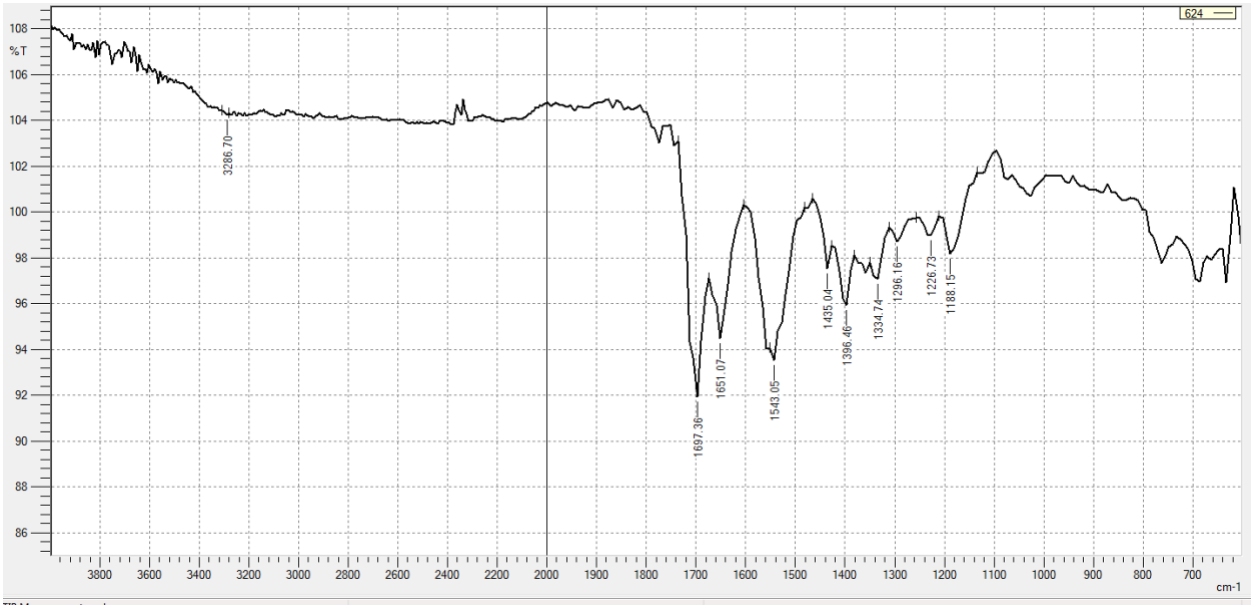

Image 2h: FTIR Spectrum of T-24 (#2)

