STUDIES OF PORPHYRIN METAL INTERACTIONS

by

Yuzuru Murata

T 1030

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Signed: Yuzuru Murata
Yuzuru Murata

Golden, Colorado
Date: May 20, 1965

Approved: George Luce
Thesis Advisor

Walter H Vernon
Head of Department

Golden, Colorado
Date: May 20, 1965

ABSTRACT

Protoporphyrin dimethyl ester was synthesized by esterification of protoporphyrin, and was used to study the iron and copper metallation reactions in chloroform, acetone and methyl alcohol. In these and other solvents, three different and quite stable protoporphyrin-iron (III) complexes characteristic of each solvent were formed. Their spectra are the chloroform type with two peaks, the acetone type with three peaks, and the methyl alcohol type with three peaks and a small hump.

The nature of these complexes was studied, one of which (the chloroform type), Fleisher and Wang (26) called a new sitting-atop type of intermediate.

The author's conclusion is that probably these complexes are neither intermediates nor new types of iron porphyrin complexes, but porphyrin di-cation (the chloroform type), monocation (the acetone type), and quasi-monocation (the methyl alcohol type). Porphyrin cations are probably a function of proton concentration as well as the basic character of solvent.

The copper metallation reaction in these solvents advanced into the formation of copper (II) metalloporphyrin without forming porphyrin cation when copper (II) acetate was used. Different, as yet unexplained, results

were obtained when copper (II) chloride was used as a source of copper.

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INTRODUCTION

Treibs reported for the first time in 1934 the existence of porphyrin compounds in organic mineral deposits (1). Since then, many papers have been published on the study of porphyrin compounds in crude oil or oil shale (2 - 5). The discovery of these porphyrin compounds has given some of the best evidence that the origin of petroleum is from living organisms. Thus, the nature of the porphyrin as it occurs in crude oil or oil shale has been of interest to petroleum chemists.

This paper basically aims at the investigation of the reaction mechanism of metal incorporation into the porphyrin nucleus, called metallation in this thesis, particularly the formation of an intermediate porphyrin metal complex.

The metallation reaction and metalloporphyrin compounds have been extensively studied both experimentally and theoretically in the various fields of chemistry (6, 10, 12).

The porphyrin nucleus is a tetradentate ligand, in which the space available for a coordinated metal has a maximum diameter of approximately 3.8Å (18) as shown in Fig. 1.

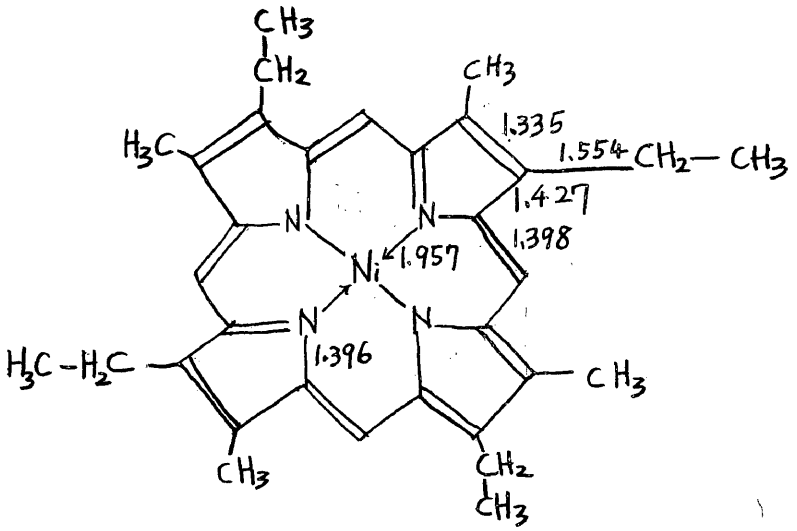


Figure 1.- Porphyrin bond length (Ni(II)-aetioporphyrin).

When coordination occurs, two protons are lost from nitrogen atoms, leaving two negative charges which are no doubt distributed about the whole inner ring. According to the classical picture, the metal ion is held in the plane of the porphyrin ring by two coordinate and two ionic bonds with the four nitrogen atoms (10).

Complexes with a number of monovalent cations are known (e.g. Na_2, K_2, Li_2 complexes); in these one of the cations sits slightly above, and the other slightly below the plane of the nucleus. When divalent metal ions (e.g. Co (II), Ni (II), Cu (II)) are chelated, the resulting tetracoordinate complex is square planar with a diameter of approximately 8.5Å and a thickness of approximately 4.7Å, and has zero residual charge. Some metal ions chelated in the porphyrin nucleus may be oxidized to the trivalent state (e.g. Fe (III), Mn (III), Co (III) complexes) and the resulting complexes have unit positive charge, and are associated with anions in the solvent medium (11, 19). Porphyrins differ from the more common chelating agents in that there is an

appreciable activation energy associated with the incorporation of the metal ion, the reaction rate being dependent upon the nature of the porphyrin, the nature of the metal ion and the solvent medium.

It is therefore necessary in discussing metalloporphyrins to distinguish between the thermodynamic stability, i.e., the equilibrium affinity of a porphyrin for a metal ion and the kinetic stability (or lability), i. e., the ease with which a complex may be formed or dissociated. Phillips deduced the order of thermodynamic stability of metalloporphyrin from consideration of spectroscopic, replacement and dissociation reaction data (20).

$$Pt\ (II) > Pd\ (II) > Ni\ (II) > Co\ (II) > Ag\ (II) > Cu\ (II) > Fe\ (II) > Zn\ (II) > Mg\ (II) > Cd\ (II) > Sn\ (II) > Li_2 > Na_2 > Ba\ (II) > K_2 > [Ag(I)]_2$$

As for kinetic stability, little quantitative information is available on the rates of the metallation of different ions under comparable reaction conditions. However, Erdman (21, 22), Corwin (23), Hobbs (24) and Fleisher (25) have reported the relative order of kinetic stability under various and incomparable reaction conditions by using various species of porphyrins. However, as explained above, this relative stability is extremely dependent upon species of metal ion, β-substituted functional group, properties of solvent medium and small amount of added agents such as detergent or chelating agent. Small amount of anionic detergent solution such as sodium dodecyl sulphate (SDS) accelerates metallation reaction rate, and the maximum rate of incorporation of Cu (II) into mesoporphyrin in the presence of 8-hydroxyquinoline (oxine) is obtained when the oxine to Cu ratio is 1:1, and is depressed as the ratio increases (19).

TABLE 1

Relative Order of Reactivity

Researcher	Porphyrin used	Solvent	Relative Order
Erdman	aetioporphyrin - I	benzene - HAc - HCl	Cu (II) > Ni (II) > Fe (III) > VO (II)
Corwin	aetioporphyrin - II	H_2SO_4 - HAc HCl - HAc	Co (II) > Ni (II) < Cu (II) $\ll$ Zn (II)
Hobbs	porphyrin* aggregate	HAc - pyridine	Ni (II) > VO (II)
Fleisher	tetrapyridyl-porphyrin	H_2O	Cu (II) > Zn (II) > Mn (II) > Co (II) > Fe (II) > Ni (II) > Cd (II)

* A mixture of porphyrins which has been obtained from a single crude oil sample.

Regarding the mechanism of metallation, Phillips and co-workers (39) suggested from their kinetic studies that reaction between metal ion and free porphyrin to form the corresponding metalloporphyrin takes place, as indicated in the reaction below, through a displacement mechanism of the SN_2 type rather than a dissociation mechanism.

$M^{+n} + PH_2 \rightleftharpoons PM^{+(n-2)} + 2H^+$ where M^{+n} represents metal ion, PH_2 a free porphyrin, and $PM^{+(n-2)}$ metalloporphyrin.

