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Synthesis of ZnTPP-IL as a Potential Photodynamic Therapy Agent

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Abstract

Photodynamic Therapy is a new technique used in cancer treatment involving the use of a photosensitizer—a porphyrin—and light exposure to kill malignant cells. In this research, ZnTPP-IL was used as a photosensitizer with white light as a possible source of treatment of NIH 3T3 fibroblast and A549 lung cancer cell lines, with preliminary DNA testing on MYC-22 and G-quadruplex samples. ZnTPP-IL was synthesized by the reaction of the porphyrin ZnTPPC with isoleucinol. The new porphyrin was then purified by column chromatography using Sephadex LH-20 and G-50. ZnTPP-IL was characterized through NMR, IR, UV-vis, and fluorescence spectroscopies. The purity of the compound was then determined using HPLC. Additionally, ROS production using ZnTPP-IL was determined. The cytotoxicity of ZnTPP-IL was then examined by analyzing MTT assay with A549 lung cancer cell lines when paired with white and red light.

Background

Introduction

Lung cancer is the second most common form of cancer affecting people in the United States. For men, there is a 1 in 17 chance that they will develop lung cancer in their lifetimes; for women, that number increases to 1 in 16. Treatments for lung cancer range from surgery, ablation, radiation, and chemotherapy to palliative care procedures, depending on the stage, lung function, and overall patient health.[1] Side effects of these traditional cancer treatments include a decrease in white blood cells, edema, hair loss, nausea and vomiting, deep vein thrombosis, memory and thinking issues, pain, and tiredness.[2] Some side effects present themselves months or even years after the treatment is administered, referred to as late side effects.[3]

Photodynamic Therapy

Photodynamic therapy (PDT) is a new cancer treatment that utilizes light, oxygen, and a drug called a photosensitizer. Photosensitizers are molecules which preferentially accumulate in malignant cells and use radiant energy or light to produce a response in the cells. The treatment is conducted by administering the photosensitizer, allowing it to accumulate in malignant cells and metabolize out of healthy cells over an incubation period, then shining light on only the affected tissues (figure 1).[4] The light absorption by the photosensitizer produces singlet oxygen which disrupts normal cell functions, ultimately leading to cell death. “Because these drugs preferentially accumulate in cancerous cells rather than healthy cells, and light is shone directly at those target tissues,

photodynamic therapy has selective toxicity and fewer side effects than do chemotherapy and radiotherapy."[5]

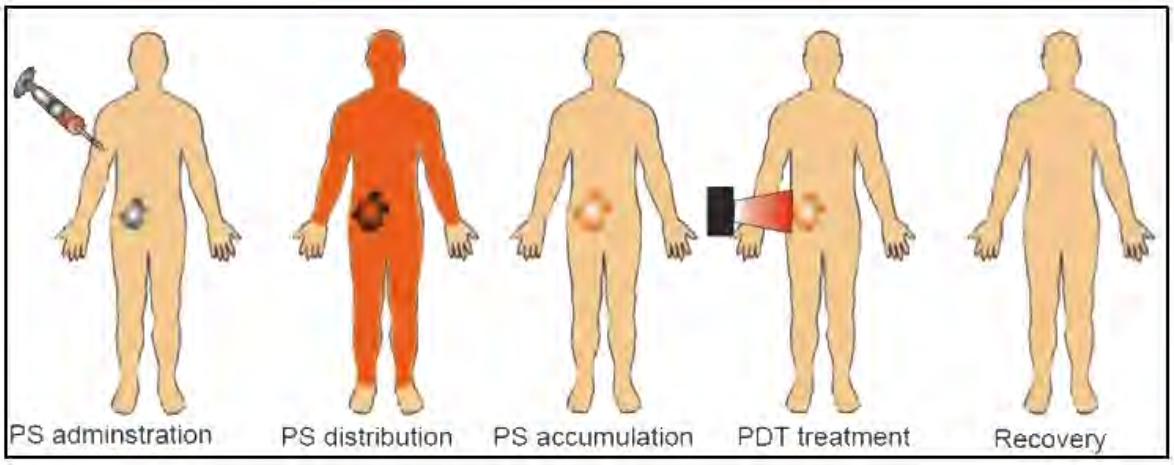

Figure 1- Photodynamic therapy process

Photosensitizers: Porphyrins

Photosensitizers that are good candidates for use in PDT are those that preferentially accumulate in the target tissue, are rapidly excreted from healthy tissue, are chemically pure and of known composition, have minimal toxicity in the dark and only cytotoxic in the presence of light, and have an absorbance with an extinction coefficient in the 600-800 nm range. The most researched photosensitizers for PDT are porphyrins, specifically those considered a hematoporphyrin derivative. The first purified hematoporphyrin derivative is known as Photofrin. This compound was effective, but extremely difficult to reproduce, so the goal for many researchers has been to find a porphyrin that can more easily replace Photofrin. One such group of porphyrins which achieves this goal is tetraphenylporphyrins, TPP (figure 2). On its own, TPP has minimal solubility, but with the addition of substituent groups, the solubility and absorbance values of the porphyrin can be modified.[6] Metallating the porphyrin core can also contribute to higher absorbance wavelengths. For PDT, this is typically achieved with iron or magnesium.[7]

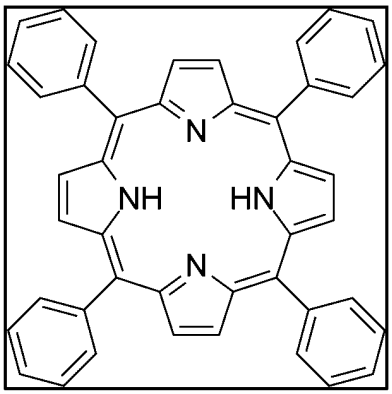

Figure 2- TPP structure

PDT Use in Medically Underserved Areas

Access to cancer treatments may be limited in rural areas of the world due to lack of medical facilities and personnel, and lack of complex surgical procedures or availability of chemotherapy/radiation. PDT may be a useful strategy for patients within the Medicare population within the U.S. as well as for patients of low socio-economic status abroad because of its inherent cost effectiveness. The issue using photodynamic therapy in the field lies in the incubation period that is required for the photosensitizer to accumulate in affected tissues. If a patient lived far from a treatment center, the challenge would be making the remainder of the treatment accessible and still at a low cost.[8] Specifically for use in rural areas of the world, the challenge lies in the relative inaccessibility to performing invasive procedures, limiting PDT use for skin cancers, oral cancers, and cancers or degenerations of the eye. In response to the issue of treatment accessibility for rural populations, there has been investigation into a portable, battery-operated device for PDT treatment using LED. In the case of cancers that do not require intraoperative light delivery, PDT can be conducted without extensive medical infrastructure. “Activation of the photosensitizer can be accomplished with relatively low cost LED light sources which can be used as an alternative to traditional medical lasers

and which can be engineered to operate in battery power in settings with unreliable electrical power supply." Continuity of care could thus be carried out via telemedicine if applicable.[9] See figure 3 for schematic of the portable device developed by Hui Liu, Liam Daly, Grant Rudd, and team at the Wellman Center for Photomedicine in Boston.

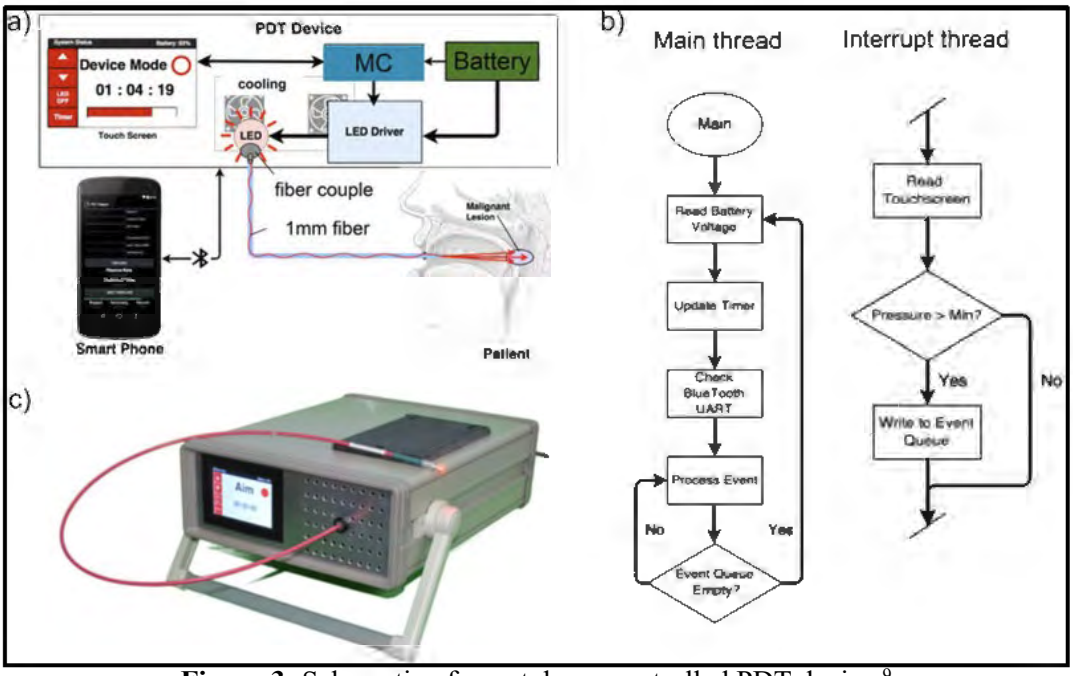

Figure 3- Schematic of smartphone-controlled PDT device.[9]

Project Introduction

The goal of this research was to synthesize novel, water-soluble porphyrins for potential use in photodynamic therapy (PDT) for the treatment of lung cancers. The porphyrin core, made up of four pyrrole subunits linked together to form an aromatic cyclic structure, can be modified on the periphery by addition of one or more side chains. The addition of these side chains allows for changes in solubility and cytotoxicity. In this research, ZnTPPC was modified by addition of (2S,3S)-2-amino-3-methyl-1-pentanol (L-isoleucinol) to form the novel ZnTPP-IL. Isoleucinol is an amino alcohol derived from the amino acid isoleucine that contains an amine group -NH_2, as well as an alcohol -OH group. It was thought that the presence of the amine and alcohol groups would increase the porphyrins solubility in water. Also, the derivation of a compound from an amino acid which is commonly found in the body was thought to prevent the novel porphyrin from having cytotoxic effects without exposure to light.