Appendix B: UV-Vis Spectra

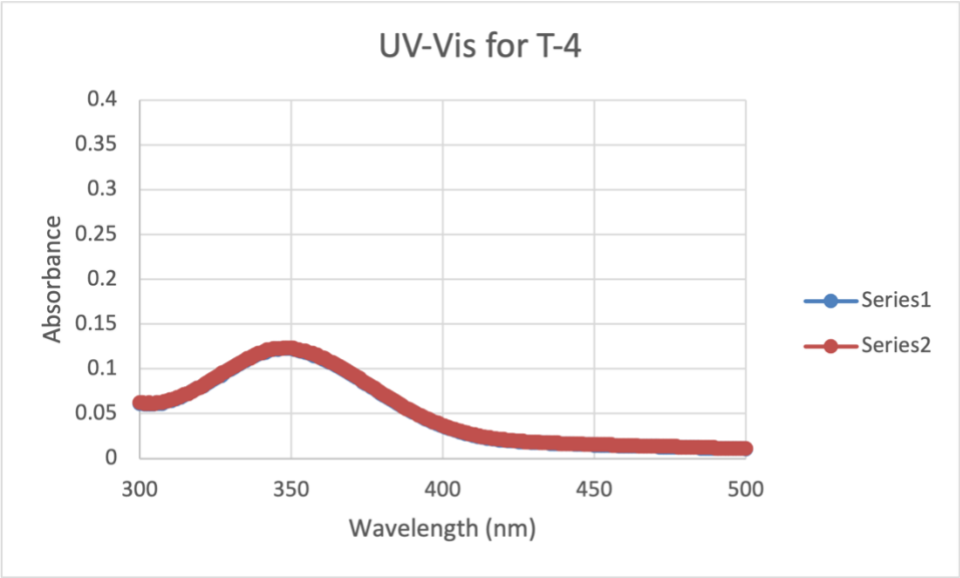

Image 3a: UV-Vis Spectrum of T-4

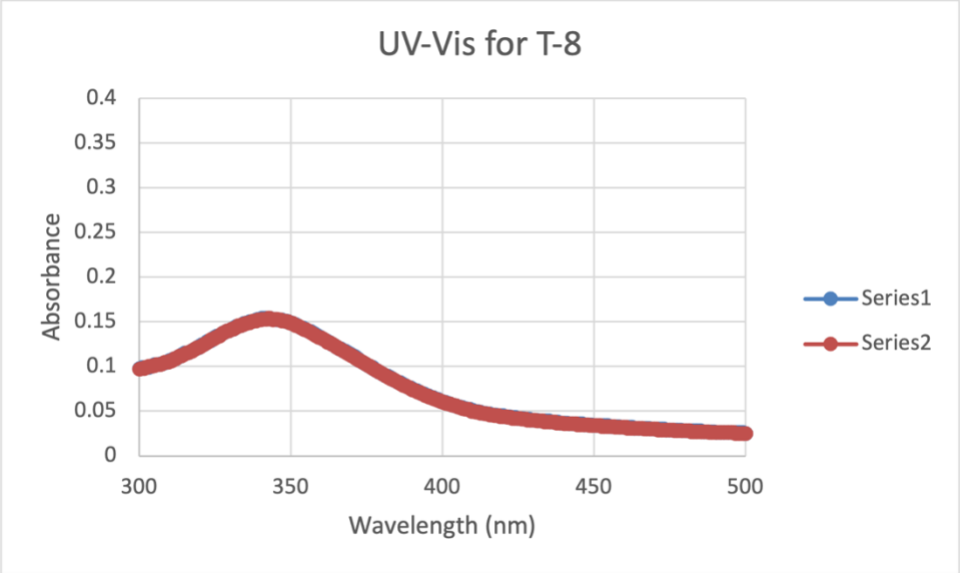

Image 3b: UV-Vis Spectrum of T-8

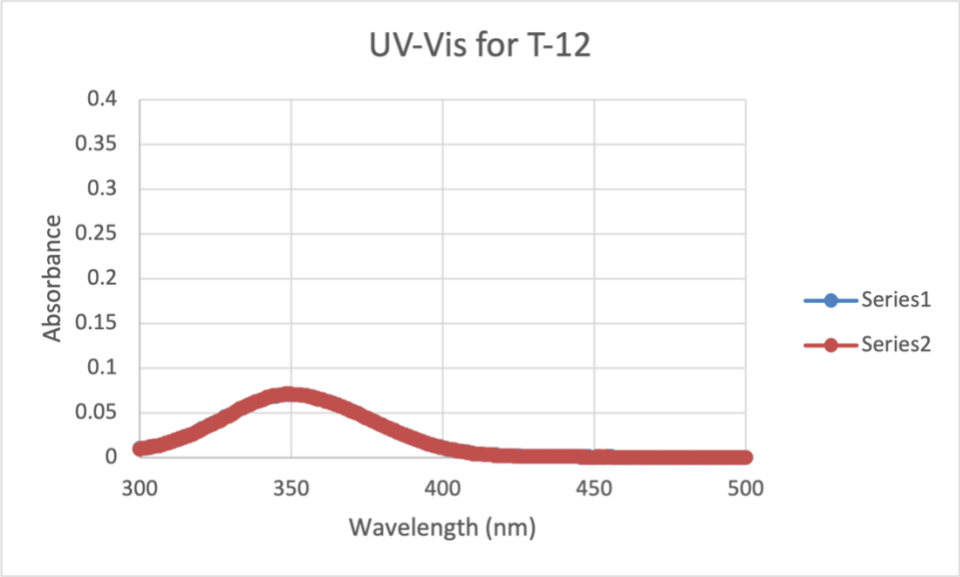

Image 3c: UV-Vis Spectrum of T-12

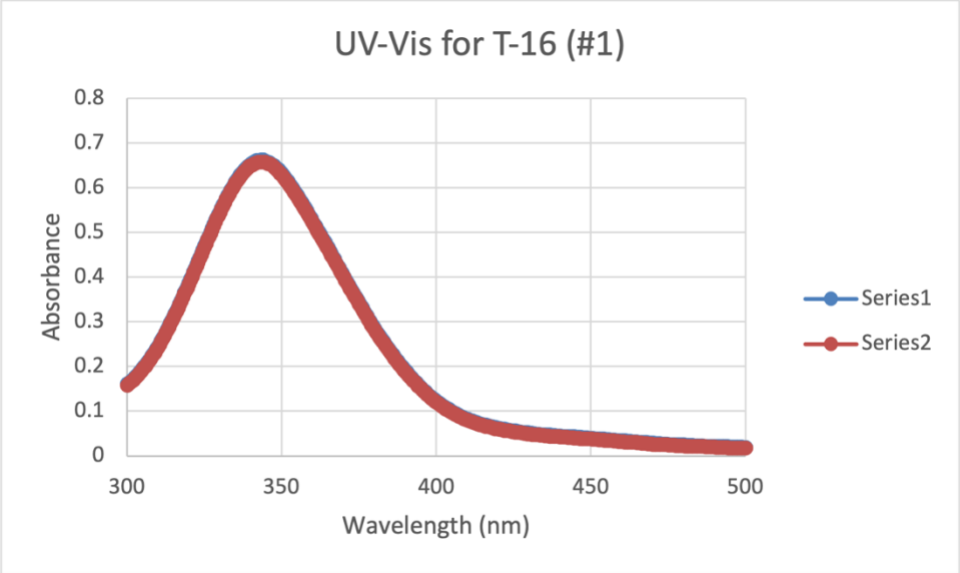

Image 3d: UV-Vis Spectrum for T-16 (#1)

