Porphyrins: Super-molecules of the Future: Porphyrin Polymers for Artificial Blood

Amani Albalawi, Sandra Ciborowski, Sara Dolloff, and Emmanuel Ojadi

***Abstract*—The synthesis of porphyrin polymers or porphyrimers provide the potential for diverse applications in several areas of life and industry. Most notable is the formulation of artificial blood, the development of photocatalysts for solar energy conversion, the development of electronic conductors and semiconductors, and the development of holographic image devices, photodynamic therapy and energy storage. Even after decades of research much of these applications are yet to be realized. Hence, we attempted to design and develop a new porphyrin polymer that may be capable of application as artificial blood. In this study, we present the synthesis and characterization of 5, 10, 15, 20-tetra-3, 5-diaminophenylporphyrin. The polymerization process will be achieved using known amide reaction techniques employing diacid chlorides such as oxaloyl chloride as the co-monomer. Various analytical techniques (UV-VIS absorption and fluorescence emission spectroscopy, GS-MS, LC-MS and IR) have been used for characterization of reaction products and intermediates.**

***Index Terms*—5, 10, 15, 20-tetra-3, 5 diaminophenylporphyrin (DAPP), polymerization, porphyrin, artificial blood, Heme, porphyrimers, porphyrin applications.**

I. Introduction

Nature impresses us with the intricacy and precision in her design and didacts us with the way she neatly applies the same basic concept to different situations. This molecule called porphyrin, is one of those compounds with unique characteristics that are utilized cleverly for different functions following structural variability through modification. Organic chemists have been redesigning porphyrin structures of their own for various purposes. Due to their great versatility and function, porphyrins have been studied by many scientists in the last thirteen decades following its discovery [1]. Research has shown the ability of porphyrins to be used as metal binders [2], dye-sensitized solar cells [3], iron metabolism [4], photodynamic therapy [5], catalysts and the potential for use as artificial blood [6]. Porphyrins consist of a basic ring skeletal structure called a porphin. This structure is a macrocyclic tetradentate ligand of four pyrrole rings joined together by four methine bridges [7]. Porphyrins can be easily identified because they display a distinctive absorption spectrum in solution. In general, the porphyrin absorption spectrum consists of a Soret band (350-450 nm), which is the major absorption band in the 400 nm region, and four smaller absorption bands for non-metal compounds (two absorption bands for metalloporphyrins) at longer wavelengths between 500 nm and 800 nm, with decreasing intensity toward the red end of the spectrum. The difference between the number of absorption bands for metal and non-metal porphyrins can be explained through symmetry. One of the most common naturally occurring metalloporphyrins is the heme found in hemoglobin. Four hemoglobin molecules constitute red blood cell. The color of the cell is due to the iron [8]. Heme is responsible for oxygen transport. Being alive is impossible without blood, the liquid complex containing millions of chemicals and cells. Scientists started to think about a proper replacement such as an artificial blood. Due to the increased demand for blood transfusion and concerns about blood-borne pathogens, development of artificial blood substitutes, is under intensive focus. Red blood cells (RBCs) isolated from donated blood are an important component widely used to save patients' lives via oxygen-carrying capacity owing to hemoglobin (Hb). Several complications associated with transfusion of RBCs in patients are categorized into noninfectious and infectious types which are of important concerns for the application of artificial blood [9]. Furthermore, cross matching and blood group typing are needed before transfusion, which is challenging in case of emergencies and when rare blood group types are necessarily required. Hence, it is essential to develop efficient RBC substitutes capable of active oxygen and carbon dioxide transfer. The most important features of an RBC substitute include its ability to transport oxygen and carbon dioxide, low cost, no need for cross matching and blood typing, lack of contamination and infectious agents, easy access, trouble-free storage conditions, extended half-life in circulation, full elimination from body, zero toxicity, non-immunogenicity, no antigenicity, and no carcinogenicity [9]. The goal in this study is to synthesize 5, 10, 15, 20-tetra-3, 5-diamino- phenylporphyrin (DAPP), which can be obtained from the nitro analog, 5, 10, 15, 20-tetra-3, 5-dinitrophenylporphyrin. DAPP is used as starting monomer for polymerization to synthesize an artificial blood. Molecular modeling (HyperChem Release 8.08 for windows 2009, Geometry optimization, Single Point Molecular Mechanics, MM-Plus, Steepest Decent; Default Parameters for torsions, stretches, van der Waals and bends) of the polymerization process starting with the oligomers: tetramer, hexamer, octamer, dodecamer, hexadecamer, etc showed unique and interesting structures which included helices as shown below (Fig. 1).