After synthesis of the compound, it was purified by column chromatography with Sephadex LH-20 and G-50. It was then characterized by UV-Vis spectroscopy, nuclear magnetic resonance (NMR), fluorescence spectroscopy, and infrared spectroscopy (IR). The final product's purity was tested using high performance liquid chromatography (HPLC), and preliminary DNA testing was done using the same method. The compound's cytotoxicity and ROS production was then tested in various light conditions via MTT assay and ROS assay on both A549 non-small cell lung cancer and NIH 3T3 fibroblast cell lines.

Methods

Synthesis of Porphyrin Compounds

Reaction 1- Formation of H_2TPPC (3)

H_2TPPC, the starting porphyrin core, was synthesized by reaction of pyrrole (**1**) with 4-carboxybenzaldehyde (**2**) in propionic acid solution (figure 4). 4-formylbenzoic acid (3.008g) was added to a 500-mL round bottom flask with a stir bar. Approximately 250 mL of propionic acid was then added to the flask, followed by 1.5mL of pyrrole. The flask was wrapped in aluminum foil and heated to reflux (approximately 15 minutes). The reaction refluxed for 1 hour before being allowed to cool to room temperature, then the flask was sealed with parafilm and placed in a -20°C freezer overnight. The reaction product was then filtered by vacuum filtration using a medium glass fritted filter. The product was rinsed with dichloromethane twice, then finally rinsed with ethyl acetate to ensure removal of impurities. The product was then allowed to airdry in the filter before being transferred to a previously oven-dried vial.

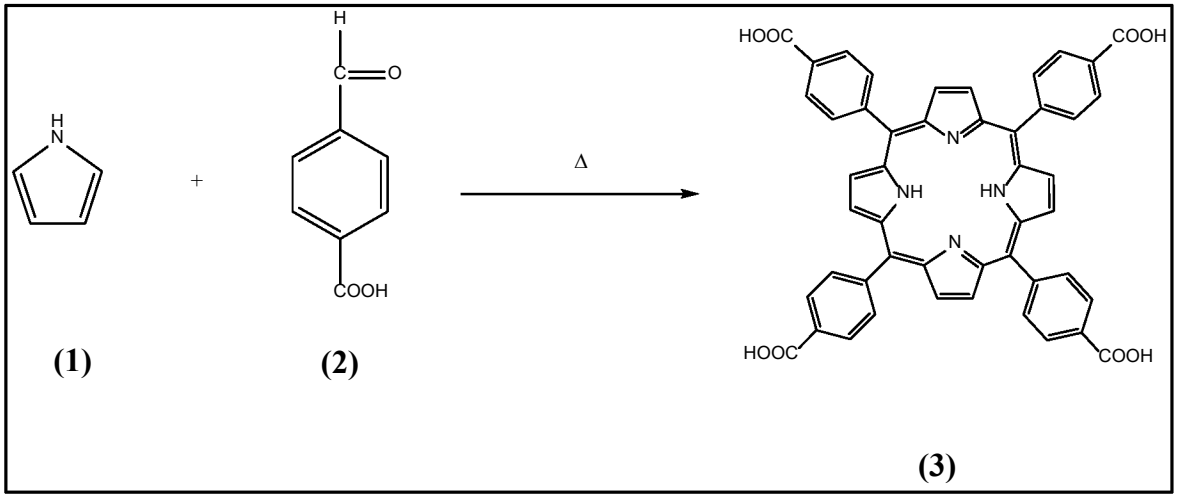

Figure 4- Reaction of Pyrrole (1) and 4-carboxybenzaldehyde (2) in propionic acid to form H_2TPPC (3).

Reaction 2- Formation of ZnTPPC (4)

The porphyrin core was metallated by addition of zinc to the inner ring of the core. $ZnCl_2$ (0.364g) and 1.012g of the previously synthesized H_2TPPC **(3)** was added to a 250 mL round-bottom flask with a stir bar and approximately 75 mL of dimethylformamide, DMF (figure 5). This reaction was allowed to heat until reflux, then refluxed for 3 hours. After 3 hours, the reaction was removed from heat and the DMF was evaporated from the flask using a rotovap. The resulting solid was then added to enough methanol to suspend the solid. The solution was then filtered using a medium glass fritted filter and rinsed with MeOH until the mother liquor coming through the filter was transparent. The mother liquor was then added to the rotovap and all methanol was evaporated from the solution. Dichloromethane was then added to the solid product and the solid was collected by filtration. The solid product was transferred to a previously oven-dried vial.

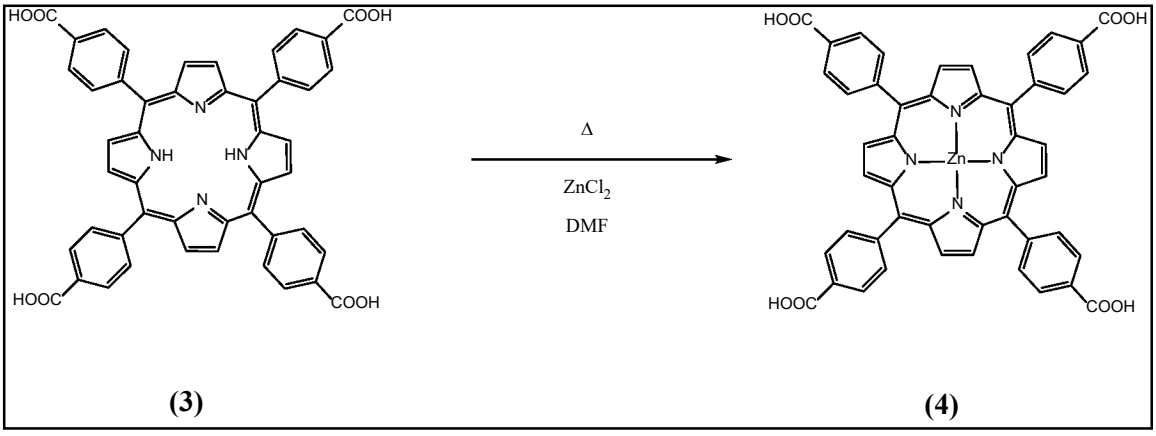

Figure 5- Reaction of H_2TPPC (3) with $ZnCl_2$ in a solution of DMF to form ZnTPPC (4).

Reaction 3- Formation of the Acid-Chloride Intermediate (5)

An acid-chloride intermediate of the porphyrin **(5)** was formed by addition of thionyl chloride ($SOCl_2$) in a DMF solution, under flow of nitrogen (figure 6). All glassware was dried in the oven, then allowed to cool in a desiccator with a rubber stopper to prevent moisture. ZnTPPC (0.15g) was added to 250 mL round-bottom flask with a stir bar. A disposable needle was then inserted into the center of the rubber septum to vent the reaction flask. Nitrogen flow was then added to the flask in the same manner, through the rubber septum. Approximately 25 mL of DMF was then added to the flask. Using a syringe, 0.15mL of thionyl chloride was injected into the reaction flask. The reaction then stirred for 1 hour under constant flow of nitrogen. Afterward, the DMF was evaporated using the rotovap then placed under vacuum overnight.

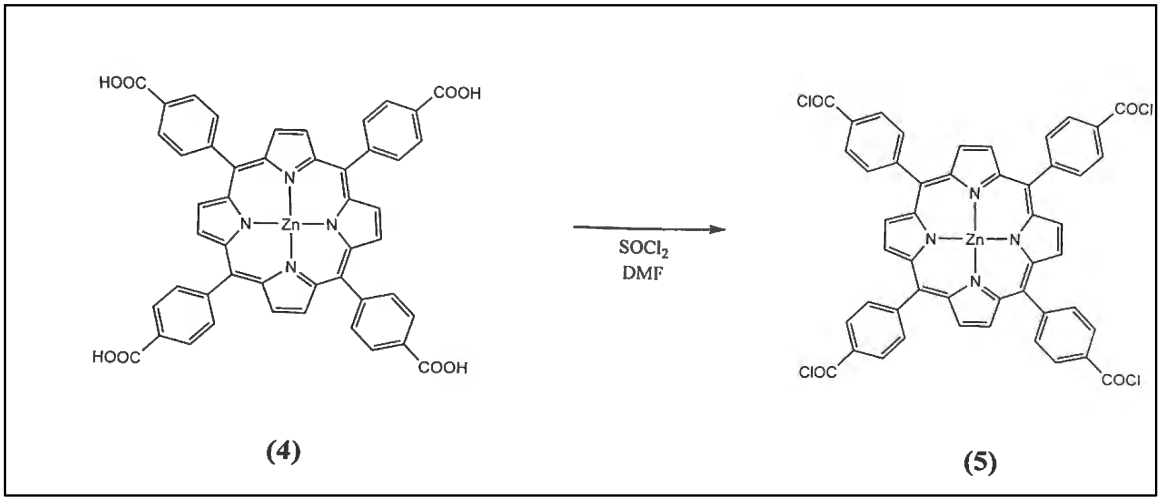

Figure 6- Reaction of ZnTPPC (4) with $SOCl_2$ to form acid-chloride intermediate (5).

Reaction 4- Formation of ZnTPP-IL (6)

ZnTPP-IL, the end-product porphyrin to be tested, was synthesized by reaction of the acid chloride intermediate **(5)** with isoleucinol (figure 7). Approximately 0.41g of isoleucinol was added to a previously oven-dried vial. The vial was then filled halfway with freshly distilled methanol and the vial was capped and shaken to dissolve the isoleucinol. The acid chloride flask from the previous reaction was removed from the vacuum and again placed under the flow of nitrogen. The isoleucinol/methanol solution was then added to the flask, and the vial was rinsed with methanol to ensure no isoleucinol remained in the vial. This reaction stirred for one hour under nitrogen flow. After one hour, the methanol was evaporated from the flask using the rotovap. Once all of the methanol was evaporated off, the resulting product's solubility was tested. A small amount of MilliQ H_2O was added to a test tube, then a clean spatula was used to place a small amount of ZnTPP-IL into the tube and stirred.