Fleisher and Wang (26) reported that their experimental results showed not only that metallation takes place through a displacement mechanism but also that the metal ion first combined with the porphyrin to form a reaction intermediate which subsequently decomposed into the normal metalloporphyrin and two hydrogen ions. This conclusion is based on the observation that protoporphyrin dimethyl ester (to be called PPM in this thesis) converted into iron (III) - protoporphyrin dimethyl ester (to be called Fe (III) - PPM

in this thesis) in the reaction with iron (III) chloride in chloroform after prolonged time. In this process, first, PPM formed a new type of complex (Fig. 2C) with absorption spectrum markedly different from that of the metalloporphyrin, and gradually changed to that of the corresponding metalloporphyrin compound (Fig. 2B). They proposed a structure with the metal sitting-atop the flat porphyrin molecule (to be referred as SAPP) (Fig. 3) for this intermediate compound deduced from spectroscopic data. As additional evidence for their hypothesis, they showed a spectroscopic similarity of this proposed intermediate metal porphyrin complex with porphyrin dication, that is, PH_4^{++} (Fig. 2D).

If alcohol or pyridine is added to the chloroform solution of this complex, the spectrum changes immediately back to that of the original protoporphyrin (Fig. 2A). This means that the binding between Fe (III) ion and protoporphyrin in the complex is weak and this complex may indeed be an intermediate in the metalloporphyrin formation. These observations suggest that the observed absorption spectrum is probably due to electronic transitions in the protoporphyrin rather than in the metal ion part of the complex. Similarly, if acetone solutions of the sitting-atop complexes of Fe (II), Fe (III), Co (II), Pt (IV), Zn (II), respectively, are diluted with water, the spectra immediately change to that of protoporphyrin dimethyl ester (Fig. 2A), as water molecules rapidly displace the organic ligand in the complex. In acetone some metal ions, Ni (II), Cu (II), Bi (III), Hg (II), Cd (II) react readily with PPM even at room temperature to form the corresponding metalloporphyrins.

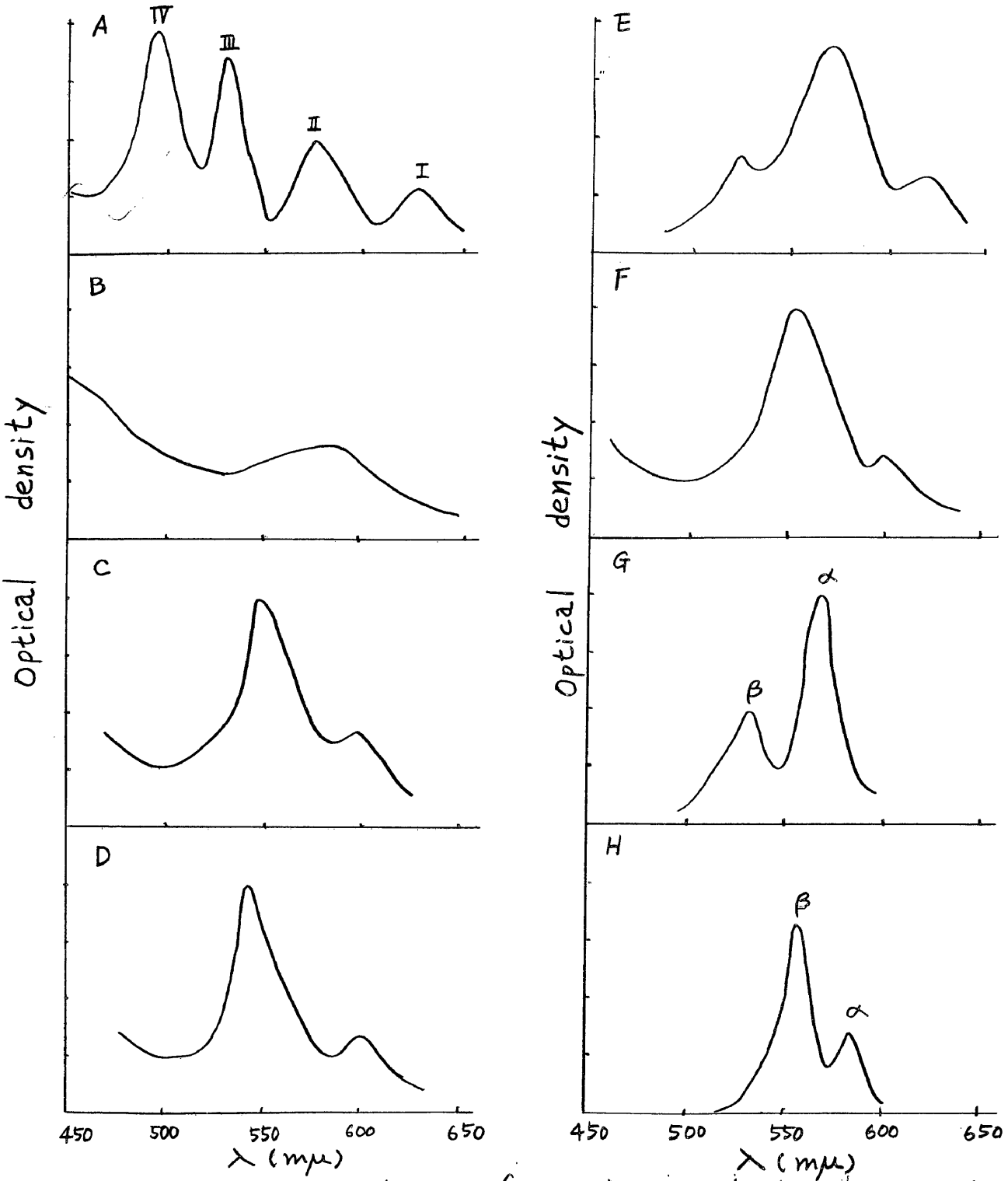


Figure 2.- Spectra of porphyrin derivatives: (A) PPM; (B) Fe(III)-PPM; (C) Fe(III)-SAPP; (D) PH_4^{++}; (E) PH_3^{+}; (F) $P^{=}$; (G) Cu(II)-PPM; (H) Cd(II)-PP; (13, 26).

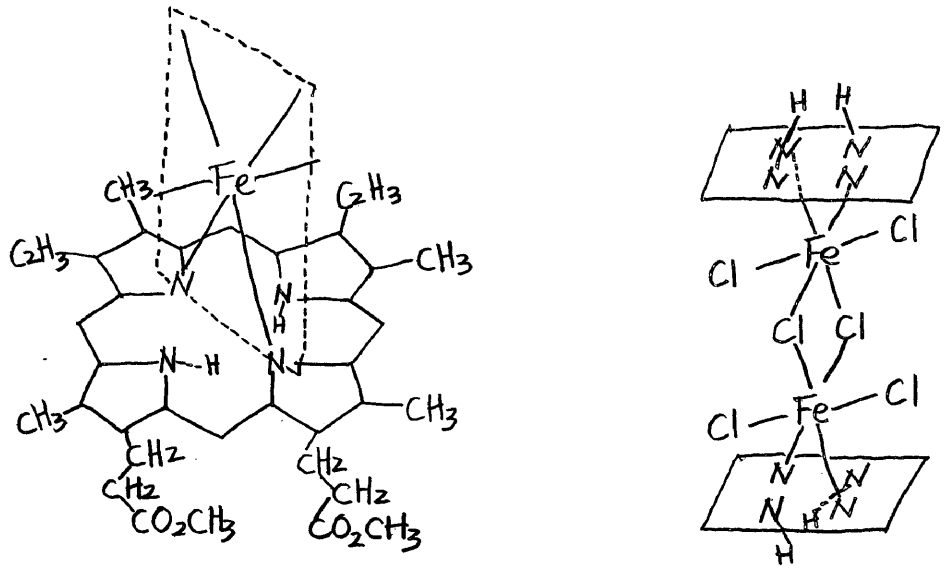


Figure 3.- Proposed sitting-atop type of structure for the reaction intermediate complex.

