EXPERIMENT 23

# Bioinorganic Coordination Chemistry: Copper(II) Tetraphenylporphyrinate

*Note: This experiment requires 2 hours for Part A and 6 hours for Part B.*

Metal ions play vital roles in many biological processes, and at least seven transition metals (iron, zinc, copper, manganese, cobalt, nickel, and molybdenum) are essential to almost all life on earth. These metals are key components of many important proteins. In some cases, the metals coordinate to the nitrogen, sulfur, or oxygen atoms in the side chains of certain amino acids that make up the protein's structure; among these "metal-binding" amino acids are histidine, cysteine, methionine, tyrosine, aspartic acid, and glutamic acid. In other cases, however, the transition metals are bound to special ligands, the most important of which are the porphyrins. Metal-bearing porphyrin complexes are called metalloporphyrins.

Porphyrins have the general structure shown in Figure 23-1; they are compounds with a central 16-membered ring consisting of four pyrrole subunits linked by one-carbon bridges. The porphyrin ring is polyunsaturated and completely conjugated; consequently, porphyrins and their complexes with transition metals are intensely colored. In metalloporphyrins, a metal atom coordinates

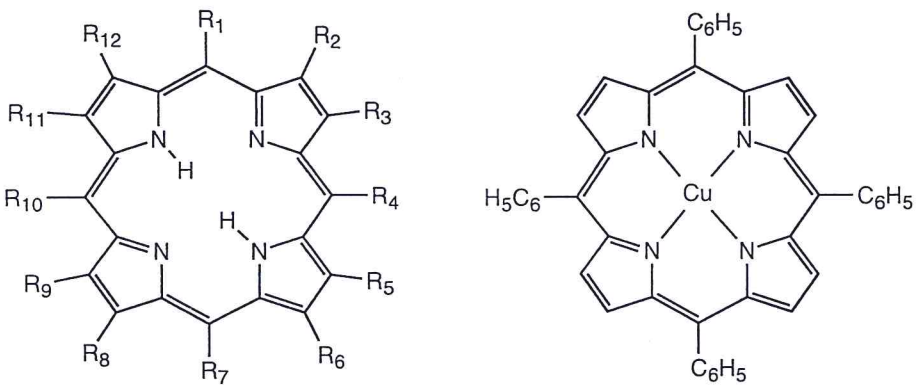



**Figure 23-1**
General structure of a porphyrin with peripheral groups $R_1$–$R_{12}$; structure of the copper(II) complex Cu(TPP).

to the four nitrogen atoms and displaces the two central hydrogen atoms. One important metalloporphyrin complex that is found in almost all animals is hemoglobin, which contains four iron-porphyrin units. In vertebrates, hemoglobin is responsible for the transport of $O_2$ from the lung to cells throughout the body. Metalloporphyin complexes perform a variety of other important biochemical functions; for example, they serve as electron-transfer relays and as oxidation catalysts.

Closely related to metalloporphyrins are the chlorins, corrins, and corphins, which have similar though not identical structures. Two important examples of such porphyrin-like molecules are chlorophyll and vitamin $B_{12}$ (see Experiment 21). Phthalocyanines, which are nitrogen-rich analogues of porphyrins, are produced and used industrially as pigments and catalysts.

Naturally occurring porphyrins generally have a variety of different organic groups on the periphery (exterior) of the ring. The synthesis of such polysubstituted porphyrin rings is a challenging task for the chemist. For many purposes, however, the characteristic chemical properties associated with metalloporphyrins are exhibited by simpler analogues with small peripheral groups. The most important examples of such synthetic analogues are the complexes of *meso*-tetraphenylporphyrin (abbreviated $H_2TPP$), where the "meso" designation means that the phenyl groups are located on the four carbon atoms that bridge between the pyrrole rings. All the other peripheral groups in $H_2TPP$ are hydrogen atoms, and the chemical formula of $H_2TPP$ is $C_{44}H_{30}N_4$. In this experiment, you will prepare $H_2TPP$ and convert it to its copper complex Cu(TPP), whose structure is shown in Figure 23-1.