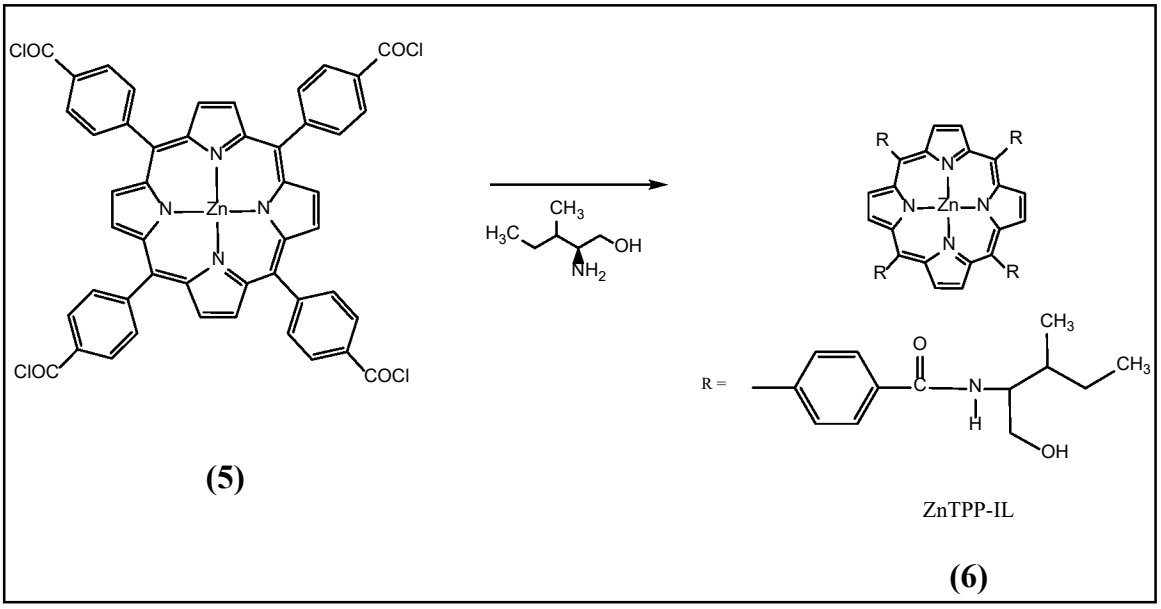

Figure 7- Reaction of ZnTPPC (5) with Isoleucinol to form ZnTPP-IL (6).

Once solubility of the product was ensured, reactions 3 and 4 were repeated three times to ensure repeatability and to obtain an adequate amount of working product. Solubility of the product was tested each time, and any trial that resulted in an insoluble product was repeated (figures 8 and 9).

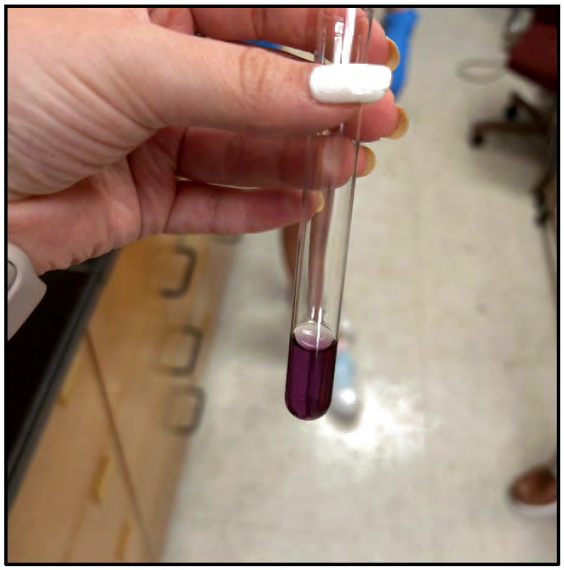

Figure 8- Porphyrin when soluble in MilliQ H_2O

Figure 9- Porphyrin when insoluble in MilliQ H_2O

Purification of ZnTPP-IL

LH-20 Column Chromatography

To prepare the gel filter column, approximately 6 scoops of LH-20 was added to a beaker, and the beaker was filled with 50:50 MeOH:H_2O and allowed to sit for 24 hours before being decanted and packed into the chromatography column. A 50:50 MeOH:H_2O mixture was added to the flask that held ZnTPP-IL to dissolve the product. A small amount of cotton was then placed into a syringe and the ZnTPP-IL solution was poured into the syringe and pushed through the cotton into a beaker. This cotton-filtered solution was then filtered through a syringe with a 0.45 μM nylon syringe filter attached. The ZnTPP-IL solution was then added to a chromatography column packed with Sephadex LH-20, 10-20 mL of solution at a time (figure 10). The column was rinsed with a small amount of MeOH:H_2O. Throughout filtration, the level of MeOH:H_2O was kept at least 1 inch above the LH-20 once the product had fully entered the LH-20 column. The color of the purified solution was closely monitored and only the desired purple product coming off the column was collected. Any impurities were discarded. The purified solution fractions were combined and were then added to the rotovap. The MeOH:H_2O was removed by evaporation leaving the solid ZnTPP-IL product.

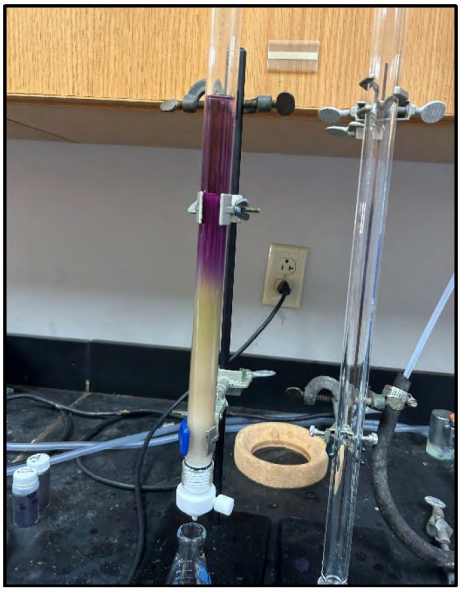

Figure 8- LH-20 Chromatography Column

Figure 9- G-50 chromatography column

G-50 Column Chromatography

To prepare the G-50 gel filter column, approximately 2 scoops of Sephadex G-50 was added to a beaker and then the beaker was filled with Milli-Q H_2O. This was allowed to sit for several hours before being packed into the chromatography column. The dried filtrate was then dissolved in Milli-Q H_2O and added to the column (figure 11). The column was rinsed with Milli-Q H_2O, and only the desired product that came off the column was collected. The rotovap was then used to evaporate the H_2O from the purified product.

Characterization

UV-Vis Spectroscopy

Conjugated double bonds are characteristic of porphyrin structures, and this π-conjugated system of electrons results in two distinct regions of peaks, one in the near-ultraviolet range and another in the visible light range. Excitation of these electrons gives rise to a Soret band (at 415 nm for un-metallated porphyrins, at 423 nm for zinc-metallated porphyrins) and several Q-bands. The intensity of these Q-bands is dependent on the type of substituents attached to the core ring.[10] To prepare the porphyrin for use in the spectrophotometer, 0.0033g of ZnTPP-IL was weighed out and placed into a 25mL volumetric flask which was then filled with deionized water. Five milliliters of this solution was then moved to a clean volumetric flask and again diluted with DI water up to the mark of the flask. Both the dilute and the more concentrated solutions were analyzed. Within the spectrophotometer, light passes through a cuvette containing the ZnTPP-IL product dissolved in DI water. The intensity of the light waves that pass through are measured at varying wavelengths and a spectrum is generated along with the absorbance at each of those wavelengths. Beer's law was then used to calculate the molar absorptivity, ε, for the compound.

The results, shown in figures 12-14, confirmed the presence of a conjugated system as well as the presence of zinc and the addition of the isoleucinol. The ZnTPP-IL solution (figure 14) shows the Soret band characteristic of zinc-metallated porphyrins at 423 nm, in contrast with the unmetallated Soret band at 415 nm shown in figure 12 of ZnTPPC. This is also verified through the comparison of figures 12 and 13, which show a transition of four Q-bands to two after metalation.[11] In tables 1-4, the Soret bands are shown in gray while the Q-bands are in white.

H_2TPPC	
λ (nm)	Molar Absorptivity Coefficient, ε ($\mu M^{-1}cm^{-1}$)
645	6.73
589	8.59
548	13.9
513	26.1
479	7.72
415	307

Table 1-UV-VIS Molar absorptivity coefficients for H2TPPC spectral peaks[12]

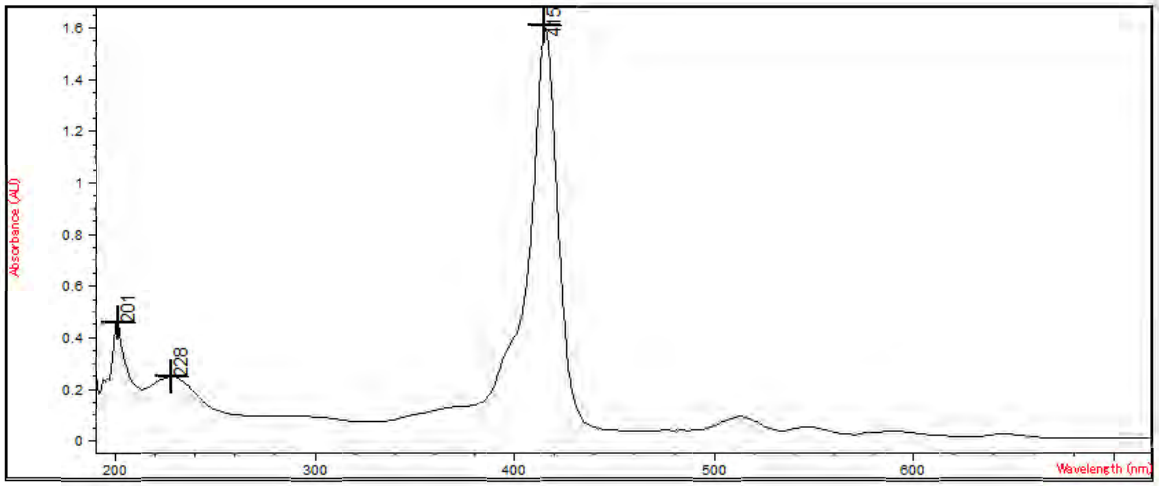

Figure 10- UV-Vis Spectrum for H_2TPPC in MeOH

ZnTPPC	
λ (nm)	Molar Absorptivity Coefficient, ε ($\mu M^{-1}cm^{-1}$)
598	2.61
557	5.74
520	1.32
424	161
405	14