Fleisher and co-workers (27,28) claimed the metalloporphyrin is formed through the collapse of the sitting-atop type of intermediate (Fig. 3).

As available experimental techniques, the spectroscopic method (8, 14) is the most useful for quantitative and qualitative analysis of porphyrins. The free porphyrins are characterized by a four-band spectrum (Fig. 2A) in the visible range (400 — 700 mμ) and a single absorption maximum (the Soret band (29)) in the near ultraviolet range. Porphyrin monocation (PH_3^+) (Fig. 2E), porphyrin dianion (Fig. 2F) and metalloporphyrin (Fig. 2G - Cu (II) - PPM, Fig. 2H - Cd (II) - PP) have each characteristic spectra different enough for analytical purposes.

Regarding the theoretical interpretation of these visible absorption spectra, porphyrin is particularly ameanable to theoretical treatment (30, 31).

The four visible bands are really two pairs of bands which would be superposed if the porphyrin nucleus were strictly square and uniformly substituted. X-ray analysis has shown that its structure is slightly distorted from square, probably because the hydrogens bound on opposite nitrogen atoms each form hydrogen bonds with an adjacent nitrogen atom. This distortion leads to the fairly constant difference of 6 - 7 K cal between the energy levels of corresponding absorption bands (I and III, II and IV). This difference is removed in the dianion, the dication and the metalloporphyrin, and in all these cases only two of these bands are found. The α - (long wavelength) and β - bands of metalloporphyrin complexes seem to be related to bands I and III, II and IV, respectively. Thus bands II, IV and β are little affected by substituents, while bands I, III and α vary considerably (11). It is interesting to note that in the stable metalloporphyrins (Cu, Co and Ni) the α - band is dominant while in the weaker complexes (Mg and Ba) the β - band is stronger, suggesting that the more thermodynamically stable the complex, the more rigid its structure (11).

In this thesis the metallation reaction with Fe (III) and Cu (II) in neutral solvents is studied. Experiments were prompted by the statement of Fleisher and Wang that the metal-porphyrin complex formed a sitting-atop type of complex and readily converted to the corresponding metalloporphyrin. Iron(III) ion was used to compare with Fleisher and Wang's results, and copper ion was selected for the reason that copper is, from kinetic lability, relatively easily incorporated into the porphyrin nucleus.

For the achievement of these objectives, the procedures for preparing both iron and copper porphyrin were examined and accomplished.

LABORATORY WORK

The laboratory work for this thesis consists of preparing reagents, accomplishing analysis, preparing porphyrin derivatives (PPM and N,N' bis - (2 - aminoethyl) protoporphyrin diamide, which is referred to as PPA in this thesis, studying the metallation reaction, and studying the nature of the porphyrin metal complex.

Analytical Method

Among several available qualitative methods for the analysis of porphyrins, visible or near ultraviolet absorption spectroscopy was used. Solubility tests also furnished auxiliary information on identification of reaction products.

Absorption Spectroscopy

Every metal free porphyrin has four characteristic absorption bands in the visible range, which are located approximately at 625 mμ, 570 mμ, 530 mμ and 500 mμ. Besides these visible absorptions, porphyrins have a very strong ultraviolet absorption band around 400 mμ, which is called the Soret peak and extremely valuable for quantitative analysis. The identification of every porphyrin can be performed by the number of peaks, the maximum absorbance of each

peak and the relative peak height, although these peaks are slightly shifted by analytical conditions such as solvent medium or solvent basicity. When the metallation reaction occurs, the visible absorption spectrum with four peaks converts to one with two peaks characteristic enough to identify metal porphyrin. The visible and ultraviolet absorption spectra were measured on a Beckman DK - 2A ratio recording spectrophotometer with the following settings:

Scan time:	5
Scale expansion:	1x
Function:	Absorbance
Time constant:	0.10
Range:	0-1 Absorbance
Sensitivity:	0.012
Lamp:	Tungsten
Detector:	1 x Photomultiplier

For the standard reference of porphyrin and metalloporphyrin determination, typical spectra are tabulated in Fig. 4 and Table 2 and 3.

TABLE 2

Porphyrin Spectra in Organic Solvents

Porphyrin	Solvent	λmax (mμ) I	II	III	IV	Soret	Type	Ref.
Protoporphyrin	Pyridine	624	576	540	506		Aetio	(32)
PPM	Chloroform	630	575	541	505	407	Aetio	(33)
	Dioxane	631	575	538	504	406	Aetio	(33)

TABLE 3

Metalloporphyrin Spectra in Organic Solvents

Porphyrin	Solvent	λmax (mμ) I	II (α)	III (β)	IV	Soret	Type	Ref.
Fe (III) - PPM	Ether	638	585h	540	512	399		(15)
Fe (III) - Aetio	Dioxane	632	580h	535	510	373 402sh		(34)
Cu (II) - Aetio	Dioxane		562	525		395		(34)
Cu (II) - Aetio	Acetic acid -Pyridine		558	525		---		(23)

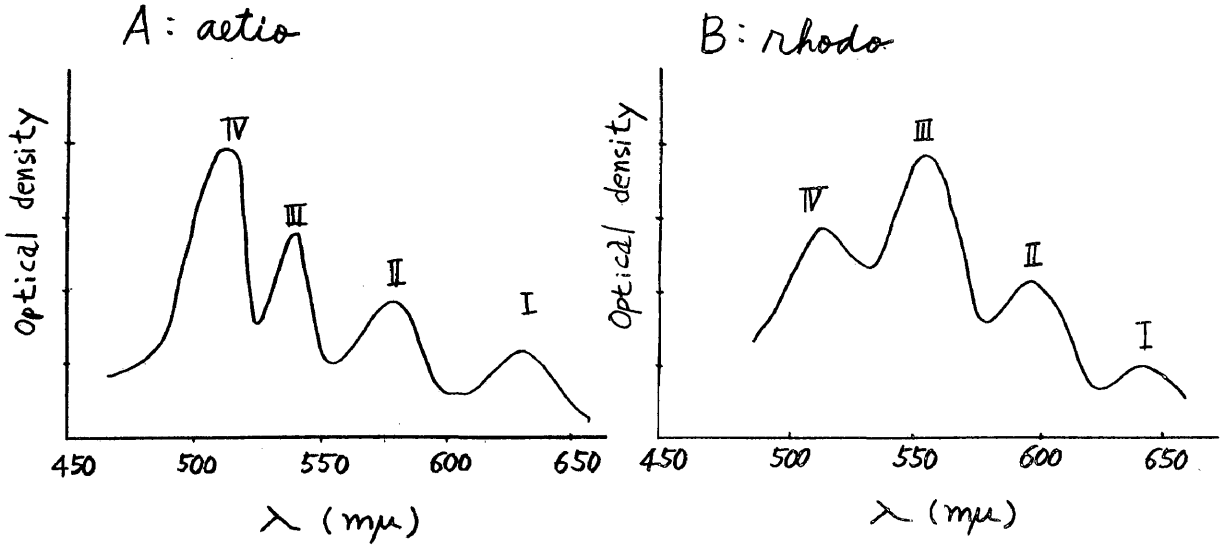


Figure 4.– Spectra of porphyrins : (A) aetioporphyrin ; (B) rhodoporphyrin.