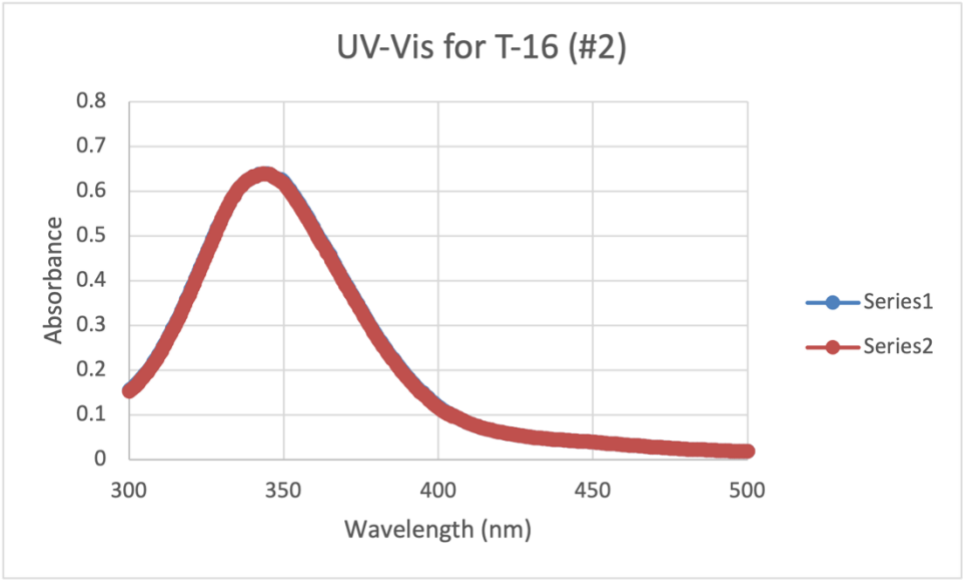

Image 3e: UV-Vis Spectrum for T-16 (#2)

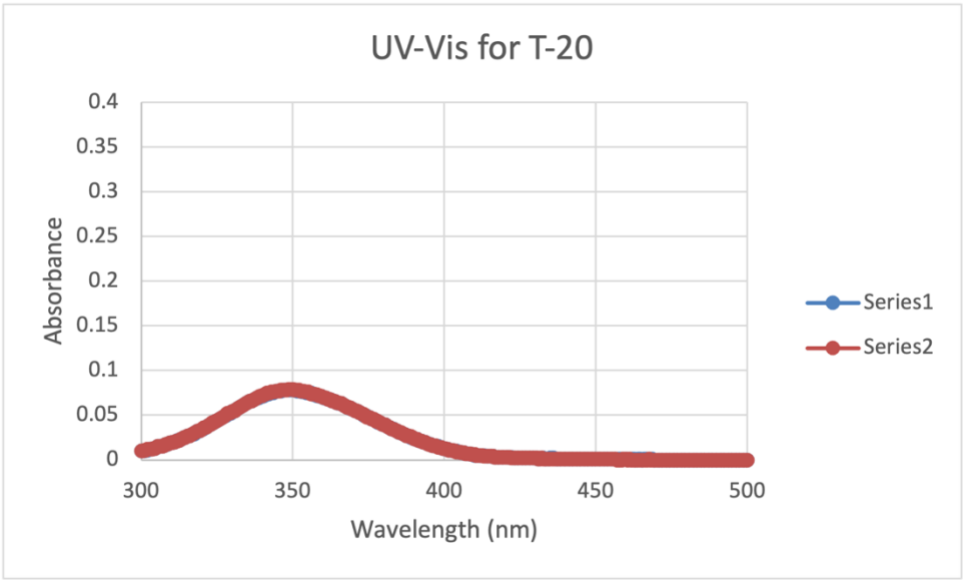

Image 3f: UV-Vis Spectrum for T-20

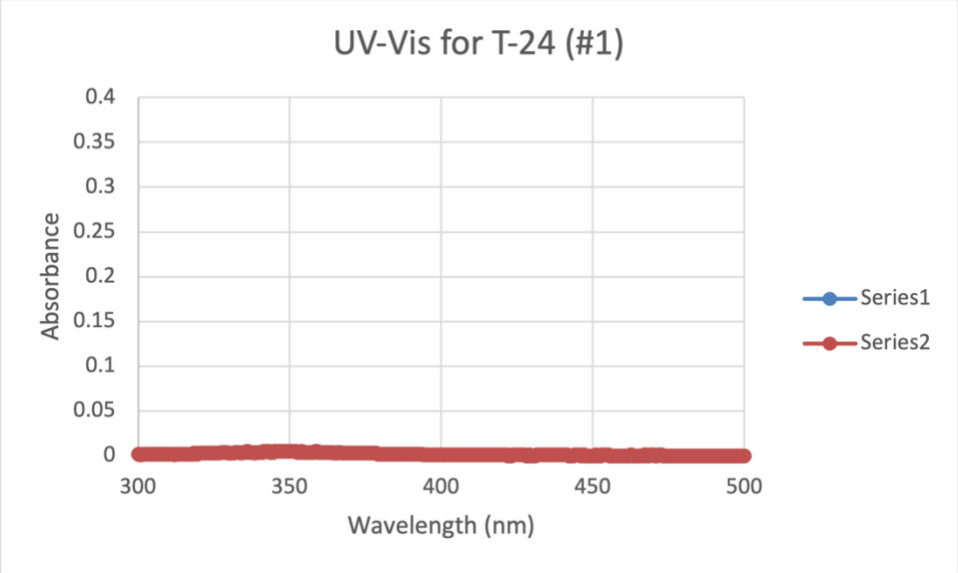

Image 3g: UV-Vis Spectrum for T-24 (#1)