Table 2- UV-VIS molar absorptivity coefficients for ZnTPPC spectral peaks

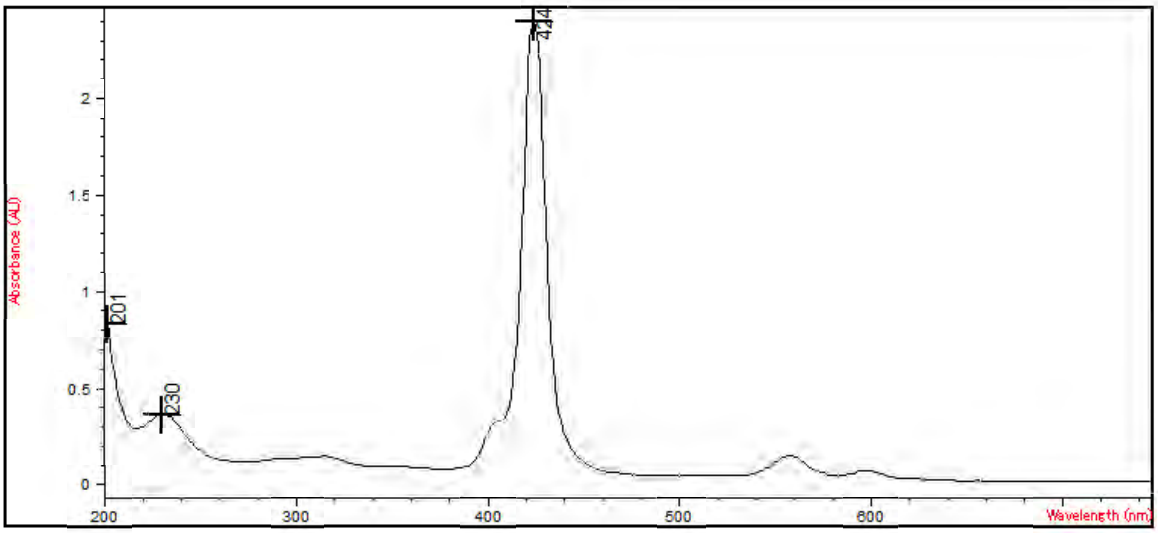

Figure 11- UV-Vis Spectrum for ZnTPPC in MeOH

ZnTPP-IL (dilute)	
λ (nm)	Molar Absorptivity Coefficient, ε ($\mu M^{-1}cm^{-1}$)
423	470.491
228	49.407
311	23.501
557	17.870
597	9.685

Table 3-UV-Vis molar absorptivity coefficients for ZnTPP-IL

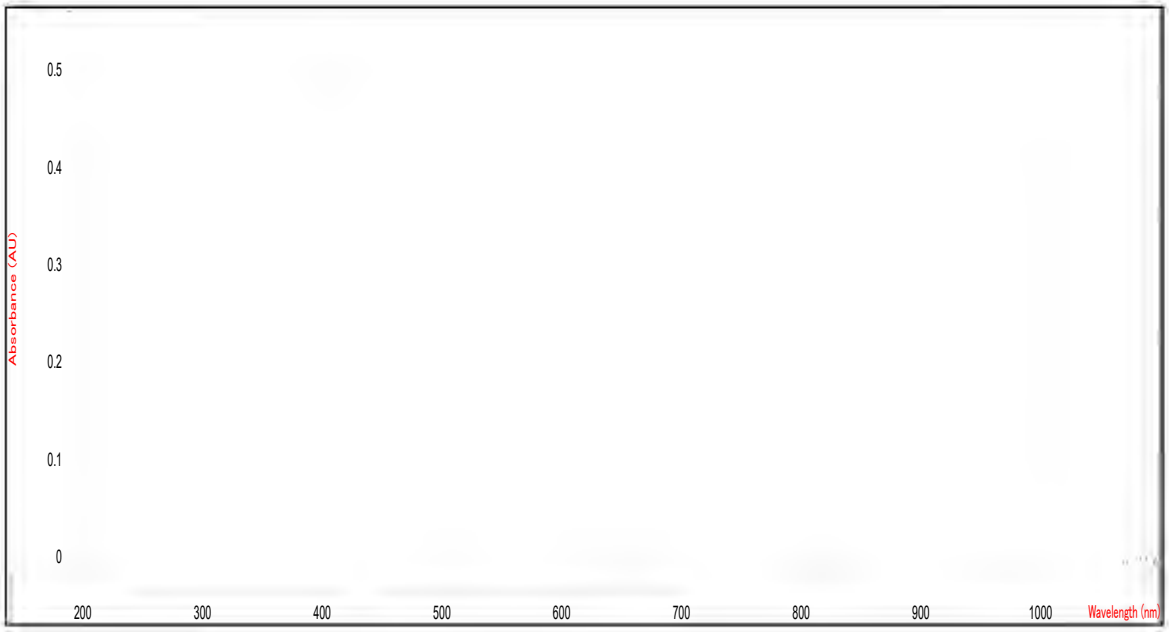

Figure 12- UV-Vis Spectrum for ZnTPP-IL in H_2O

Nuclear Magnetic Resonance (NMR)

Nuclear Magnetic Resonance Spectroscopy (NMR) is a type of spectroscopy that uses a magnetic field to characterize compounds on the basis of their nuclei's spin and magnetic dipoles. When placed in a magnetic field, the nuclei resonate at specific frequencies that are characteristic of that molecule. That frequency is referred to as the "chemical shift" and from those shifts, the chemical groups and bonding pattern within a compound can be identified. The specific chemical shift identifies the type of group, while the intensity of the peak reveals the quantity of that specific group.[13] For the purposes of this research, the chemical shift values are referenced against resonance of the tetramethylsilane (TMS) peak at 0 ppm.[14]

^{1}H NMR was used to characterize the final porphyrin, ZnTPP-IL, as well as the starting amine, isoleucinol. Water eliminated fourier transform (WEFT) was used to eliminate the water signal to allow for better visualization for the ZnTPP-IL spectrum. Chemical shifts were expected to be seen in the 6.5-8.0 ppm region characteristic of the porphyrin's aromatic macrocyclic structure, while the aliphatic proton shifts are expected in the 0.5-5ppm region. Figures 15 and 16 show the resulting spectra.

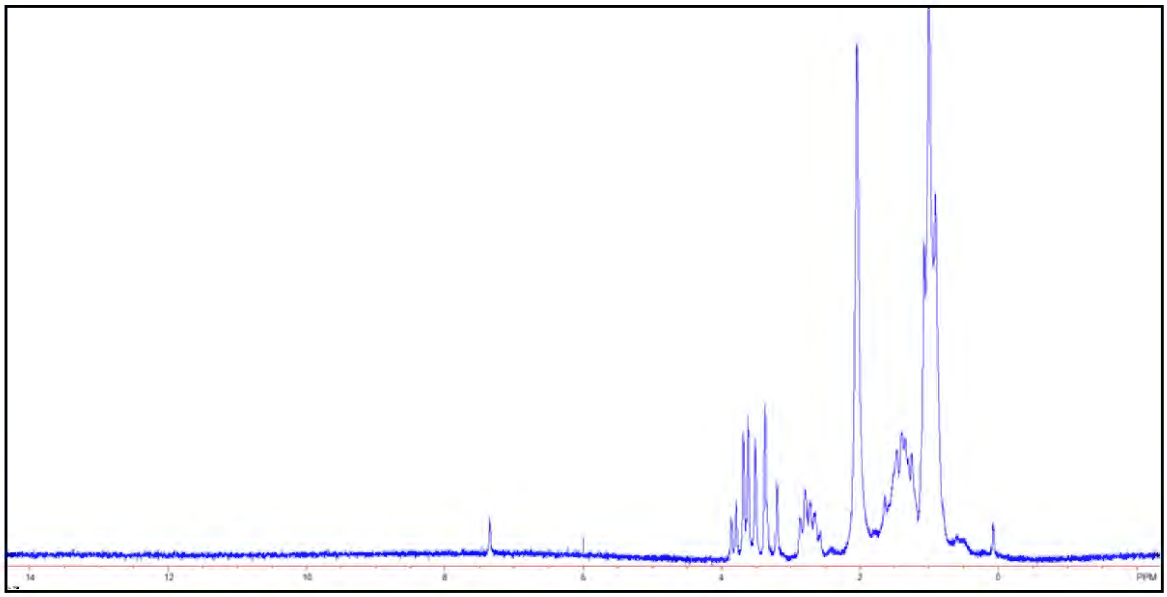

Figure 15- ^{1}H NMR Spectrum of Isoleucinol in $CDCl_3$

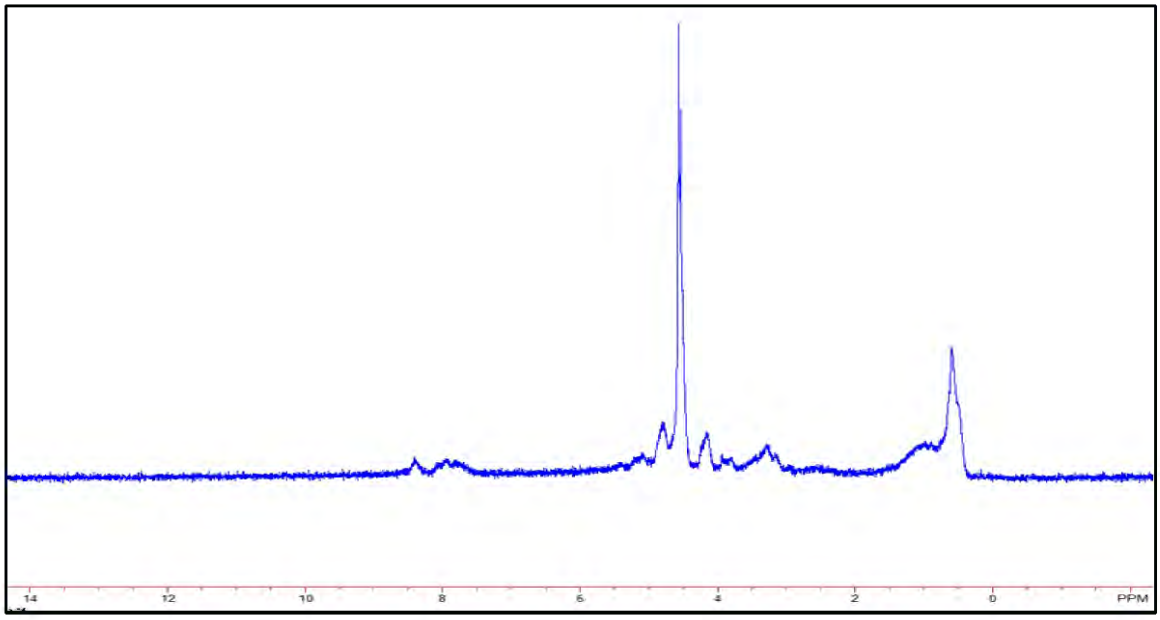

Figure 16- ^{1}H NMR spectrum of ZnTPP-IL WEFT in D_2O

Infrared Spectroscopy (IR)