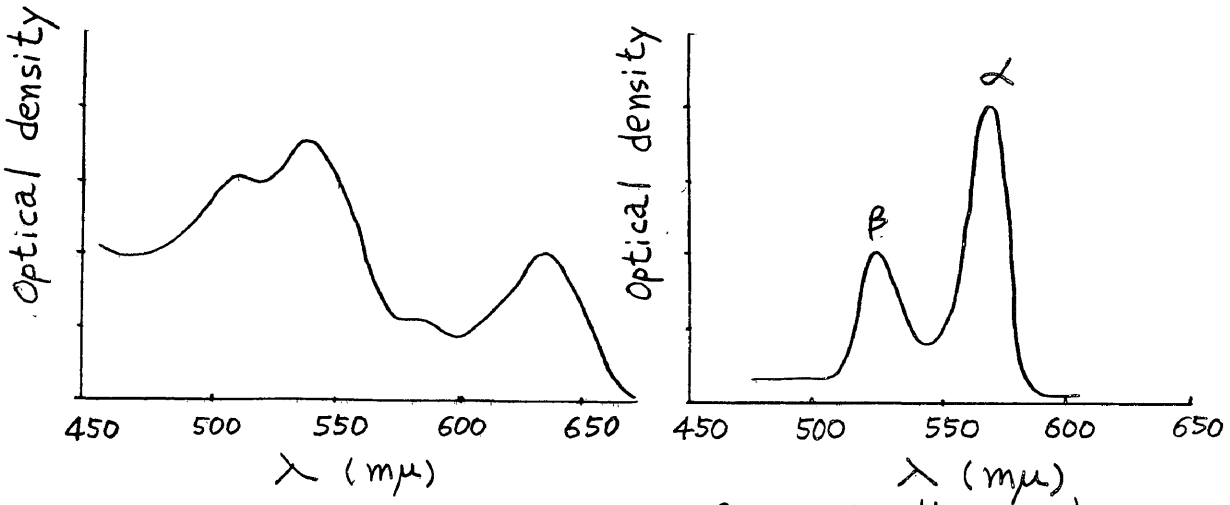


Figure 5.– Spectra of metalloporphyrins: (A) Fe(III)–aetioporphyrin in dioxane; (B) Cu(II)–aetioporphyrin in dioxane.

Solubility Test

Certain porphyrins have some degree of amphoteric character which is derived from carboxylic acid groups and basic nitrogen atoms in the porphyrin nucleus. Thus, generally, porphyrins with carboxylic acid side chains have high solubility in basic solvent, but lose this solubility when acidic side chains are masked by esterification. As will be shown later, the following experiment was verified at each state by simple solubility test.

Synthesis of N, N' - bis - (2 - aminoethyl) protoporphyrin diamide

Protoporphyrin (insoluble in benzene) $\longrightarrow$ [esterification]

$\longrightarrow$ PPM (soluble in benzene) $\longrightarrow$ [amidization]

$\longrightarrow$ PPA (soluble in water)

General classification of solubility is listed in Table 4.

Unlike most organic bases, crystalline porphyrins and porphyrin esters are not readily dissolved in dilute mineral acids; however, they usually dissolve in concentrated acids which are used as reaction solvents. Transfer of porphyrin used in these experiments from aqueous acid to ether can be accomplished in 4N hydrochloric acid and ether mixture.

TABLE 4

Porphyrin Solubility

	H_2O	H_2O detergent	aq. base	concd. H cl	HAc H_2CO_2 H_2SO_4	ether dioxane	CCl_4 $CH\ cl_3$	benzene	pyridine acetone	alcohol
A	X*	◎	○	◎	◎	○	◎	X	◎	○
B	X	◎	X	◎	◎	◎	◎			
C	◎	◎	○	◎	◎			X		
D	X	◎	X	◎	◎	◎	◎	◎	◎	○

◎: very soluble; ○: soluble; X: insoluble.

A: porphyrin with carboxylic acid side group; ex., protoporphyrin mesoporphyrin, uroporphyrin* (quite soluble in H_2O)

B: porphyrin with neither acidic nor basic side groups; ex., aetioporphyrin, phthalcyanine

C: porphyrin with basic side groups; ex., $\alpha,\beta,\gamma,\delta$-Tetra - (4 - pyridyl) - porphyrine (27)

D: porphyrin ester

Reagents

The reagents which did not require laboratory preparation are listed in this section with their chemical quality and source of supply. Because some species of metal ions form complexes with porphyrins and because the trace presence of peroxide may decompose small quantities of porphyrin, care must be taken to exclude metal ions, peroxides and other impurities from the solvents used. Therefore, except for extra pure reagents, solvent purification is obligatory.

Solvents

Methyl Alcohol: Technical grade methyl alcohol was purified by the following procedure. 10 g. of dry magnesium and 1 g. of iodine were placed in a 3-l. flask fitted with a double-surface reflux condenser. 100 ml. of methyl alcohol was added down the condenser and the mixture was warmed on a water-bath until all magnesium was converted to magnesium methoxide. Then 2 l. of methyl alcohol was added, and the mixture was refluxed for 30 min. Methyl alcohol was then distilled with exclusion of moisture, collecting the 58.5 — 59.5° fraction at 625 mm. Hg.

Ethyl Ether: ACS grade of ethyl ether was purified in order to remove peroxides. Ethyl ether was washed with a fresh solution of iron (II) sulfate in very dilute sulfuric acid; then the ethyl ether was washed three times with water, dried over calcium chloride and filtered.

Benzene (technical grade), chloroform (technical grade), pyridine (reagent grade), acetone (technical grade), iso-prophyl alcohol (technical grade),

glacial acetic acid (reagent grade), tetrahydrofuran (reagent grade), thiophene (reagent grade) were used after simple distillation.

Ethylenediamine: Ethylenediamine, (98%), produced by Eastman Organic Chemicals, was used as supplied.

Ferric chloride: Ferric chloride was ACS grade produced by Baker Chemical Co.

Cupric chloride: This material was reagent grade, produced by Allied Chemical Co.

Cupric Acetate: Cupric acetate was reagent grade produced by Baker Chemical Co.

Protoporphyrin: Protoporphyrin was the product of Bios Laboratories, Inc., and spectro-pure. Microscopic examination indicated high crystallinity.

Other common laboratory reagents such as sulfuric acid, hydrochloric acid or sodium hydroxide are reagent grade and used as such.

All water used is dimineralized water by ionic-resin exchange.

Preparations

Protoporphyrin dimethyl ester and N_1N^1-bis - (2 - aminoethyl) protoporphyrin diamide were synthesized to be used for the study of the metallation reaction.

Synthesis of Protoporphyrin Dimethyl Ester

The carboxylic side chains of porphyrins may be esterified at toom temperature or below with an alcohol saturated with gaseous hydrogen chloride or containing concentrated sulfuric acid (35). The following procedures were followed in esterification of protoporphyrin.

First, 5% (w/v) of sulfuric acid - methyl alcohol solution was prepared as follows. 19 ml. of absolute methyl alcohol and 0.6 ml. of sulfuric acid (s.g. 1.84) were mixed. Then 1.5 mg. of protoporphyrin was weighed into this solution and maintained in a dark place at room temperature for 24 hours. After esterification was completed, 25 ml. of chloroform was added to extract prepared PPM. Sulfuric acid was neutralized by two additions of 10 ml. of 2N sodium carbonate solution. This step was done extremely carefully by adding sodium carbonate solution drop by drop so as not to decompose the porphyrin. The chloroform layer was coloured reddish brown. The extract was washed with 10 ml. of water twice in order to remove remaining sodium carbonate, and then chloroform was evaporated to dryness in a vacuum evaporator. The PPM thus prepared was dissolved in 80 ml. of benzene. The physical properties of PPM are tabulated in Table 5, 7, and shown in Fig. 6 with the results of another prepared sample.

TABLE 5

Physical Properties of Prepared PPM

	Sample 1	Sample 2
M. P.	225 — 226°	
	Etio-type	Etio-type
Spectrum in	I (631 mμ), II (575 mμ)	I (631 mμ), II (575 mμ)
Chloroform	III (541 mμ), IV (505 mμ)	III (540 mμ), IV (505 mμ)
Solubility	soluble in benzene	soluble in benzene

Synthesis of N, N' - bis - (2 - aminoethyl) Protoporphyrin Diamide

A water-soluble porphyrin was prepared in order to test the occurrence

of metallation and to test pH dependence of the formation of porphyrin dication. The synthetic procedure is referred to as Fleisher and Wang's method (26).