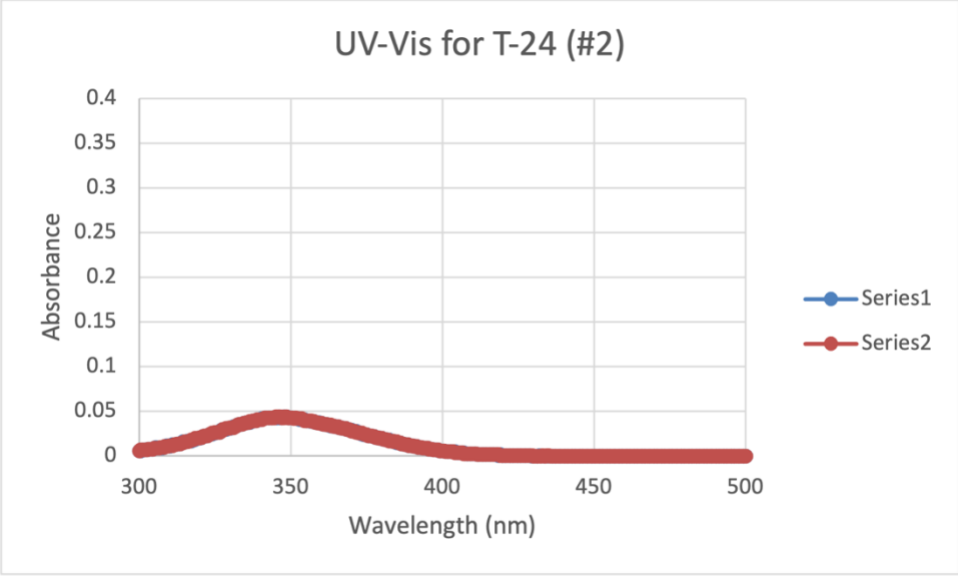

Image 3h: UV-Vis Spectrum of T-24 (#2)