Infrared radiation causes changes in a molecule as a result of molecular vibrations. Measurements of the way the bonds vibrate give rise to IR spectra. Bond length and stretch vary from molecule to molecule, and so the frequency at which a particular bond absorbs this infrared radiation also varies. The IR spectrum measures these frequencies in wavenumbers, cm^{-1}. Individual, pure compounds can be identified by analysis of their spectra. The region of 1430-910 cm^{-1} is known as the fingerprint region and contains multiple, sometimes identical, absorption bands. The region 4000-1430 is simpler and is used for identification of molecules based on their absorption bands.[15]

Both ZnTPP-IL and isoleucinol were analyzed by IR spectroscopy. For isoleucinol, two distinct bands are expected to be seen at 3500 and 3400 cm^{-1} for the amine, while a broad stretch is expected to be seen for the -OH groups of the porphyrin at *ca*. 3300 cm^{-1}. This is verified by the results shown in figures 17 and 18. When coupled, the spectrum also shows a broad -OH stretch around 3200 cm^{-1}, as well as the presence of three amide peaks *ca*. 1700-1400 cm^{-1}.

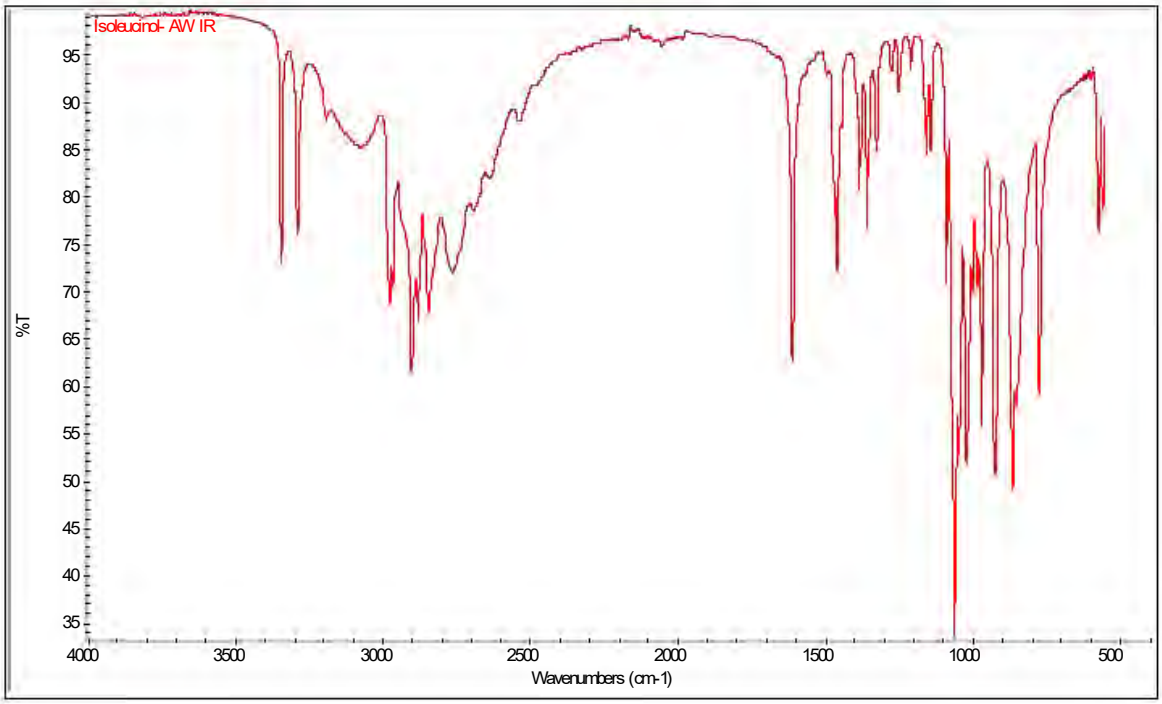

Figure 17- IR spectrum of Isoleucinol

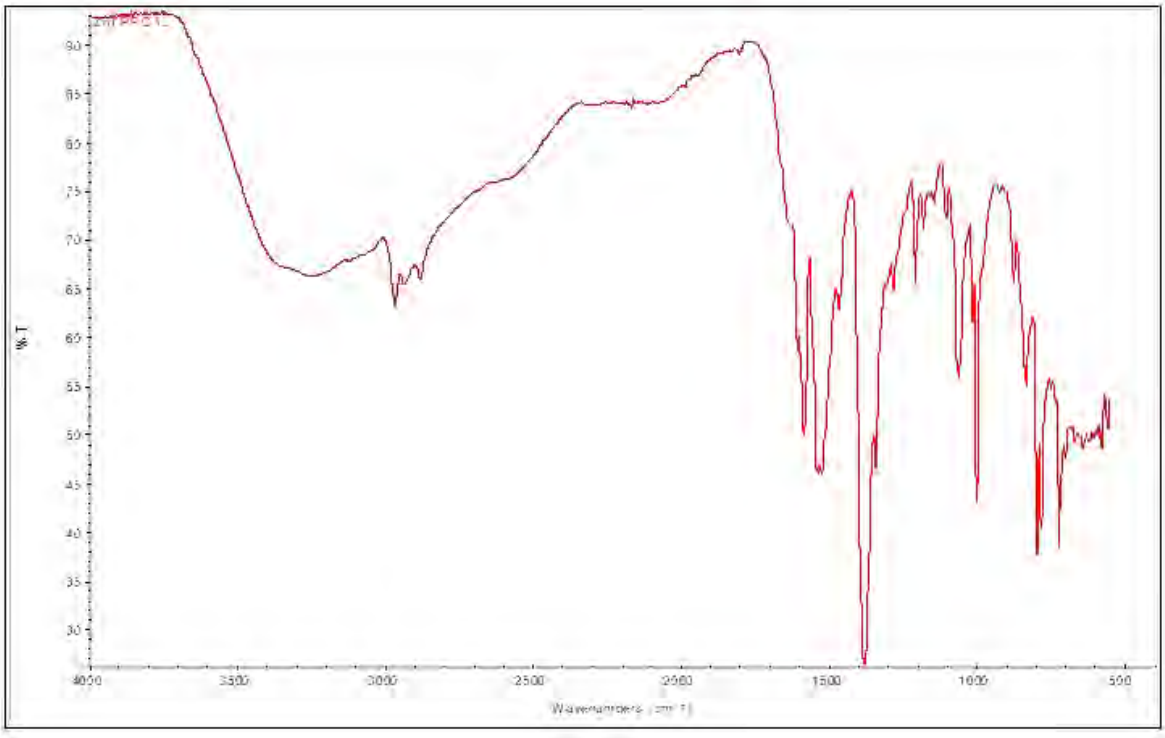

Figure 18- IR spectrum of ZnTPP-IL

Fluorescence Spectroscopy

Fluorescence spectroscopy is an imaging technique that characterizes both the excitation and emission wavelengths of compounds that are thought to fluoresce. Porphyrins are thought to fluoresce close to the red-light region.[16] Figure 19 shows the fluorescence of ZnTPP-IL under UV light. Upon excitation, electrons are excited to a higher energy state, which for zinc-metallated porphyrins occurs at a wavelength of 423 nm as shown via UV-Vis spectroscopy. Once in this excited state, the electrons begin to relax and return to ground state, which emits a photon.[17] For ZnTPP-IL, the wavelength of this photon emission was expected to be in the range of 570-710 nm. Figure 20 shows the resulting spectrum of ZnTPP-IL, with the excitation peak at 423 nm and the emission peak at 606 nm.

Figure 19- Fluorescence of ZnTPP-IL under UV light

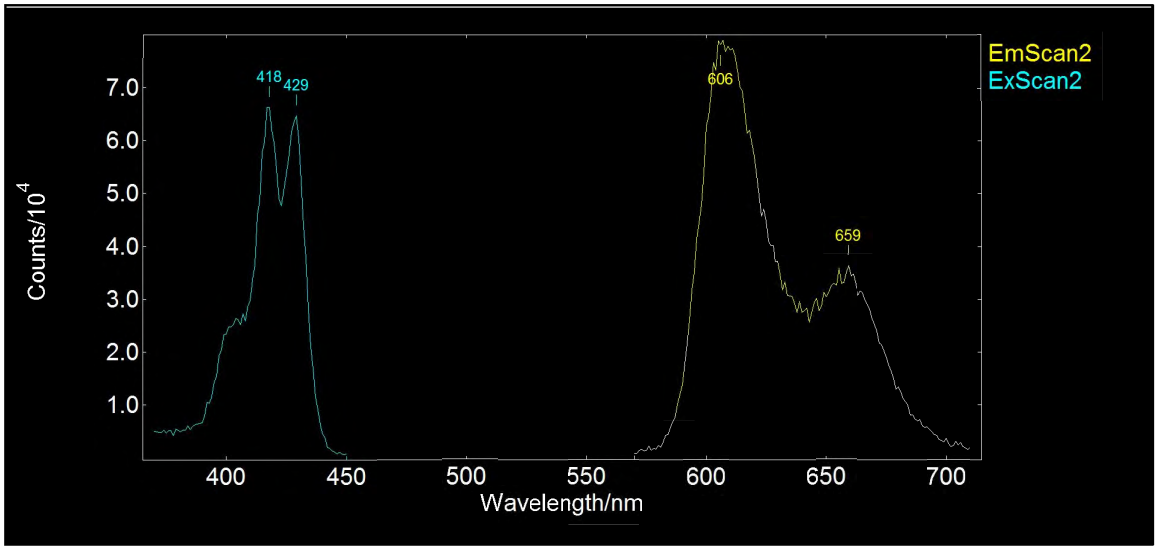

Figure 20- Fluorescence Spectrum of ZnTPP-IL

High Performance Liquid Chromatography

High Performance Liquid Chromatography (HPLC) is a method of chromatography used to separate and identify the purity of compounds. It is used as an indicator of the purity of a sample by measuring the areas of the peaks, where a totally pure peak would represent 100%. High pressure is used to generate the flow of a solvent through packed columns. Different compounds will move at different rates through these columns and generate different peaks on the resulting chromatogram.[18] A concentrated sample of ZnTPP-IL was tested with 100% acetonitrile solvent, a flow rate of 1.00 mL/min using a Hamilton PRP-1, 5 μM 4x150 mm column. The sample was dissolved in H_2O and the purity of the sample was determined to be 96% (resulting HPLC trace in figure 21).