1.6 mg. of protoporphyrin and 25 ml. of ethylene diamine were placed under draft in a round bottomed flask with gas-inducing side arm. Before heating, nitrogen gas was introduced for about 5 min. to replace air and to prevent the porphyrin from being oxidized. The nitrogen gas was previously washed by passing through chromium (II) chloride - concd. sulfuric acid. The flask was placed in a water bath for reflux at 85 - 90°. After 5 hrs. reflux under nitrogen stream at the rate of 2-bubbles per sec., excessive ethylene diamine was evaporated to dryness in rotary vacuum evaporator at room temperature. The product was completely water soluble. Then the product was washed by 10 ml. of benzene and 25 ml. of methyl alcohol-chloroform (vol. ratio 1:1) in this order to remove unreacted protoporphyrin or byproduct [1]. The physical properties of product, PPA, are listed in Table 6 and shown in Fig. 7.

TABLE 6

Physical Properties of Prepared PPA

M. P.	242 - 243°
Spectrum in water	Etio-type; I (620 mμ), II (566 mμ), III (538 mμ), IV (504 mμ)
Solubility	Very soluble in water

[1] Before the product was washed as above, the spectrum was recorded. (Fig. 7A).

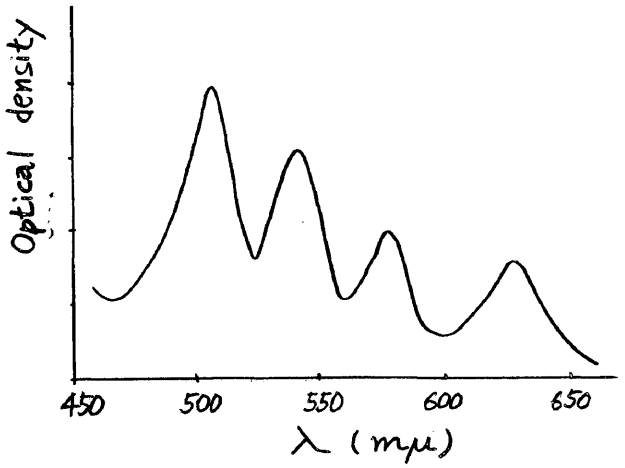


Figure 6. – Spectrum of prepared PPM in chloroform.

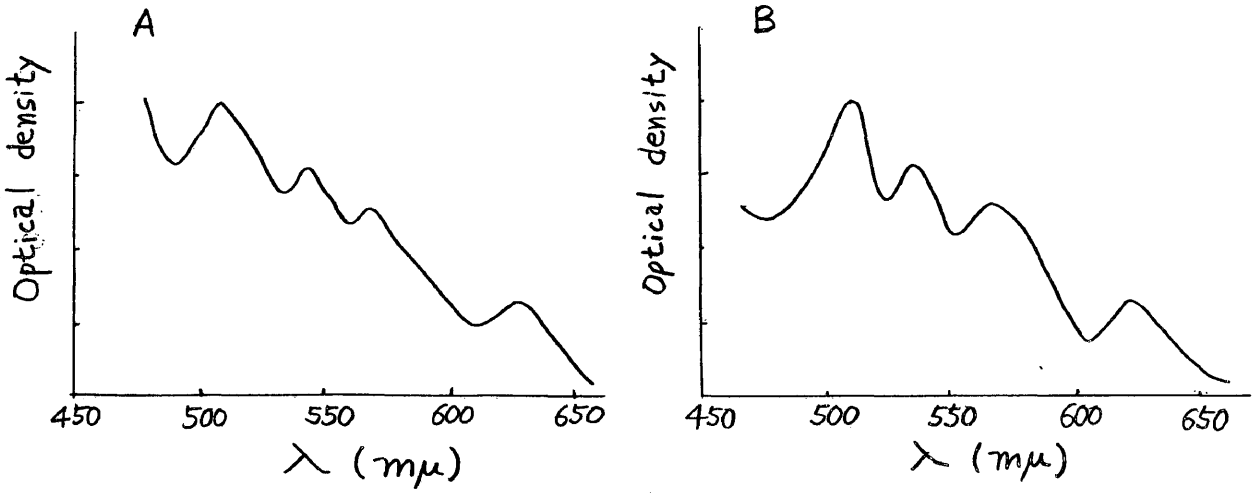


Figure 7. – Spectra of prepared PPA : (A) impure PPA ; (B) purified PPA.

TABLE 7

Spectra of Prepared PPM in Organic Solvents

	λ max (mμ)					
	I	II	III	IV	Soret	Type
Benzene	632	576	540	505	408	Aetio
Acetone	627	574	535	502	402	Aetio
Methylalcohol	628	574	538	503	400	Aetio

Metallation Reaction

Fleisher and Wang's method (26) was used to prepare Fe (III) - PPM and Cu (II) - PPM. In order to synthesize standard Fe (III) - PPM, the iron (II) sulfate method was used (36).

Iron (III) Metallation (Synthesis of Haemin)

Iron (II) Sulfate Method. 20 ml. of previously prepared PPM (concentration 36.6×10^{-6} M in benzene) was measured and dried in rotary vacuum evaporator at room temperature. 1 ml. of pyridine, 50 ml. of glacial acetic acid and 1 ml. of saturated aqueous iron (II) sulfate were added quickly in this order into a round bottomed flask with side arm. A stream of nitrogen gas (oxygen and moisture free) was passed for about 5 min. to replace air, and then the solution was kept in water bath at 80 - 85°. After 15 min. reflux under nitrogen stream, the mixture was placed in a beaker and allowed to stand in air for about 10 min. to allow oxidation to the Fe (III) complex to occur. Then, reddish brown Fe (III) - PPM was extracted with ether and residual porphyrin was removed by extraction with 6N of hydrochloric acid. This extracting solvent was prepared as follows. 10 ml. of 6N hydrochloric

acid and 70 ml. of ether was mixed thoroughly. The concentration of hydrogen ion was adjusted (pH 4). When Fe (III) - PPM was added, quick shaking was necessary not to form a white scum in the ether layer. Ether layer became reddish brown while hydrochloric acid layer was greenish yellow. After separation, Fe (III) - PPM in ether showed spectrum in Fig. 8A and Table 8, which is identical to the reference spectrum in Fig. 5A and Table 3.

TABLE 8

Spectra of Prepared Fe (III) - PPM (Haemin)

	λ max (mμ)				
	I	II	III	IV	Soret
Ether	642	587h*1	540	508	---
Chloroform	640	590h	541sh*2	510	385

*1: hump;
*2: shoulder

Fleisher and Wang's Method

10 ml. of PPM (concentration 36.6×10^{-6} M) was taken and evaporated to dryness in the rotary vacuum evaporator. The following solvents were used for the metallation reaction; chloroform, acetone and methyl alcohol.

10 ml. of each solvent was used to dissolve the dried PPM sample. The concentration of PPM was 0.37 μM per 10 ml. of solvent. Ca. 1 mg. of iron (III) chloride (3.7 μg-mole) was added to each solution. Right after mixing, a new type of iron (III) - protoporphyrin dimethyl ester complex (to be referred to as PPM - Fe (III) complex) was formed, whose spectra were markedly different from those of Fe (III) - PPM obtained in Fig. 8 or Fe (III) - Aetio referred in Fig. 5. Besides each of the three solvents gave definitely

different spectra as shown in Fig. 9 and Table 9. None of these three PPM - Fe (III) complexes displayed any change of spectra for 24 hrs. at room temperature. The one thing that calls upon the writer's interest is the surprising resemblance of the spectra of the acetone type to PH_3^+ shown in Fig. 2E and of the chloroform type to PH^{++} shown in Fig. 2D. The spectrum of chloroform type is exactly the same as that presented by Fleisher et al (26) (Fig. 2C).