Appendix C: FL-S Spectra

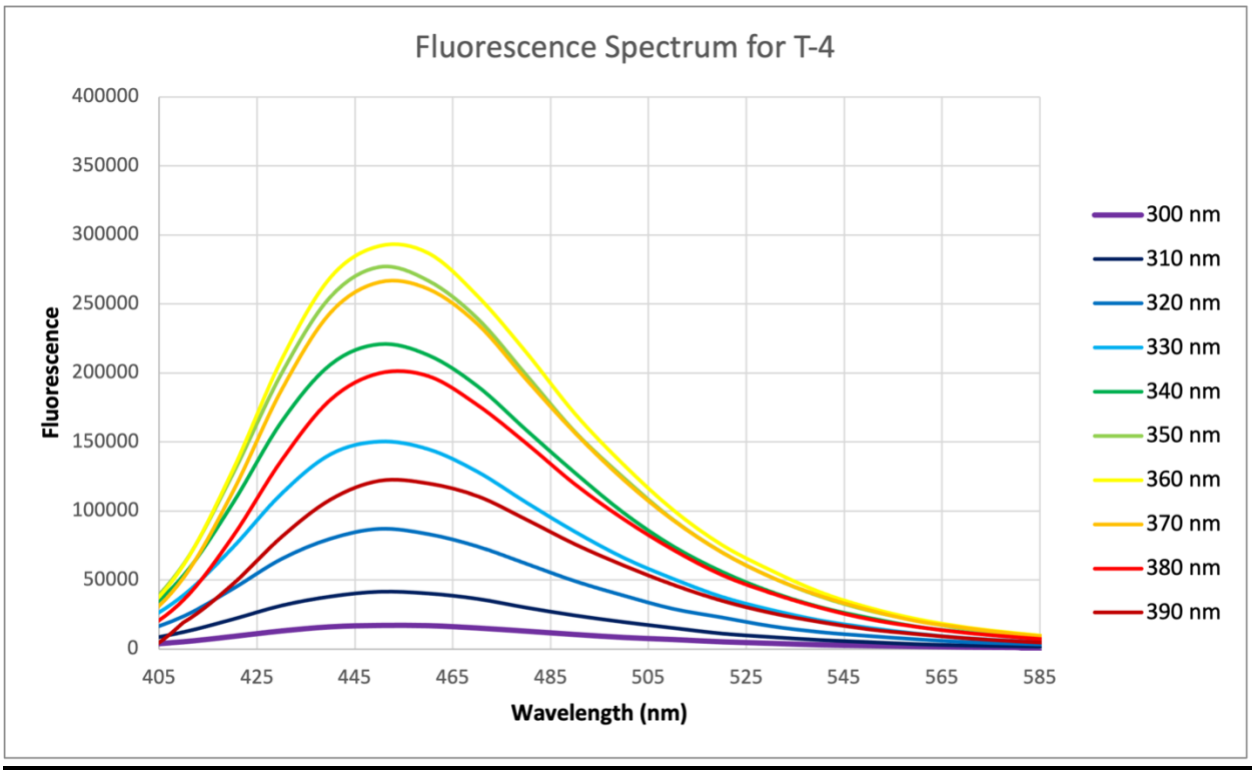

Image 4a: Fluorescence Spectrum for T-4

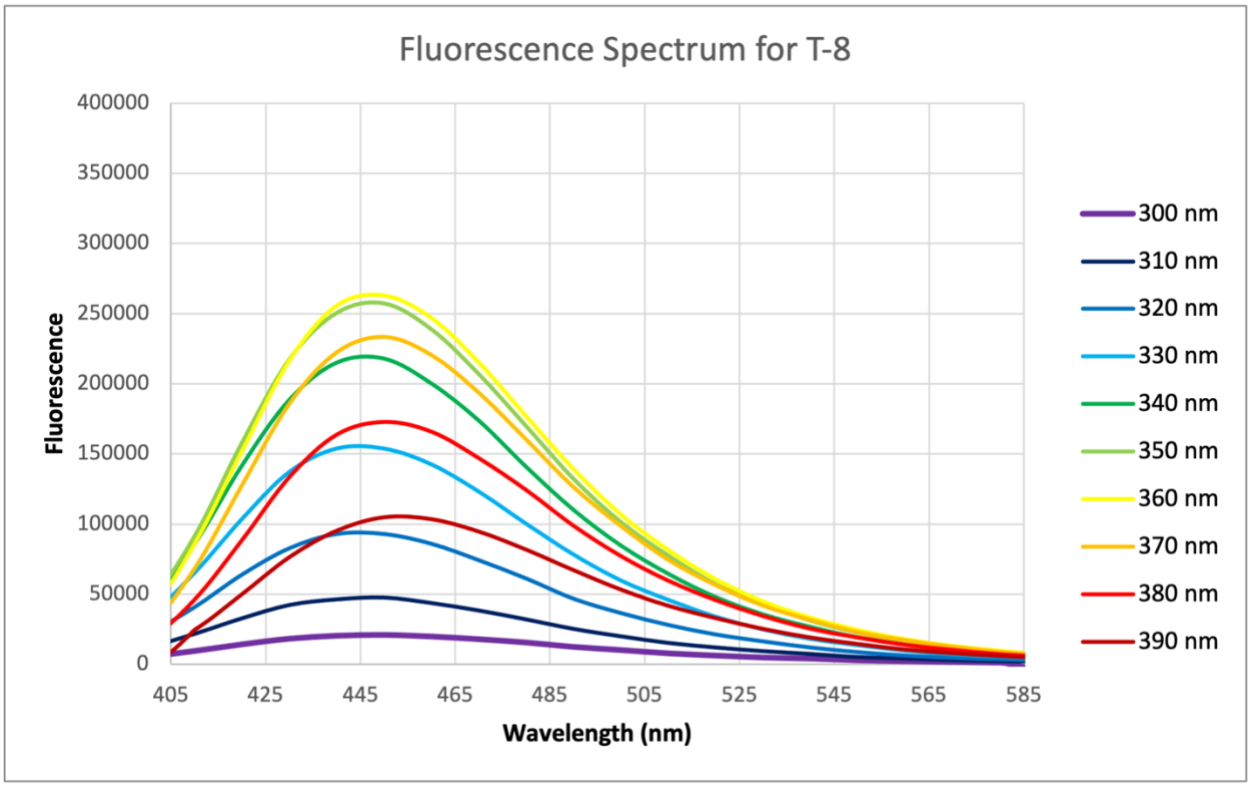

Image 4b: Fluorescence Spectrum for T-8