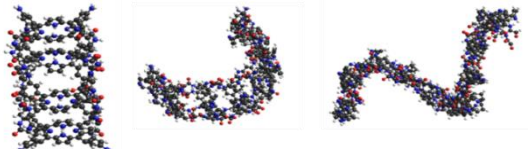

Fig.1. Modeling of the Porphyrimers.

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II. Materials and Methods

A. Materials

3, 5-Dinitrobenzyl alcohol (98%, Lot # 0011AJ), was purchased from Sigma-Aldrich. Pyridinium chlorochromate (98%, Lot # 042261Q), and octanoic acid (98+%, Lot #05223LD) were purchased from Aldrich Chemical Co. Acetone (reagent ACS/USP/NF grade, Lot # C1401133), Methanol (general use HPLC-UV grade, reagent ACS/NF grade, Lot#C1005249), methylene chloride (general use HPLC, reagent grade ACS, Lot # PB006278DCM),and Hydrochloric Acid (reagent grade ACS, Lot # PB006069HAG) from Pharmco-AAPER were used without further purification. Sodium Sulfate (99%, Lot # A0317o15) and Pyrrole (99%, Lot # A0337381) were from Acros Organic was distilled before use. Silver nitrate (ACS reagent grade, Lot #4804875) was from Ricca Chemical Co.

B. Synthesis of 3,5-Dinitrobenzaldehyde

3,5-Dinitrobenzaldehyde has been previously synthesized by the reduction of 3, 5-dinitrobenzoyl chloride using dry diglyme [10]. Due to the highly involved procedure, a new route was developed. 3,5-Dinitrobenzaldehyde was obtained from the oxidation of 3,5-dinitrobenzyl alcohol by pyridinium chlorochromate (PCC), a mild oxidizing agent, in a suitable solvent. 3,5-Dinitrobenzyl alcohol (5.0122 g) was dissolved in 50 mL acetone. Pyridinium chlorochromate (6.0659 g) was also dissolved in 50 mL acetone. The acetone solutions of the reagents were poured into a 250-mL round bottom flask containing molecular sieves and a stirring bar. The flask was attached to a reflux condenser, and heated by a mantle connected to a variac. The mixture was refluxed for three hours, variac set at 45 V (approximately 55^{o}C). Tollens test was performed on the reaction mixture to check for presence of aldehyde. A small sample of product mixture was added into the test tube, and allowed to react. Silver plating on the glass walls indicated that aldehyde was present. The aldehyde was oxidized by silver (I) to generate a carboxylic acid and silver metal, which coated the surface of the glass tube. The product mixture was cooled and then rotary evaporated to remove acetone. Methylene chloride was added to dissolve the product and filtered twice to remove the sieves and any insoluble reagent or solid by-product. The filtrate was further rotary evaporated to remove the methylene chloride, leaving behind a dark sludge material. The sticky tar is due to the pyridine byproducts. Hot distilled water (approximately 600 mL at 80^{o}C) was added to remove the pyridine from the sludge material. Sonication was applied to help break up the compound and adding the hot water in aliquots. The sludge broke up into a green powder, insoluble in water. Suction filtration was applied to give the product residue. The product yield was 79.7%. product was characterized using IR Spectrum and ITQ-700 GC-MS.