TABLE 9

Spectra of PPM - Fe (III) Complex

Solvent	λ max (mμ)				
	I	II	III	IV	Soret
Chloroform	602	558			412 363
Acetone	610	562	536		396sh
Methyl Alcohol	608	566	534	502h	402

Copper Metallation

In a similar fashion, 0.38 μM of PPM and 10 ml. of the same three solvents were treated. Both copper (II) chloride and copper (II) acetate were used as a source of copper (II) ion, because there are some examples in which the metallation reaction is affected by the specie of metal-salt. Both copper salts were weighed to the nearest 1 mg; therefore, the amount of copper (II) chloride was ca. 5.9 μg-mole used and that of copper (II) acetate was ca. 5.0 μg-mole used.

The copper metallation reaction was fast enough to allow the investigator to follow the rate of the reaction as shown in Fig. 10, 11, 12 and

Table 10, 11, 12. In chloroform, soon after copper (II) acetate was added, the order of the height of peaks altered from I < II < III < IV to I < IV < III < II. As time passed, the first and the fourth peaks decreased in intensity while the second and the third peaks increased in intensity. The intensity of the second peak was remarkably augmented at the early stage of metallation whilst the third peak did not increase as much. Forty min. after the reaction started, the first and the fourth peaks had almost disappeared to remain just as small humps. After that, no discernable changes occurred. The resulting two sharp peaks, their relative height and respective maximum absorbance, are believed to be enough evidence to conclude that the copper metallation reaction to form Cu (II) - PPM occurred.

In acetone (Fig. 11) and methyl alcohol (Fig. 12), the metallation reaction also appeared to occur. The relative rate of the metallation were the following order: methyl alcohol > chloroform > acetone. The only striking difference from the metallation reaction in chloroform was that a fairly intense broad hump was to be found about 700 mμ. In acetone, the height of this hump (peak 1) was less than half of the highest peak (peak II); however, in methyl alcohol it was almost the same degree of absorbance as the highest one.

When copper (II) chloride was used as a source of copper, quite different results emerged. In solvent, acetone, the Cu (II) - PPM may have been formed (Fig. 13A), but the peaks are shifted to shorter wavelength. The reaction should be investigated further. In solvent, chloroform, a definite new spectrum appeared (Fig. 13B). It has some of the characteristics of the acetone type PPM - Fe (III) complex.

Studies of the Nature of the PPM - Fe (III) Complex

The results of the preceding metallation reaction of iron (III) indicated that PPM - Fe (III) complex in each solvent might be relatively stable intermediate complex as presented by Fleisher et al or another stable complex. Therefore the following tests were made to study the nature of the PPM - Fe (III) complex. They are the test for the conversion of PPM - Fe (III) to Fe (III) - PPM complex, the resemblance of the spectra with PH_4^{++} and PH_3^{+}, and the strength of bond of PPM - Fe (III) complex.

Conversion Test of PPM - Fe (III) Complex to Fe (III) - PPM (Haemin)

PPM - Fe (III) complex was kept at room temperature in chloroform for five days, during which time no change of spectrum occurred. This solution was transferred to an oil bath at 50-55°. After four days, there was still no spectral sign of reaction (Fig. 9A). Dimethyl formamide was added to a sample from this solution. Immediate reversion of the complex to the original metal free porphyrin took place. After 13 days in the oil bath, the spectrum indicated a small amount of decomposition (Fig. 14). After 15 days, the porphyrin (PPM - Fe (III) complex) was decomposed and dark brown decomposition products spotted the inside of the flask. The spectra of hemin (Fe (III) - PPM) as shown in Fig. 2B did not result from this experiment. This experiment was repeated under the same reaction condition with the same results, (Fig. 9A, Table 13).

In acetone and in methyl alcohol, the complex was stable even after 16 days (acetone) and 17 days (methyl alcohol) (Table 14). After nearly half a year, the sample in methyl alcohol showed some decomposition and the sample in

acetone had decomposed. No Fe (III) - PPM was formed. The difference in spectra of PPM - Fe (III) complex in acetone and in methyl alcohol are the weak absorbance (501 mμ) which the acetone type did not exhibit and the relative intensities in the second and the third peaks (Fig. 9B and 9C). When pyridine was added to PPM - Fe (III) complex in both of the solvents (10 days' sample), immediate reversion to free porphyrin occurred.

Preparations of PPM Dication (PH_4^{++})

10 ml. of PPM in benzene was evaporated to dryness in rotary vacuum evaporator and dissolved in 10 ml. of chloroform. One drop (ca. 0.05 ml.) of IN hydrochloric acid was added to ca. 4 ml. of this solution, the remarkably similar spectrum to that of PPM - Fe (III) complex in chloroform was obtained (Fig. 15). The maximum absorbance (604 mμ, 560 mμ) were shifted to the longer wavelength by 4 mμ, but relative intensity was nearly the same.

Reversion of PPM - Fe (III) complex

Equal volume of the following solvents were added to benzene solution of PPM - Fe (III) complex. Pyridine, dimethyl formamide (DMF), dimethyl sulfoxide (DMS), iroprophyl alcohol, tetrahydrofuran (THF), thiophene, acetonitrile, ethyl mercaptan and nitromethane.

Pyridine, DMF and DMS caused the spectra to revert to that of the original free porphyrin. Isoprophyl alcohol and THF caused the spectra to change to the similar spectrum of the PPM - Fe (III) complex in methyl alcohol. Acetonitrile caused a spectral conversion to the acetone type, and thiophene, ethyl mercaptan and nitromethane gave no change of spectra (Table 15).

Auxiliary Tests

pH dependence of PPA Spectra

Previously prepared PPA in ethylene diamine was evaporated to dryness in rotary vacuum evaporator and dissolved in 25 ml. of water (concentration: 56.8×10^{-6} M). pH of this sample was 10.0 and showed aetio type spectrum (Fig. 16A). As the pH was decreased by adding IN hydrochloric acid, spectrum gradually converted as indicated in Fig. 16B. Four very broad peaks were observed when the pH was 4.5 (Fig. 16C). A part of this sample was added to ca. 1 mg. of iron (III) chloride. More broad spectrum appeared (Fig. 16D). When the pH of the solution was decreased to 1.9, the spectrum changed to that of PH_4^{++}. A remarkable similarity of the spectrum with that of PPM - Fe (III) complex in chloroform or benzene was noticed. There are two distinct absorptions which have maximum intensity at 591 mμ and 551 mμ (Fig. 16E). These absorbances are shifted to shorter wavelength by approximately 10 mμ in comparison with PPM - Fe (III) complex in benzene.

The Iron Metallation Reaction by Anhydrous Iron (III) Chloride

The source of iron (III) chloride used in the preceding metallation reaction (Fleisher's method) had water of crystallization ($FeCl_3 \cdot 6H_2O$). So the iron metallation reaction with anhydrous iron (III) chloride was attempted within a moisture free glove bag. Three 100-ml. beakers which contained ca. 20 ml. of phosphorous pentaoxide, respectively, were placed in the glove bag for 10 hrs. with other materials to remove moisture. Fine particle of anhydrous iron (III) chloride was added to each sample: PPM in chloroform, benzene, acetone and methyl alcohol. The same spectra as the preceding

experiments appeared (Fig. 9 and Table 16). This indicates that the presence of water of crystallization has no effect on the formation of PPM - Fe (III) complex.