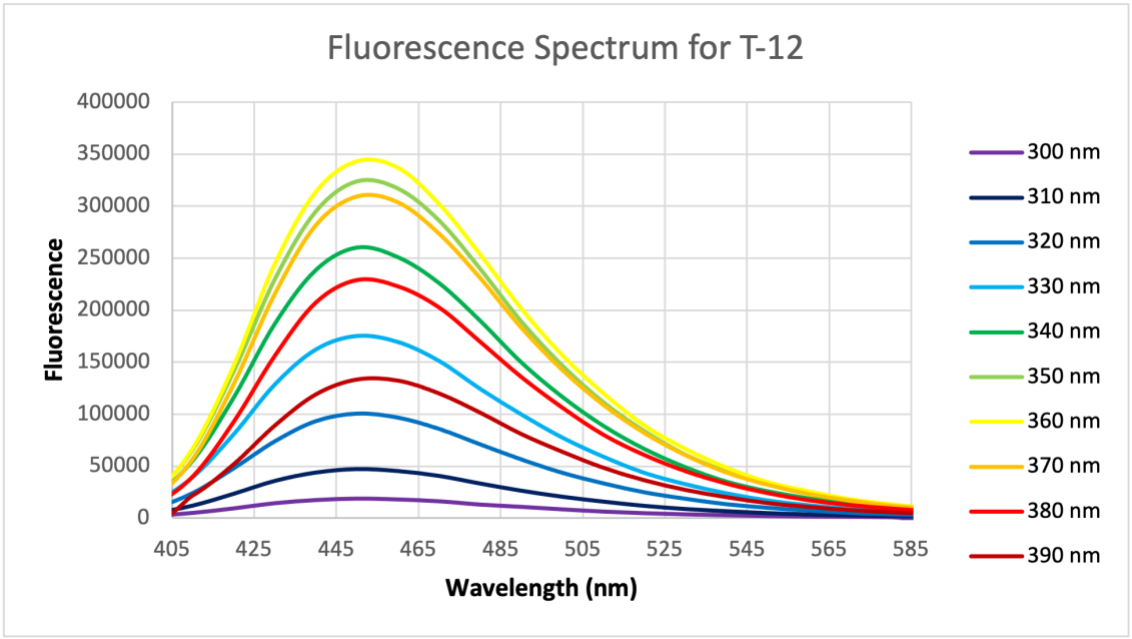

Image 4c: Fluorescence Spectrum for T-12

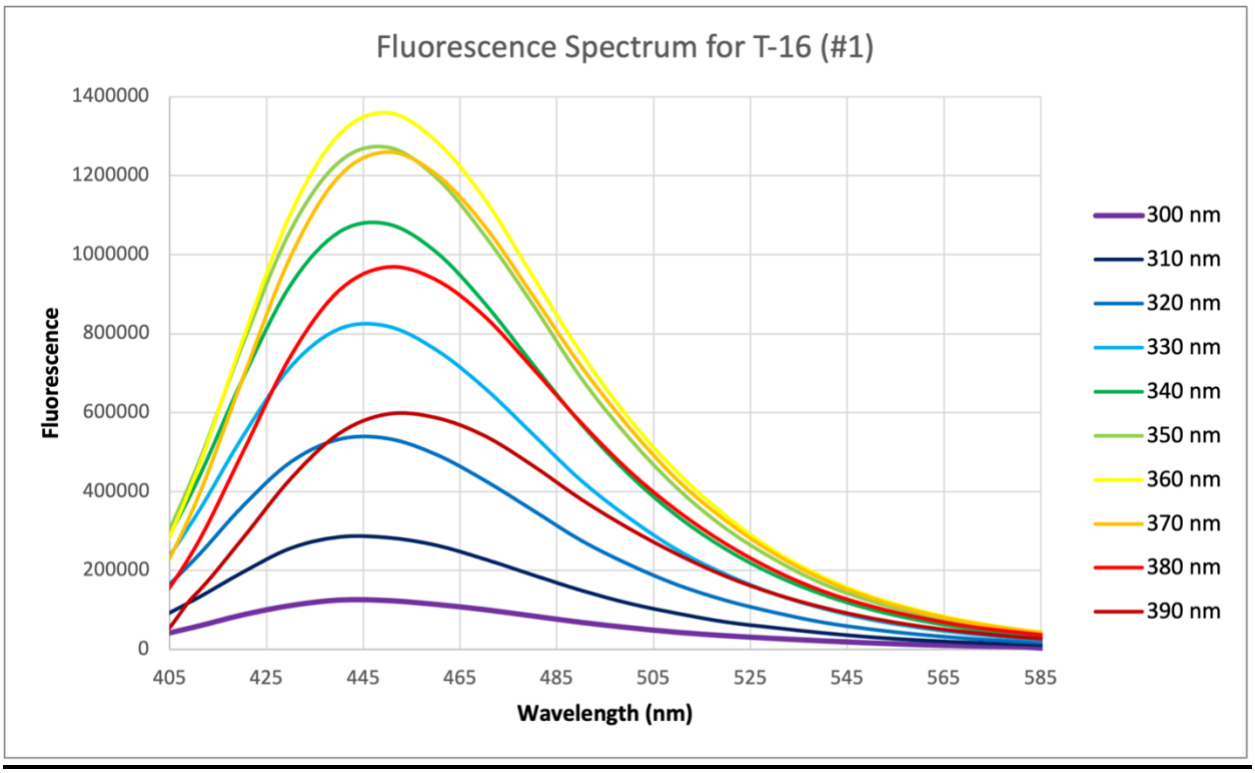

Image 4d: Fluorescence Spectrum for T-16 (#1)