This experiment begins with the preparation of $H_2TPP$ by the condensation of four molecules each of benzaldehyde and pyrrole. This reaction does not give a high yield but the starting materials are inexpensive and the product is easily isolated. The low yields of this reaction illustrate the difficulty of assembling a large ring in a "one-pot" reaction. In some cases, but not this one, the yields of such cyclization reactions can be improved by adding metal ions to the solution, which assist in the assembly of the macrocycle by binding to the reactants and orienting them in such a fashion to favor formation of the ring.

The stoichiometry of the reaction of benzaldehyde and pyrrole is as follows:

$$4\,C_6H_5CHO + 4\,C_4H_4NH + \tfrac{3}{2}\,O_2 \longrightarrow C_{44}H_{30}N_4 + 7\,H_2O$$

The mechanism of the cyclization reaction is known in some detail: It involves formation of a carbocation by addition of a proton to benzaldehyde, followed by electrophilic attack of the carbocation at the $\alpha$ position of pyrrole. Loss of water generates a new carbocation, which attacks a second pyrrole ring. These steps are repeated, and eventually a nonconjugated macrocycle is formed. Oxidation of this macrocycle by $O_2$ generates the fully conjugated porphyrin ring.

Because the oxidation step is not quantitative, however, one of the principal contaminants is *meso*-tetraphenylchlorin ($H_2TPC$), a compound that contains two more hydrogen atoms than $H_2TPP$. The chemical structure of $H_2TPC$ is shown in Figure 23-2; the extra hydrogen atoms are present on the $\beta$-carbon atoms of one of the pyrrole rings:

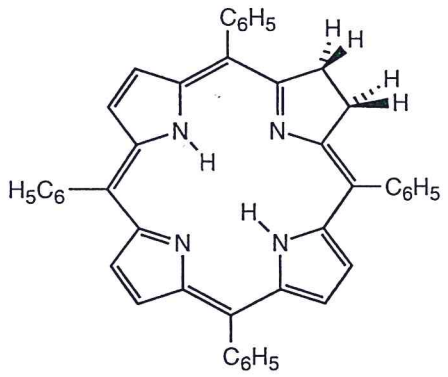



**Figure 23-2**
Structure of *meso*-tetraphenylchlorin ($H_2TPC$).

Tetraphenylporphyrin and its metal complexes are best separated from tetraphenylchlorin impurities by chromatography.

The conversion of $H_2TPP$ to Cu(TPP) is achieved by the addition of an excess of copper(II) acetate. The copper(II) center replaces the two nitrogen-bound protons at the center of the $H_2TPP$ ring:

$$H_2TPP + Cu(O_2CCH_3)_2(H_2O) \longrightarrow Cu(TPP) + 2\,HO_2CCH_3 + H_2O$$

The insertion of a metal ion into the porphyrin ring is sometimes very slow even though the reaction is very favorable thermodynamically. This somewhat surprising behavior reflects the rigidity and steric constraints of the macrocycle, which make it difficult for the porphyrin nitrogen atoms to approach a metal center that is already surrounded by other ligands (such as water or acetate groups).

**Thin-Layer and Column Chromatography**

One of the goals of this experiment is to illustrate standard chromatographic techniques that are used in the isolation of pure compounds from complex reaction mixtures. Thin-layer chromatography (TLC) will be used to test the purity of small amounts of the Cu(TPP) sample and to explore the chromatographic conditions necessary to purify it. Once these conditions are established, column chromatography will be utilized to separate larger amounts of Cu(TPP) from copper(II) tetraphenylchlorin and other impurities.

Separations effected by both TLC and column chromatography are based on the tendency of molecules to bind to certain solids called adsorbents. The adsorbents most frequently used are silica gel and alumina. Silica gel, which will be used as the adsorbent in this experiment, is to a first approximation a hydrated form of silicon dioxide, $SiO_2 \cdot x\,H_2O$. It does, however, contain significant amounts of other inorganic salts whose amounts vary from one silica gel preparation to another. For TLC, the silica gel is often mixed with plaster to help bind the gel to a glass support.

When silica gel is heated in strongly acidic or basic solutions, it acquires acidic or basic properties. Acid-treated silica gel strongly adsorbs (or binds) basic compounds such as amines, whereas base-treated silica gel adsorbs acidic compounds. The adsorption properties of silica gels also depend on their water content. If the gels are strongly heated under vacuum, water is driven off the silica gel leaving sites where other polar molecules strongly adsorb. Less strongly adsorbing silica gel can be prepared by adding back small amounts of $H_2O$ to occupy some of the adsorption sites. By altering the water content, it is therefore possible to control the degree to which silica gel binds various compounds.