C. Synthesis of 5, 10, 15, 20 – tetra - 3, 5-Dinitro-Phenylporphyrin

This porphyrin was first synthesized in 1997 [11]. However, the original procedure was not followed. This study embarked on a different route to obtain DNPP, resulting in a time-effective procedure. 10 mL of ocatanoic acid was refluxing in 25mL 3-neack round bottom flask for 10 minutes. 3, 5-Dinitobenzaldehyde (0.5006 g) was added to the acid slowly. Distilled pyrrole (0.2 mL) was then added to the reaction and allowed to reflux for approximately forty -five minutes with variac set at 50 V. Once cool, the solution was filtrated using methanol as a wash to remove any tar that was produced. The filtrate containing the desired product and octanoic acid impurity. The octanoic acid and the product was neutralized using 2.0 M potassium hydroxide solution. Potassium hydroxide solution was added dropwise to the acid solution. The product instantly precipitated out as the acid neutralized. The porphyrin product was obtained from the centrifugation for 10 minutes at 4000 rmp. The eques layer was decanted off and the black powder dissolved in DCM, using sodium sulfate to remove any moisture presence. The solvent was rotary evaporated; giving DNPP percent yield of 43.6%. the product was characterized using a Shimadzu UV-3600 which confirmed the presence of the porphyrin. LC-MS also was used in the characterization of the porphyrin.

D. Synthesis of 5, 10, 15, 20 – tetra - 3, 5-Diaminophenylporphyrin

(0.025 g) of 5,10,15,20-tetra-3,5-dinitrophynelporphyrin was placed in thick walled,100 mL, 3cm diameter test tube containing concentered HCL and (0.05 g) of Sn. The mixture was shaken and then placed in hot water bath at 70^{o}C for 20 minutes. Sodium hydroxide solution was then added dropwise to neutralize the acid. This was extracted using DCM. The product was characterized using a Shimadzu UV-3600 spectrometer.

III. Result and Discussion

A. Characterization

The mass spectrum of 3,5-dinitrobenzaldehyde was taken in acetone. The gas chromatograph and mass spectrum are shown below (Fig. 2.). Table I relates to (Fig. 2), giving the mass to charge ratios and their correlating fragmentation structures. This spectrum shows that the desired product was obtained. An Aldrich library search confirmed that structure and the fragmentation are accurate.

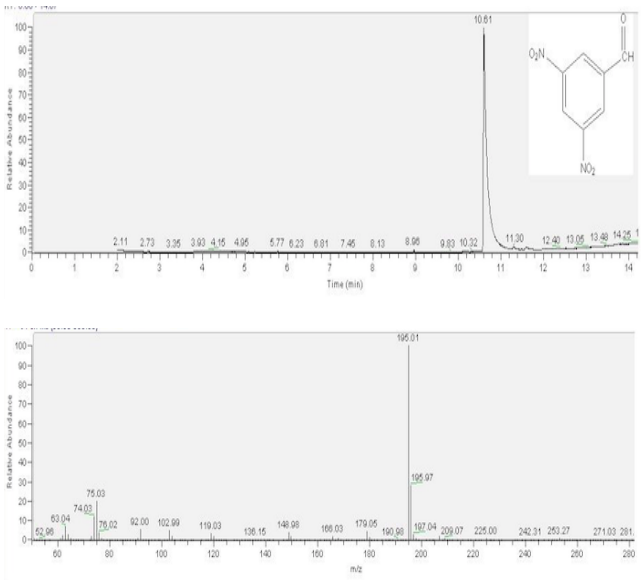

Fig. 2. GC-MS Analysis of Aldehyde using ITQ-700 GC-MS. Solvent is acetone.

TABLE I: MASS SPECTRUM ANALYSIS FOR 3,5-DINITROBENZALDEHYDE

Mass/charge (m/z)	Fragmentation
195.97	$C_6H_3(NO_2)_2COH$
148.98	$C_6H_3NO_2CO$
102.99	C_6H_3CO
75.03	C_6H_3

The infrared spectrum of 3, 5-dinitrobenzaldehyde was taken in acetone, using KBr salt plates. The spectrum, (Fig. 3) gives the carbonyl stretch at 1714 cm^{-1}, proving that the desired product was synthesized.

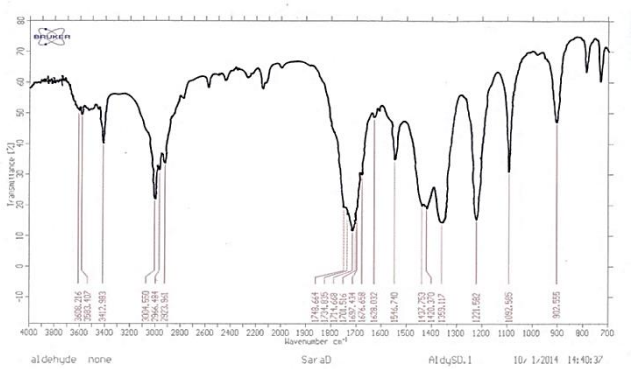

Fig. 3. Infrared Spectrum of 3,5-dinitrobenzaldehyde.