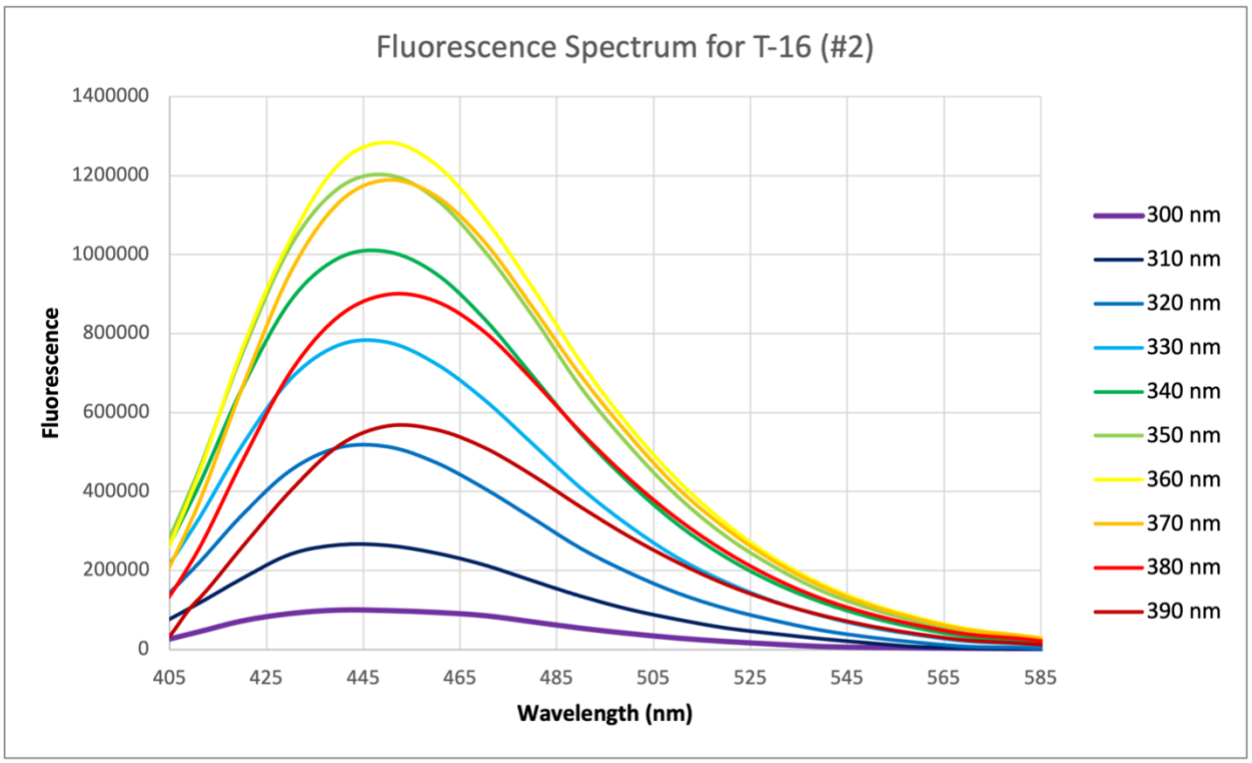

Image 4e: Fluorescence Spectrum for T-16 (#2)