EXPERIMENTAL RESULTS

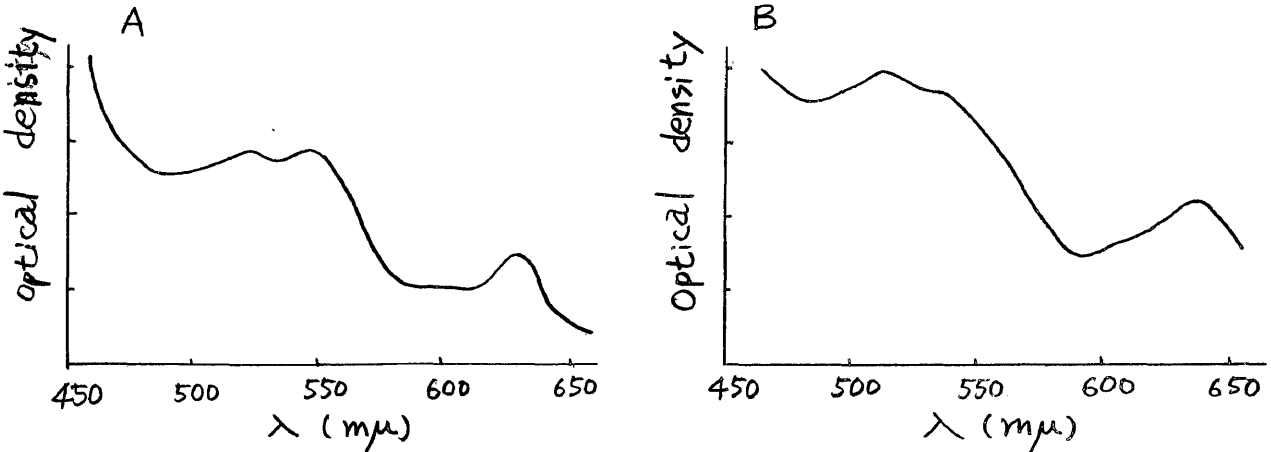


Figure 8.— Spectra of prepared Fe(III)-PPM (Haemin): (A) ether ; (B) chloroform.

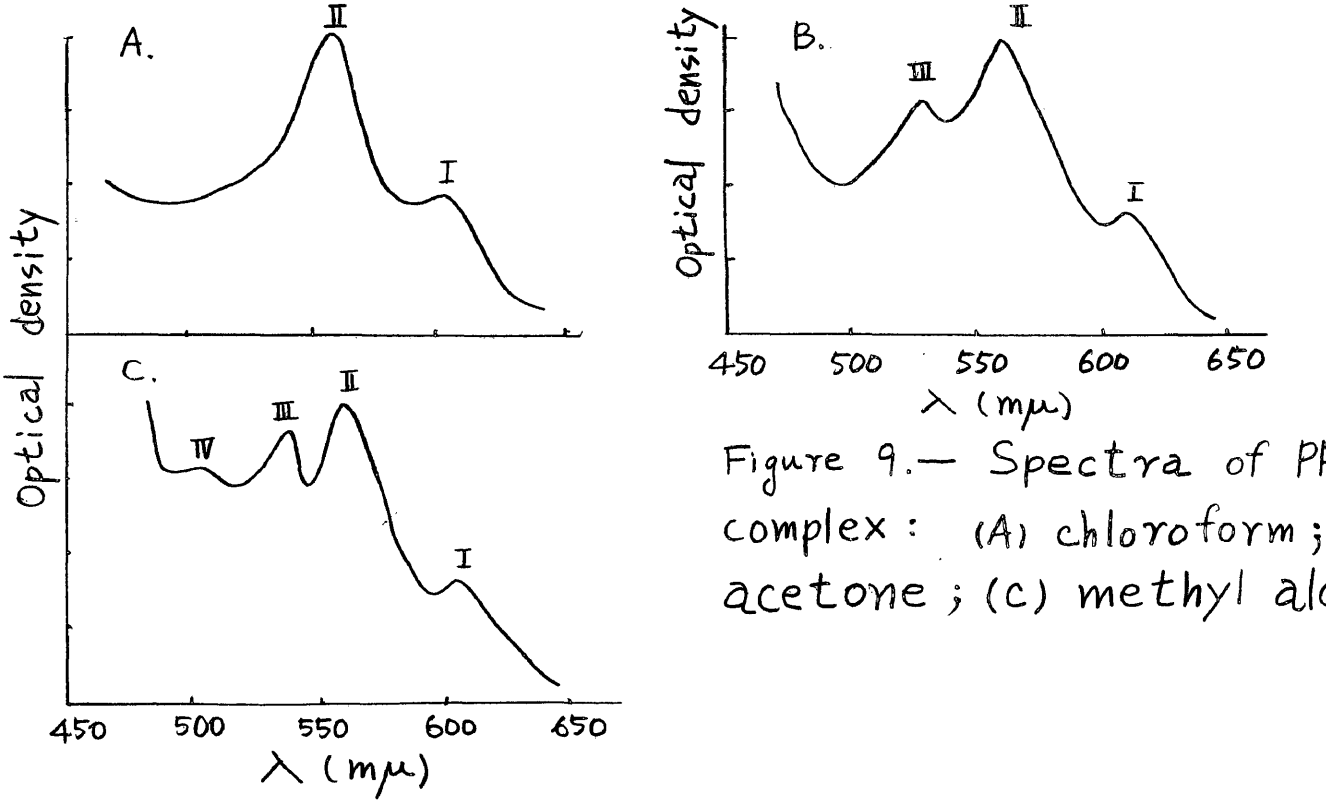


Figure 9.— Spectra of PPM-Fe(III) complex : (A) chloroform ;(B) acetone ; (C) methyl alcohol