In TLC, a thin layer (0.1–2 mm thick) of the adsorbent is spread onto a flat surface. The TLC plates can be purchased commercially and can also be prepared by coating microscope slides. A small amount of the sample to be separated is dissolved in a small volume of a suitable solvent. It is essential that the sample to be tested be completely dissolved. With a capillary, a spot (3–5 mm in diameter) of the solution is placed about 8 mm from one end of the TLC plate. The plate is allowed to dry and then the procedure is repeated to add sample to the same spot. The spot should be kept small for maximum separation of the components. The TLC plate is then placed in a bottle that contains a few milliliters of solvent (Fig. 23-3). The solvent level must be lower than the spot on the

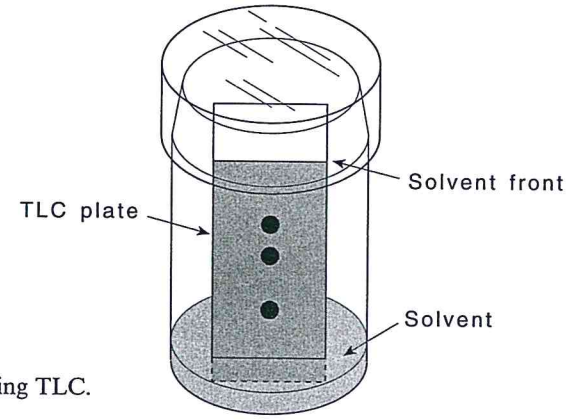


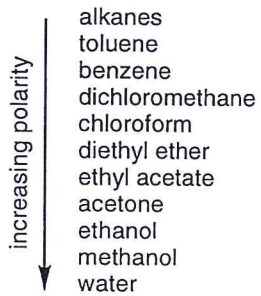

**Figure 23-3**
Separation using TLC.

plate. The bottle is capped and left undisturbed while the solvent rises up the silica gel by capillary action. If the proper solvent is chosen, the mixture will begin to move up the plate behind the solvent front. Ideally, different compounds present in the mixture will move up the plate at different rates, and thus will be separated.

When the solvent has moved about three-fourths of the way up, the plate should be removed from the bottle. If the compounds in the mixture are colored, it will be obvious if a separation has occurred. If some of the compounds are colorless, their locations on the TLC plate can usually be established by placing the air-dried TLC plate in a bottle containing a few crystals of iodine. The iodine sublimes and adsorbs in the areas where the compounds are located. Thus, dark brown spots on the plate indicate the locations of the components of the original sample. Alternatively, some TLC plates contain a fluorescent indicator:

Upon illumination with an ultraviolet (UV) light, the air-dried plate will glow in all places except the spots where compounds are located.

The separation of mixtures into their components by chromatography mainly depends on differences in the adsorption tendencies and solubilities of the components. Compounds that are weakly adsorbed and that are readily soluble in the solvent will move or elute quickly; in contrast, compounds that are strongly adsorbed and that are poorly soluble in the solvent will elute slowly. Finding the solvent that effects the best separation of the components is not easy, but generally a solvent that dissolves the desired compound moderately well will allow the compound to move up the plate; it can only be hoped that the impurities do not migrate at the same rate as does the compound of interest. If the solvent that was chosen does not separate the components of the mixture, other solvents either more or less polar than the first should be tried until a solvent that gives a separation is found. The polarities of some common chromatographic solvents increase in the following order:




It is sometimes convenient to use mixtures of solvents. For example, mixtures of ethyl acetate in dichloromethane often succeed in effecting a useful separation where other solvents do not.

At this point it is probably obvious that the successful choice of adsorbent and solvent is an art that is learned largely by doing chromatographic separations. The references at the end of this experiment do offer, however, many hints on how to use these techniques effectively.

Having established the solvent or solvent mixture that will separate the sample on TLC plates, it is hoped that the same solvent can be used to separate larger quantities of the sample on a silica gel chromatography column. Generally, this is possible. It is necessary, however, to use a much larger silica gel particle size for column chromatography (80–200 mesh) than that used in TLC (finer than 200 mesh).