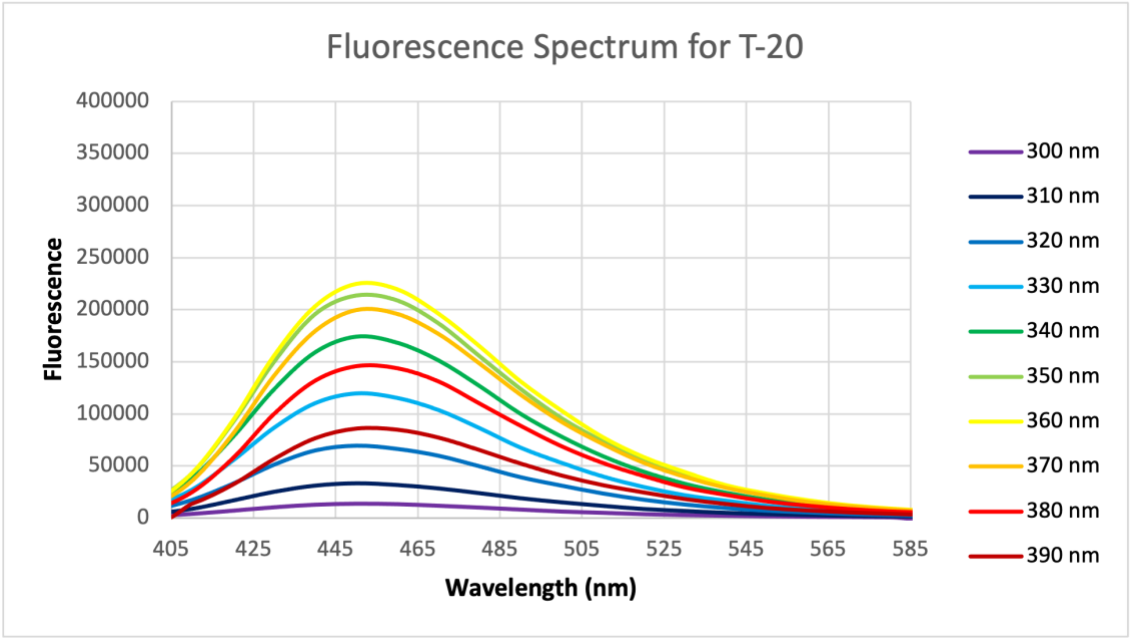

Image 4f: Fluorescence Spectrum for T-20

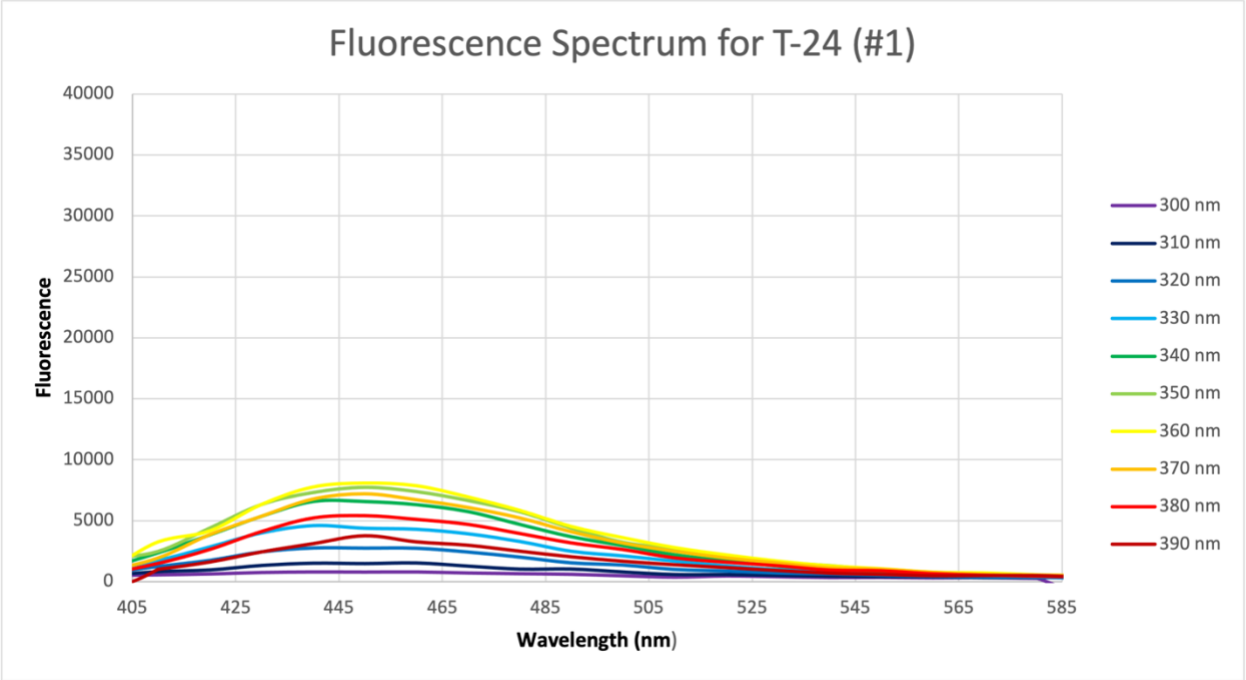

Image 4g: Fluorescence Spectrum for T-24 (#1)

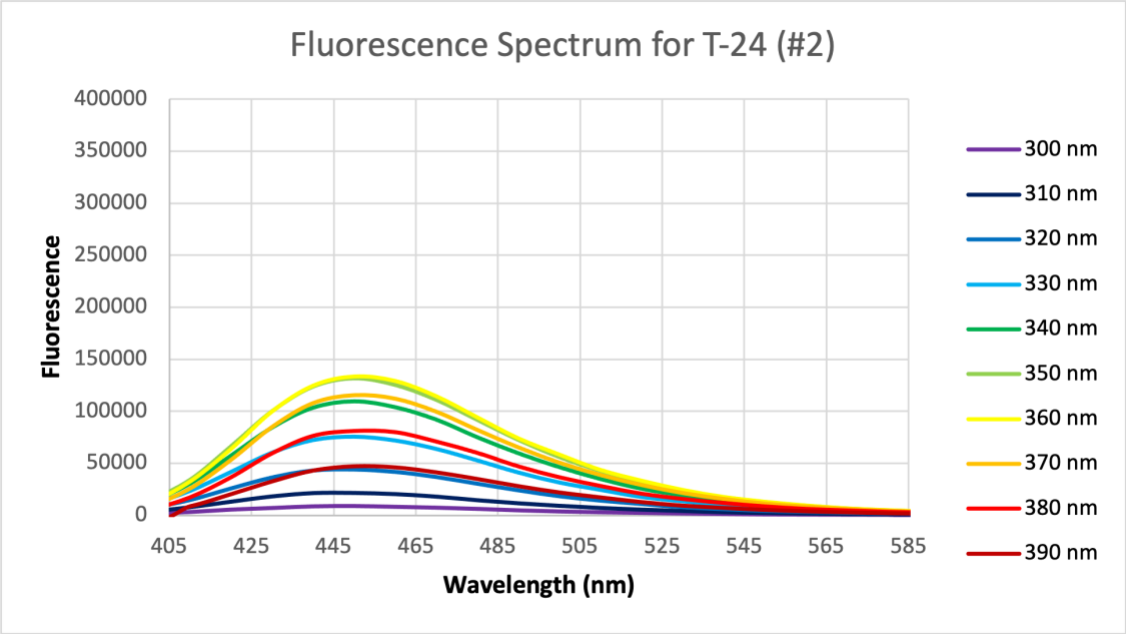

Image 4h: Fluorescence Spectrum for T-24 (#2)

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Special Thanks: Thank you Dr. Mei and future Dr. Michael Tetteh