Both 5, 10, 15, 20-tetra-3, 5-dinitrophenylporphyrin and 5, 10, 15, 20-tetra-3, 5 diaminophenylporphyrin were characterized using UV-VIS spectroscopy which results are presented in Fig. 4 and Fig. 5, identifying both the Soret and Q-bands.

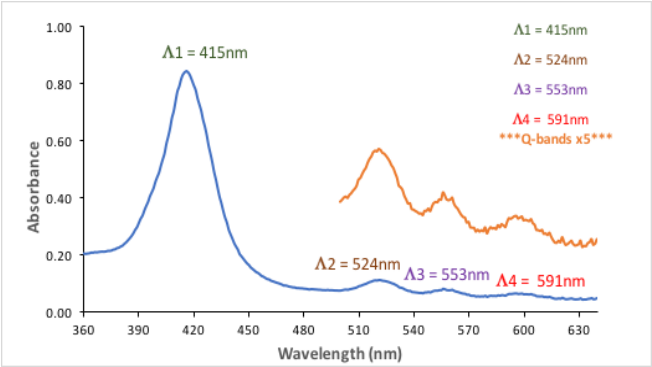

Fig. 4. UV-Vis spectrum of 5,10,15,20-*tetra*-3,5-dinitro phenylprophyrin.

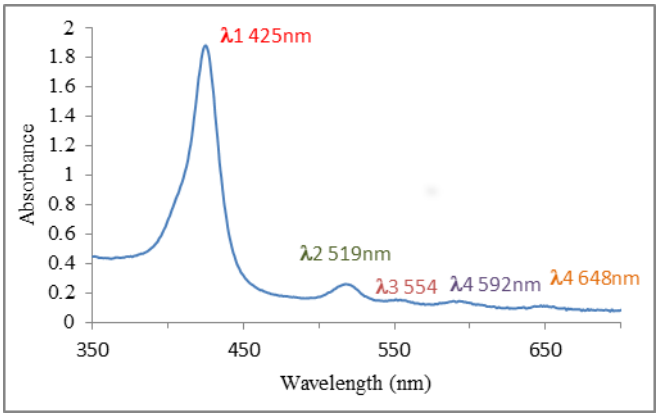

Fig. 5. UV-Vis spectrum of 5,10,15,20-tetra-3,5-diaminophenylprophyrin.

The change in the spectra of both compounds shows a red shift from DNPP to DAPP in the soret band with corresponding blue shift in the Q bands as shown Table II which agrees well with structural data relating Type I and Type II porphyrin molecular spectra interpretations [12].

TABLE II: COMPARISON OF DNPP AND DAPP PEAK ASSIGNMENTS

DNPP	DAPP
λ 415	λ 425
λ 524	λ 519
λ 553	λ 554
λ 591	λ 550

The fluorescence emission spectrum of DAPP is presented in Fig. 6 below.

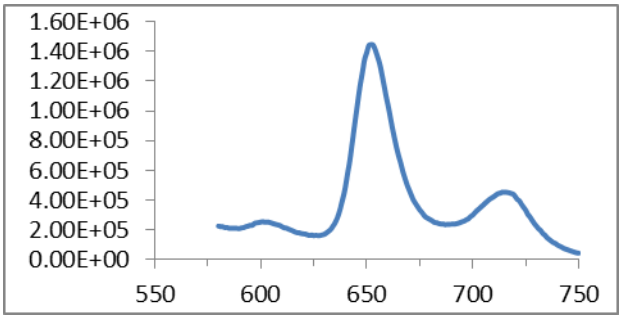

Fig. 6. The Fluorescence Emission of the DAPP.

IV. CONCLUSION

This project is a study in progress. The precursor monomer is prepared and the next steps are synthesis of the polymer.

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