The chromatography column (Fig. 23-4) consists of a glass tube that typically is about 3 cm in diameter and 30 cm long. First, a small glass wool plug is pushed to the bottom of the column, and a 5-mm layer of sand is added. A slurry of the silica gel in the solvent to be used in the separation is then poured onto the sand. The column is drained until the solvent level is the same as the top of the silica gel, and then a mixture of the sample and silica gel (both suspended in a few milliliters of the solvent) is added to the column. (Alternatively, the sample may

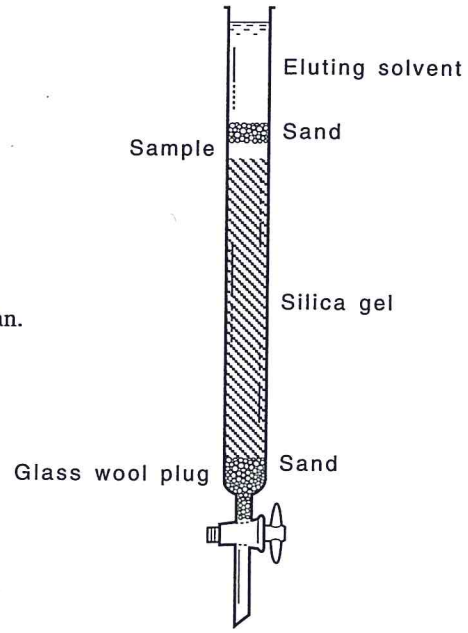



**Figure 23-4**
Chromatography column.

be dissolved in a small volume of the solvent and added to the column.) A 5-mm layer of sand is then added to the top of the silica gel.

From this point on, the solvent level should never fall below the top of the silica gel, because channels in the column will result, and the solution will pass down the channels without properly percolating through the adsorbent. The solvent is added to the column, and the movement of the compounds down the column begins. This process is called *elution*. The rate at which solvent is passed through the column is called the flow-rate; slow flow-rates give better separations than do fast flow-rates.

If the compounds in the sample are colored, it is easy to determine which fractions should be collected to obtain the desired products. If the compounds are colorless, fractions may have to be collected at regular volume intervals and examined for the presence of the desired compounds spectrophotometrically or by other techniques. Whereas the initial eluting solvent may elute one or more of the compounds, other compounds may require (as noted by TLC) more polar solvents to move them down the column. If a change in solvent is required, it is best to introduce it gradually by using first a mixture of the initial and the subsequent solvent and then finally the pure new solvent. Sometimes an abrupt change of solvent leads to the evolution of large amounts of heat when the new solvent adsorbs to the silica gel. Thermal expansion of the solvent creates channels in the adsorbent that can destroy the efficiency of the column.

The fractions eluted from the column that contain the desired compounds may simply be evaporated to dryness to give the pure product. Evaporation sometimes does not give a crystalline solid, and in such cases recrystallization of the material usually gives purer and better looking product.

Column chromatography has numerous variations. Although silica gel and alumina ($Al_2O_3$) are the most common adsorbents, many others have also been used. For materials that decompose at room temperature, chromatographic separations can be carried out in cooled, jacketed columns. Air-sensitive compounds have been chromatographed in an atmosphere of nitrogen or argon. All of these variations, however, are basically chromatography, and it is these basics that will be practiced in this experiment.

## EXPERIMENTAL PROCEDURE

### Part A

**_meso_-Tetraphenylporphyrin, $C_{44}H_{30}N_4$ ($H_2TPP$)**

Place a Teflon-coated stirbar in a 100-mL one-neck round-bottom flask, and add 40 mL of propanoic acid (sometimes called propionic acid). Fit the flask with a reflux condenser (see Fig. 23-5), and use a small amount of silicone grease on the joint. Leave the top of the reflux condenser open to air. Heat the acid to reflux with a rheostat-controlled heating mantle. When the propanoic acid begins to boil vigorously, add a mixture of 1.65 mL (15.75 mmol) of benzaldehyde and 1.0 mL (14.4 mmol) of pyrrole by pouring this solution down the reflux condenser. Measure out these liquids with a syringe or pipet. (*Note:* Freshly distilled pyrrole gives a higher yield of product but is not required.) Rinse the pyrrole and benzaldehyde down the condenser with 10 mL of propanoic acid. Continue to reflux the solution for 30 min, and then remove the heat and let the flask cool for a few minutes. Filter the dark brown mixture through a medium-porosity glass frit (see Figure 13-1). Rinse the mixture with a few mL of methanol until the washings are clear and purple crystals are left on the frit. Allow the crystals to dry by pulling air through them for a few minutes. Collect the purple crystals (do not scrape the