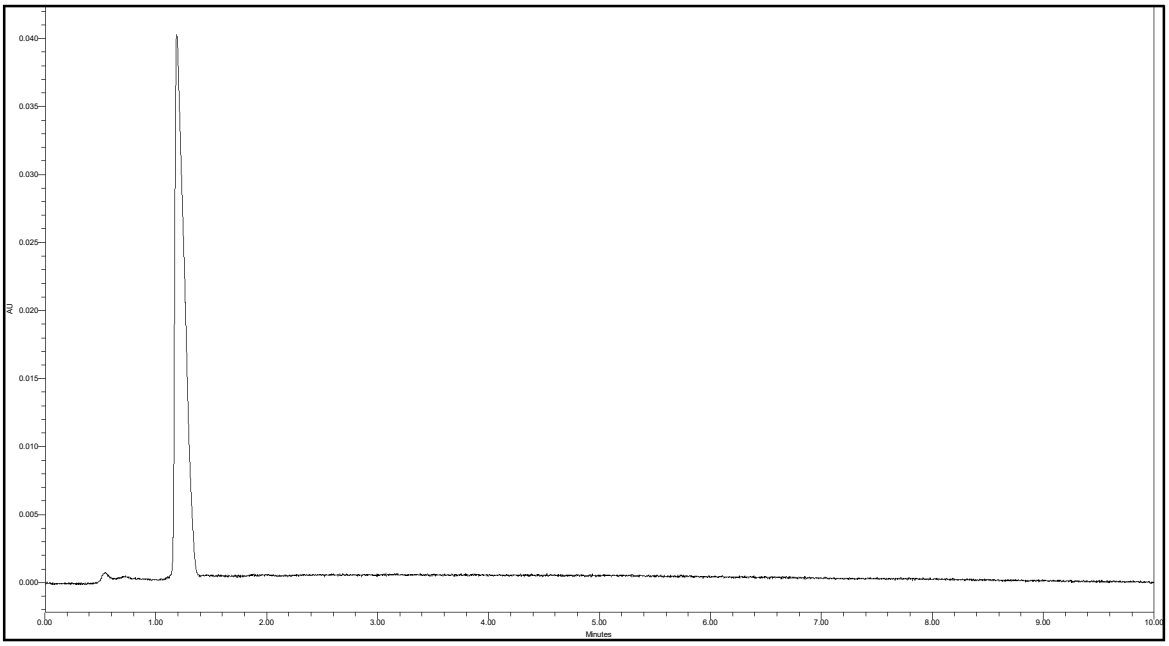

Figure 21- HPLC trace of ZnTPP-IL

DNA Testing Using HPLC

When the porphyrin is activated to a higher energy level by exposure to light, it forms reactive oxidative species (ROS) in the form of singlet oxygen. This singlet oxygen reacts with G-quadruplex DNA, disrupting the normal function of the cells.[19] C-MYC is a proto-oncogene containing G-quadruplex DNA sequences, one of which is MYC-22. The C-MYC gene is amplified in lung, breast, and colon carcinomas.[20] MYC-22 DNA was treated with ZnTPP-IL and exposed to white light then analyzed via HPLC to examine fragmentation. The DNA was exposed to white light for 22 minutes or kept in the dark. The DNA exposed to white light showed fragmentation, while the DNA kept in the dark showed no fragmentation (figures 22-24).

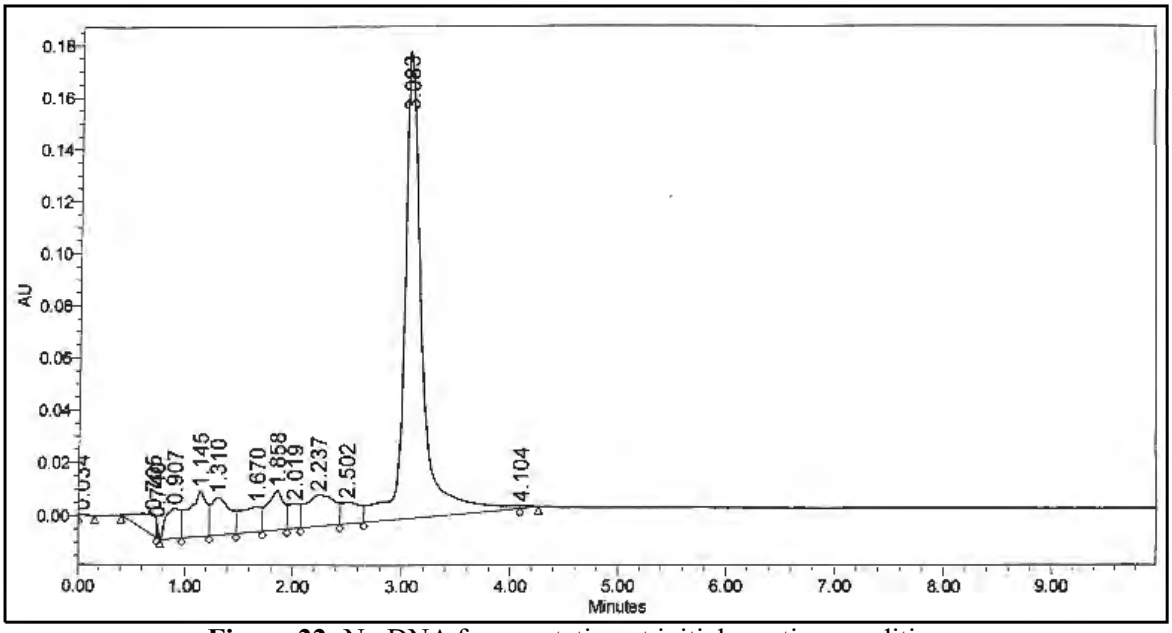

Figure 22- No DNA fragmentation at initial reaction conditions

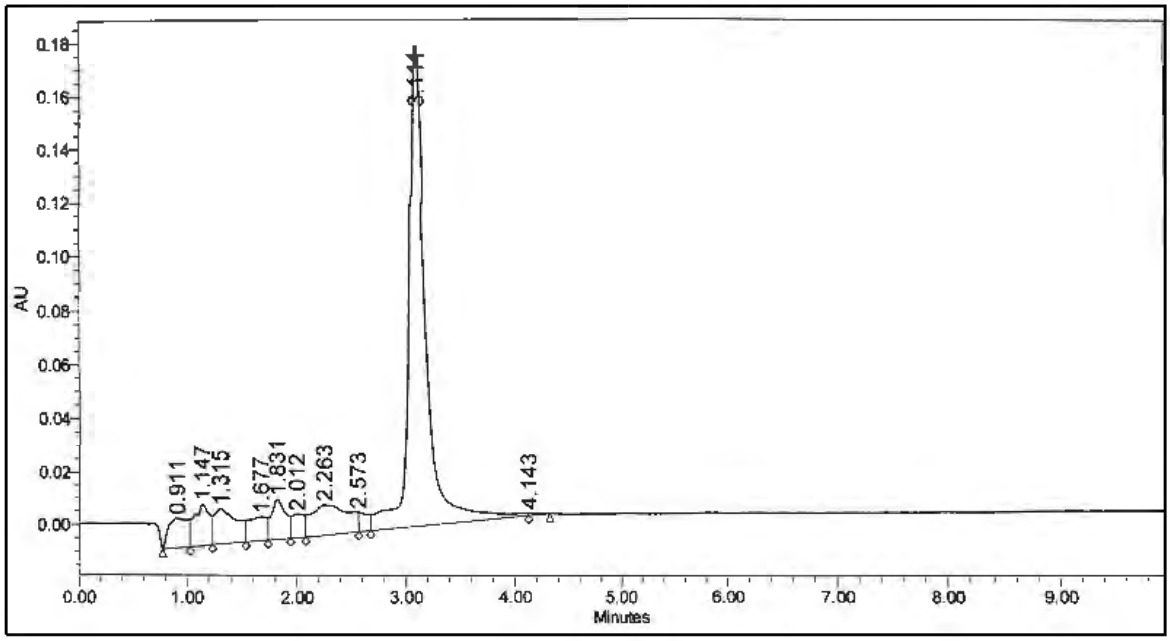

Figure 23- No fragmentation of DNA kept in the dark.

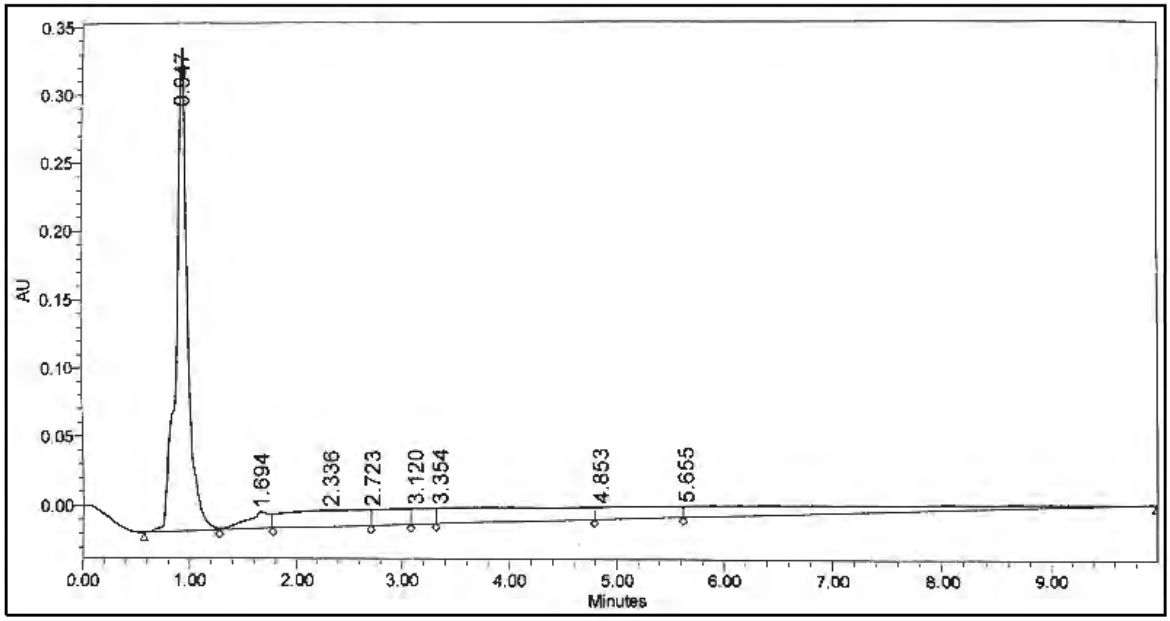

Figure 24- Cleavage of MYC-22 DNA sample after 22 minutes of white light exposure.

Cell Studies

Radical Oxidative Species (ROS) Assay

When porphyrins are used as photosensitizing agents in photodynamic therapy and are exposed to light, a photon of that light is absorbed, and an electron in the target area is excited. This produces an excited state which can then participate in an electron transfer process with triplet oxygen to form singlet oxygen, an ROS that is unstable and toxic to cells.[21]An ROS assay was performed with varying concentrations of porphyrin for two purposes: 1) to ensure ROS production was not present in cells with porphyrin that had not been exposed to light, 2) to quantify ROS production in those that had been exposed to light.

For the assay, 100µL of NIH 3T3 Fibroblast cells, $2x10^5$, were added to two 96-well plates with 100 µL of DMEM growth media and allowed to incubate for 24 hours in a 5% CO_2 incubator. After 24 hours, ZnTPP-IL in DMSO solutions of concentrations 100µM, 30 µM, 10µM, 3µM, and 1µM were made and added to each of the well plates. A blank and a DMSO control was also added to the well plates. One plate was then exposed to white light for 22 minutes while the other was wrapped in aluminum foil. Both plates were placed back in the incubator for 24 hours. After this incubation period, 100 µL of 10 micromolar di(acetoxymethyl ester)-6-carboxy-2',7'-dichlorodihydrofluorescein diacetete (CDCHF-DA) in HEPES buffered saline (HBS) supplemented with 10 mM glucose was added to the cells before being read with the plate reader. The results are shown in figure 25. The results are favorable as a significant difference was observed in ROS production between the light and dark plates, indicating

minimal photosensitizer activation when ZnTPP-IL is kept in the dark and noticeable ROS production when activated by light.

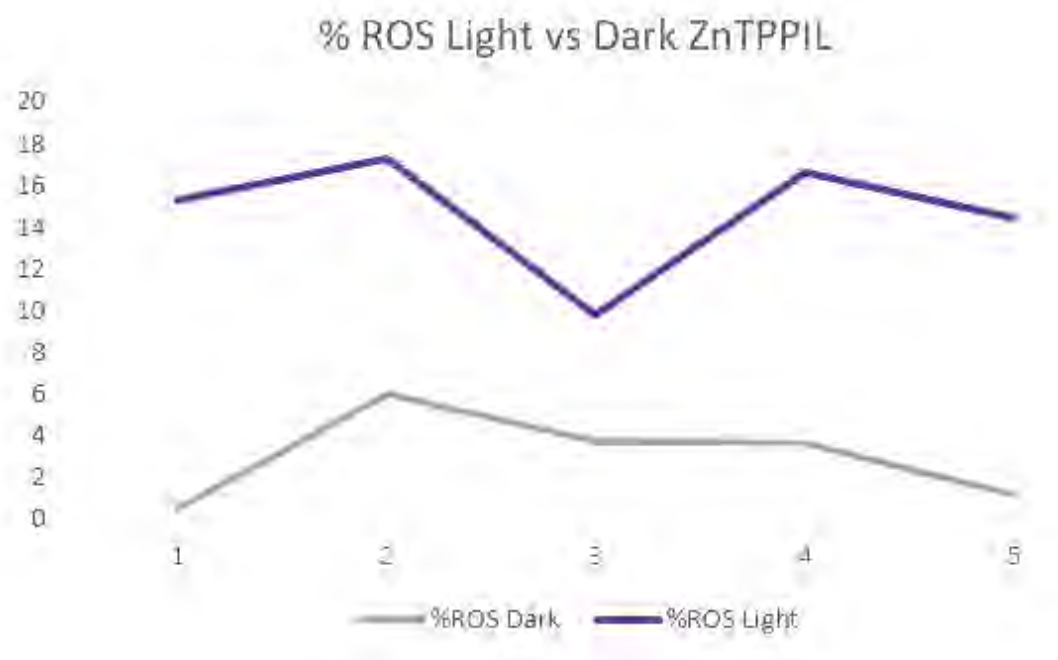

Figure 25- ROS Assay Results

MTT Assay for Cytotoxicity

In order to test cytotoxicity of the ZnTPP-IL, A549 lung cancer cells were cultured in three 96-well plates with DMEM growth media and placed in a 5% CO_2 incubator for 24 hours. After 24 hours, ZnTPP-IL in DMSO solutions of concentrations 100μM, 30 μM, 10μM, 3μM, and 1μM were made and added to each of the well plates. A blank and a DMSO control was also added to each of the well plates. One plate was then exposed to white light for 22 minutes, another to red light for 22 minutes, and the third was wrapped in aluminum foil. All plates were placed back in the incubator for 24 hours. After 24 hours, the solution was aspirated from each of the wells and 100 μL of DMEM growth medium was added to each well and returned to the incubator for 72 hours.

Afterward, the DMEM was aspirated from the wells and 100 μL of 10% MTT solution was added to each of the wells. The plates were then returned to the incubator for 4 hours. After 4 hours, the MTT was aspirated and 100 μL of DMSO was added to the cells and was placed on the orbital shaker for several minutes. The microplate reader was then used to read the plates at 570 nm with a 630 nm correction. The results are shown in figure 26. When exposed to red light, ZnTPP-IL had a lethal dose, the concentration where 50% of the cells are killed, LD_{50} of 1 μM. When exposed to white light, the LD_{50} was found to be 30 μM (Figure 26).

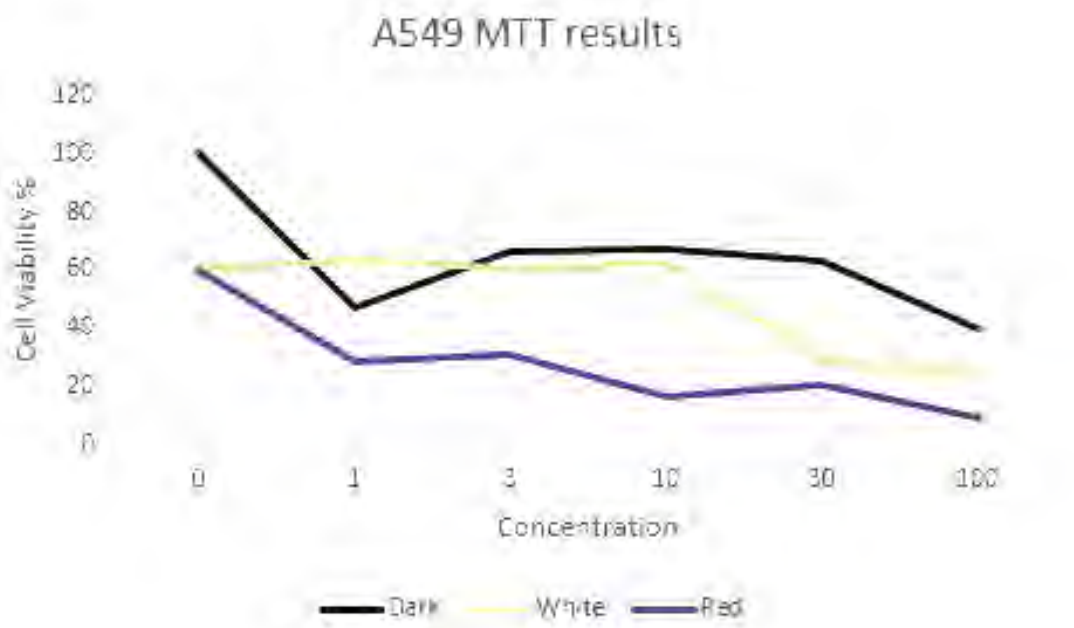

Figure 26- MTT Assay results

Conclusions

ZnTPP-IL, a novel water-soluble porphyrin, was synthesized for potential use as a photosensitizing agent in photodynamic therapy. Isoleucinol, a free amine derivative of an amino acid, was successfully attached to the porphyrin core. This compound showed minimal cytotoxicity when kept in the dark and had an LD_{50} of 1 μM when exposed to red light, and 30 μM when exposed to white light. This result is promising because the porphyrin should not be active and killing cells when not exposed to a light source. When exposed to white light, the porphyrin had extensive cytotoxic effects, but not as many as when exposed to red light at the same concentrations. Further testing would be needed to determine if the porphyrin ZnTPP-IL has cytotoxic effects when exposed to white light at higher concentrations.

If used as a photosensitizing agent in PDT, implementation of this method as a treatment for lung cancer could limit surgical invasiveness and side effects that are typically seen with more traditional cancer treatments. It could also have long-reaching effects in medically underserved, rural areas where there is limited access to healthcare and a greater percentage of patients of low socio-economic status.