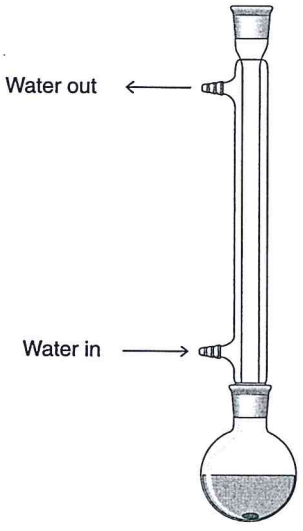



**Figure 23-5**
Apparatus for preparing $H_2TPP$.

frit too vigorously or your sample will become contaminated with powdered glass). Record the yield. Discard the wash solutions by pouring them into the containers provided for them.

Record the proton nuclear magnetic resonance ($^1$H NMR) spectrum of your product. To do this, place approximately 15 mg of your sample in a 5-mm diameter NMR tube and add about 1 mL of $CDCl_3$ (containing a small amount of tetramethylsilane, TMS, standard). The solution should fill the lower 4 cm of the NMR tube (using less $CDCl_3$ will lead to problems shimming the magnet). Record the spectrum from $\delta +10$ to $\delta -5$ (relative to TMS at $\delta$ 0), expand and plot any complex regions, and integrate the spectrum. Now add about 3 drops of $D_2O$, shake the two phase $CDCl_3$–$D_2O$ mixture vigorously for about 15 seconds, and again record the NMR spectrum.

## Part B

**(Tetraphenylporphyrinato)copper(II), Cu(TPP)**

The reaction set up is the same as in Part A (Fig. 23-5). Place 0.1 g (0.16 mmol) of $H_2TPP$ in a 100-mL one-neck round-bottom flask along with a stir bar. Add 20 mL of *N*,*N*-dimethylformamide (DMF) and stir the mixture (it is not necessary for all of the $H_2TPP$ to dissolve). To the dark purple solution, add 0.16 g (0.80 mmol) of hydrated copper(II) acetate, $Cu(O_2CCH_3)_2 \cdot H_2O$. Fit the flask with a reflux condenser and use grease to lubricate the joint. Bring the reaction mixture to reflux with a rheostat-controlled heating mantle. Allow the reaction to proceed for 30 min.

Because the copper complex is nonfluorescent, conversion to the copper complex can be confirmed by checking for complete quenching of the porphyrin fluorescence under long-wavelength UV light. Spot some of the reaction solution on a non-fluorescing TLC plate with a Pasteur pipet, and examine the plate with a long-wavelength UV light. If the conversion is not complete (i.e., if the spot still glows red—even around the edges), add additional copper(II) acetate to the reaction solution and reflux the mixture for 10 min; then redo the spot test. Alternatively, the conversion to the copper complex can be followed by UV–vis spectroscopy. Dip a capillary tube (open at both ends) into the reaction mixture and then dip the tube containing a little of the reaction solution into a cuvette containing pure dichloromethane. Complete conversion is indicated by the disappearance of the bands at 650 and 592 nm.

When the reaction is complete, cool the reaction mixture to room temperature in an ice–water bath for 5–10 min, and then add 50 mL of distilled water to precipitate the porphyrinic material. Transfer the slurry to a separatory funnel, and extract it three times with 25 mL of dichloromethane. Collect the organic (bottom) layer each time. Discard the aqueous layer that remains in the funnel (it typically is pale blue), and then pour the combined organic extracts back into the separatory funnel. Add 50 mL of distilled water, shake the mixture, and then drain out the organic (bottom) layer. Concentrate the organic layer to dryness on a rotary evaporator. Discard the aqueous wash solution that remains in the separatory funnel.