Future Work

A continuation of this project should include further investigation into the cytotoxicity of ZnTPP-IL by testing a narrower range of concentrations to confirm the true LD_{50} of ZnTPP-IL in both red and white light conditions. Cell studies examining the effects when the cells are in hypoxic conditions should also be conducted. If favorable cytotoxicity results continue to be found, tumor recurrence using these methods should also be tested *in-vivo* in mice.

A cyanine porphyrin was synthesized at the conclusion of this research in order to examine the effect of a green color porphyrin rather than the traditional purple color seen with porphyrins such as ZnTPP-IL. The structure of this cyanine compound is seen in figure 27. The absorbance values of green wavelengths of light correspond to a penetration depth that is at least 1 mm deeper than that of purple wavelengths (figure 28). The compound was synthesized and purified then solubility in water was ensured. Further synthesis, characterization, and cell studies should be done with this cyanine compound to determine its cytotoxicity.

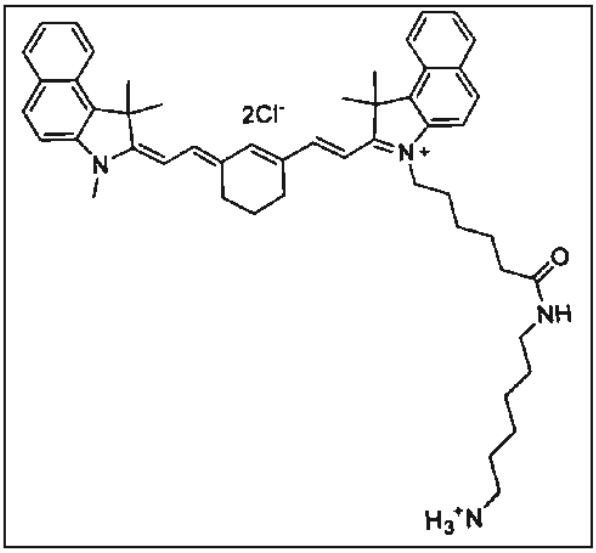

Figure 27- structure of cyanine compound

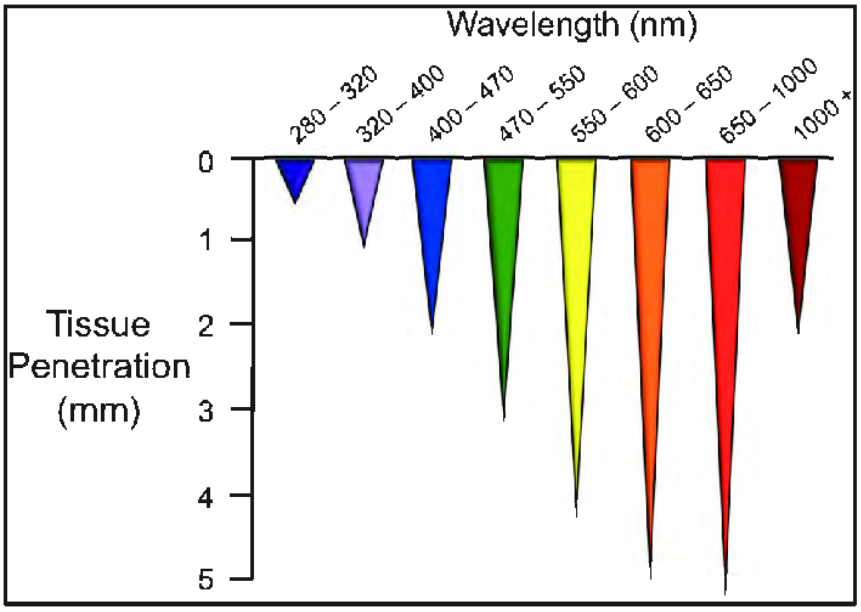

Figure 28- Tissue penetration depth corresponding to wavelength of visible light.[22]

Bibliography

1. Key Statistics for Lung Cancer. *American Cancer Society* (2024). https://www.cancer.org/cancer/types/lung-cancer/about/key-statistics.html

2. Side Effects of Cancer Treatments. *Centers for Disease Control and Prevention, Division of Cancer Prevention and Control* (2023). https://www.cdc.gov/cancer/survivors/patients/side-effects-of-treatment.htm

3. Mayo Clinic Staff. Cancer Survivors: Late effects of cancer treatment. *Mayo Foundation for Medical Education and Research (MFMER)* (2022). https://www.mayoclinic.org/diseases-conditions/cancer/in-depth/cancer-survivor/art-20045524

4. Mroz, Pawel & Yaroslavsky, Anastasia & Kharkwal, Gitika & Hamblin, Michael. (2011). Cell Death Pathways in Photodynamic Therapy of Cancer. *Cancersorg* (G.K. 3. 2516-2539. 10.3390/cancers3022516

5. Jennifer Miller. Photodynamic Therapy: The sensitization of cancer cells to light. *Journal of Chemical Education*. Vol. 76, 5. (May 1999), 592-594

6. Michael R. Detty, Scott L. Gibson, Stephen J. Wagner. Current Clinical and Preclinical Photosensitizers for Use in Photodynamic Therapy. *Journal of Medicinal Chemistry* V. 47, 16. (July 2004, 3897-3911)

7. Nayan J. Patel, Yihui Chen, Penny Joshi, et al. Effect of Metalation on Porphyrin-Based Bifunctional Agents in Tumor Imaging and Photodynamic Therapy. *Bioconjugate Chem*. 2016, 27, 667−680. DOI: 10.1021/acs.bioconjchem.5b00656

8. Shayan Cheraghlou, Hao Feng, Jeffrey M. Cohen. Trends in the Access and Cost of Photodynamic Therapy Among Medicare Beneficiaries in the United States, 2012-2017. *JAMA Dermatology*, 156(9). (2020). DOI: 10.1001/jamadermatol.2020.2427

9. Hui Liu, Liam Daly, Grant Rudd, et al. Development and Evaluation of a Low-Cost, Portable, LED-Based Device for PDT Treatment of Early-Stage Oral Cancer in Resource-Limited Settings. *Lasers Surg Med*, 51(4). (2019). Doi:10.1002/lsm.23019

10. Giovannetti, R. The Use of Spectrophotometry UV-Vis for the Study of Porphyrins. *InTech*, 87-92 (2012). https://cdn.intechopen.com/pdfs/37656/InTech-The_use_of_spectrophotometry_uv_vis_for_the_study_of_porphyrins.pdf

11. Kim, Boris F., Bohandy, Joseph (1981). Spectroscopy of Porphyrins. *Johns Hopkins University Applied Physics Laboratory* Vol. 2, 153-155. https://secwww.jhuapl.edu/techdigest/Content/techdigest/pdf/V02-N03/02-03-Kim.pdf

12. J.E. Bradshaw, K.A. Gillogly, L.J. Wilson, K. Kumar, X. Wan, M.F. Tweedle, G. Hernandez, R.G. Bryant New Non-Ionic Water-Soluble Porphyrins: Evaluation of Manganese(III) Polyhydroxylamide Porphyrins as MRI Contrast Agents,". *Inorganica Chimca Acta*, 275-276 (1998) 106-116

13. Simpson, Andre J., Simpson, Myrna J., Soong, Ronald. (2012). Nuclear Magnetic Resonance Spectroscopy and its Key Role in Environmental Research. *Environ. Sci. Technol*.2012, 46, 11488−11496. dx.doi.org/10.1021/es302154w

14. Gjuroski I, Furrer J, Vermathen M. Probing the Interactions of Porphyrins with Macromolecules Using NMR Spectroscopy Techniques. *Molecules*. 2021 Mar 30;26(7):1942. doi: 10.3390/molecules26071942. PMID: 33808335; PMCID: PMC8037866
15. Introduction to Infrared (IR) Spectroscopy. *Royal Society of Chemistry*. 2020. https://edu.rsc.org/resources/infrared-ir-spectroscopy/4010243.article#:~:text=Infrared%20radiation%20causes%20parts%20of,the%20ends%20of%20these%20bonds
16. Karolczak, Jerzy, Kowalska, Dorota, Lukaszewicz, Adam, et al. Photophysical Studies of Porphyrins and Metalloporphyrins: Accurate Measurements of Fluorescence Spectra and Fluorescence Quantum Yields for Soret Band Excitation of Zinc Tetraphenylporphyrin. *J. Phys. Chem. A* 2004, 108, 21, 4570–4575. April 29, 2004. https://doi.org/10.1021/jp049898v
17. P, Surat. "What is Fluorescence Spectroscopy?". *News-Medical*. 13 February 2024. <https://www.news-medical.net/life-sciences/What-is-Fluorescence-Spectroscopy.aspx>.
18. What is HPLC (High Performance Liquid Chromatatography)?. *Waters.* (n.d). https://www.waters.com/waters/en_US/HPLC---High-Performance-LiquidChromatography-Explained/nav.htm?cid=10048919&locale=en_US
19. Meng Cheng, Yun-Xi Cui, Jing Wang, Jing Zhang, Li-Na Zhu, and De-Ming Kong. G-Quadruplex/Porphyrin Composite Photosensitizer: A Facile Way to Promote Absorption Redshift and Photodynamic Therapy Efficacy. *ACS Applied Materials & Interfaces* 2019 11 (14), 13158-13167 DOI: 10.1021/acsami.9b02695
20. Dang CV. c-Myc target genes involved in cell growth, apoptosis, and metabolism. *Mol Cell Biol*. 1999 Jan;19(1):1-11. doi: 10.1128/MCB.19.1.1. PMID: 9858526; PMCID: PMC83860.
21. H. Kolarova, P. Nevrelova, K. Tomankova, P. Kolar, R. Bajgar, and J. Mosinger. Production of reactive oxygen species after photodynamic therapy by porphyrin sensitizers. *Gen. Physiol. Biophys*. (2008), 27, 101–105
22. Ruggiero, Emmanuel & de Castro, Silvia & Habtemariam, Abraha & Salassa, Luca. (2016). Upconverting Nanoparticles For The Near Infrared Photoactivation Of Transition Metal Complexes: New Opportunities And Challenges In Medicinal Inorganic hotochemistry. *Dalton Trans*.. 45. 10.1039/C6DT01428C