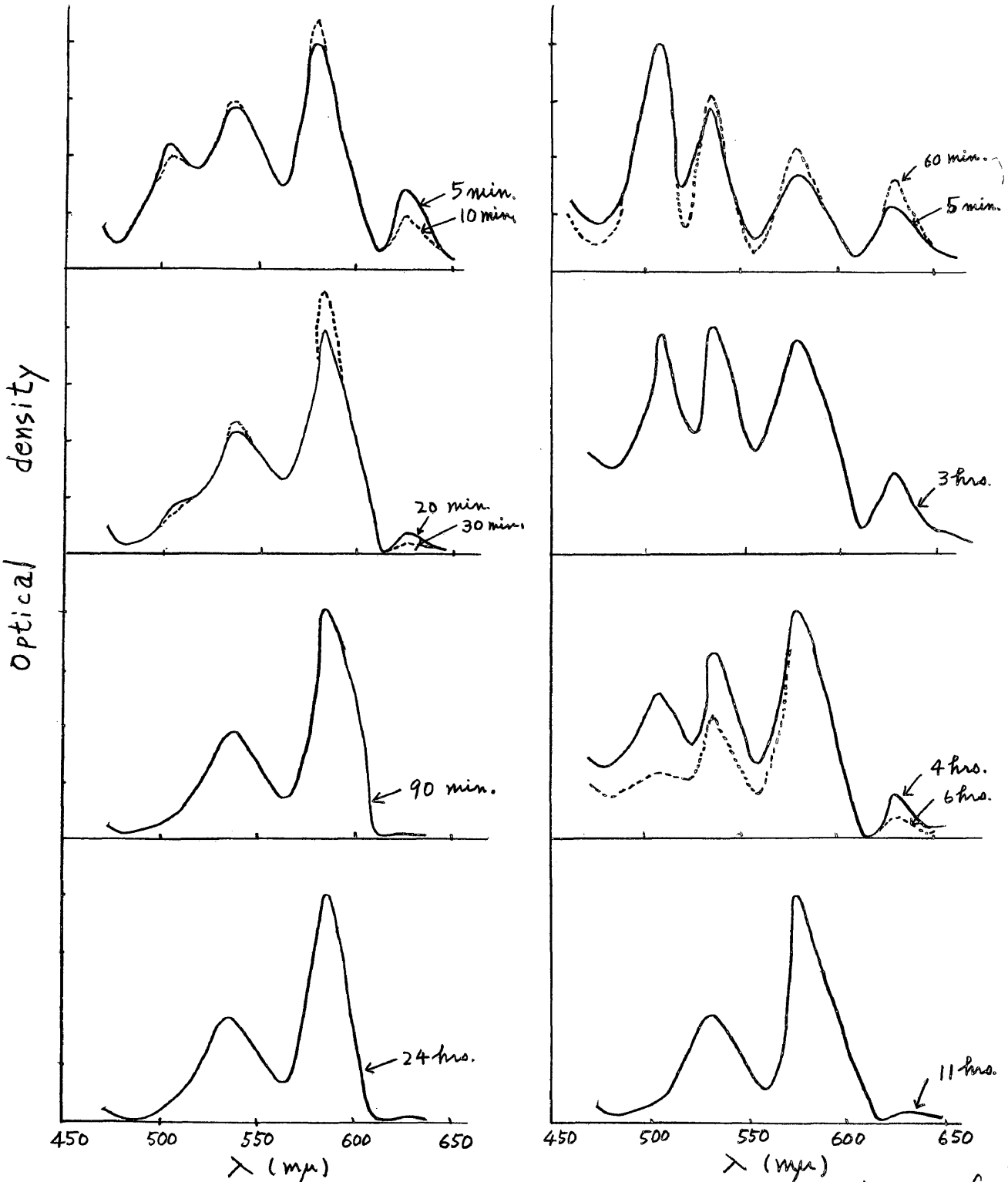


Figure 10.– Spectra of copper metallation in chloroform ($Cu(Ac)_2$).

Figure 11.– Spectra of copper metallation in acetone ($Cu(Ac)_2$).

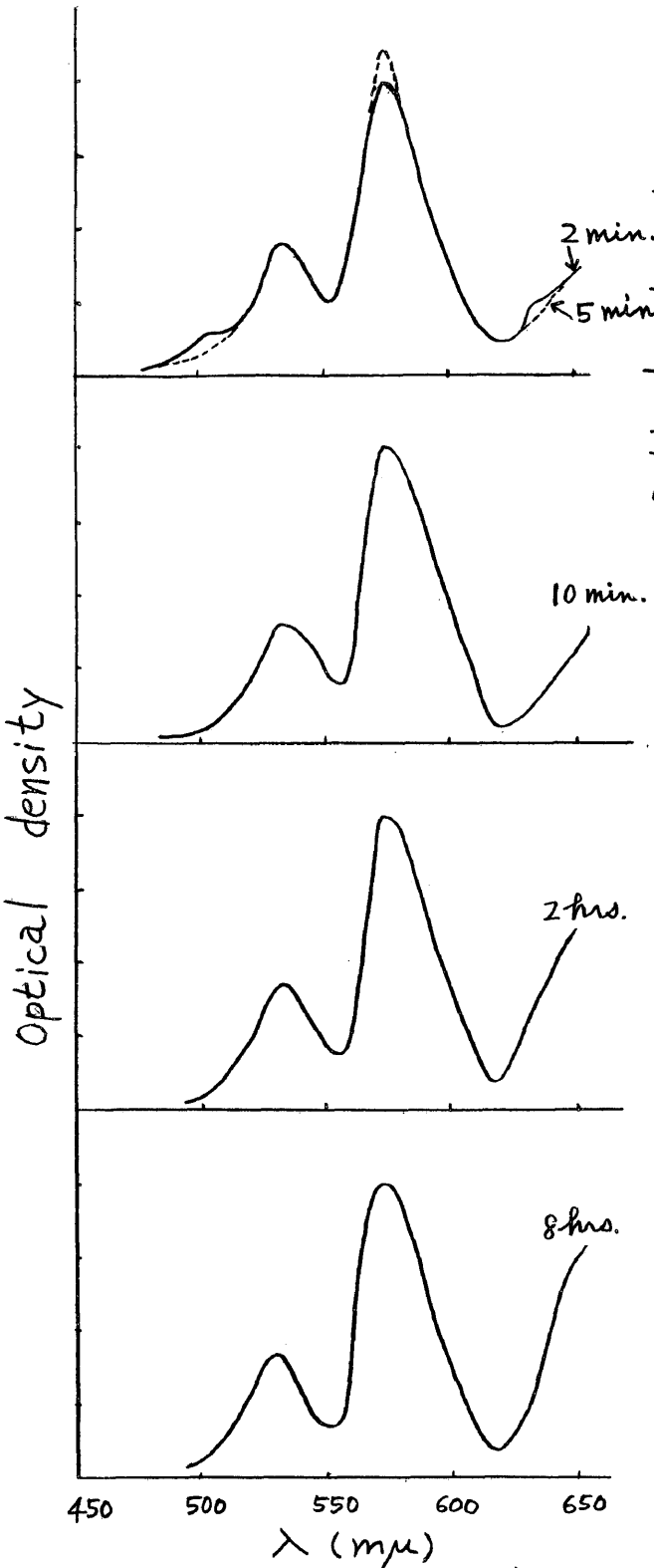


Figure 12.– Spectra of copper metallation in methyl alcohol ($Cu(Ac)_2$).

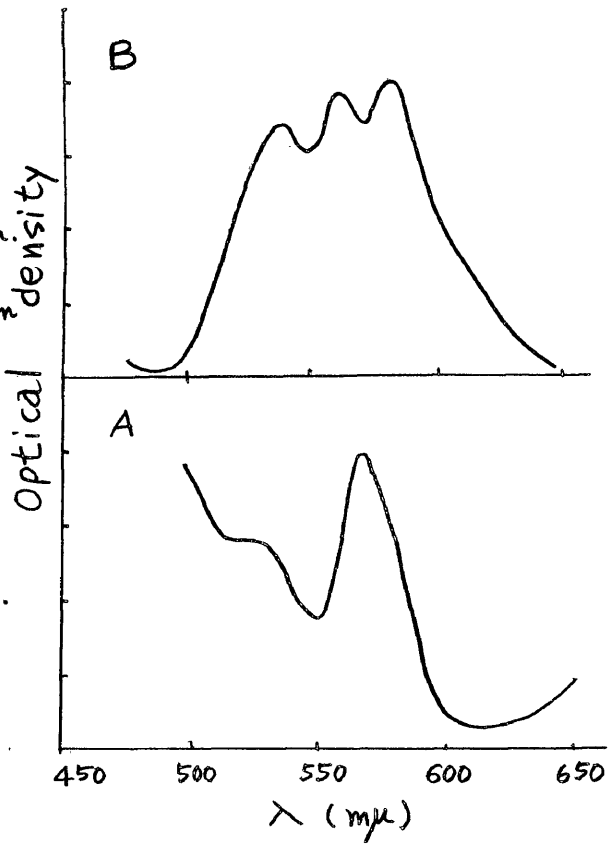


Figure 13.– Spectra of copper metallation ($CuCl_2$): (A) acetone; (B) chloroform.

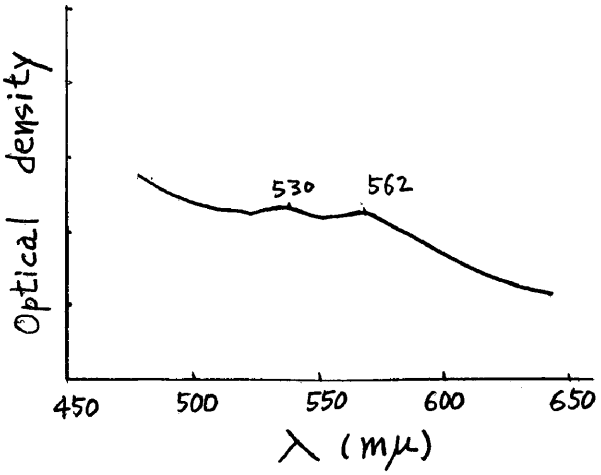


Figure 14.– Spectrum of decomposing PPM-Fe(III) complex.

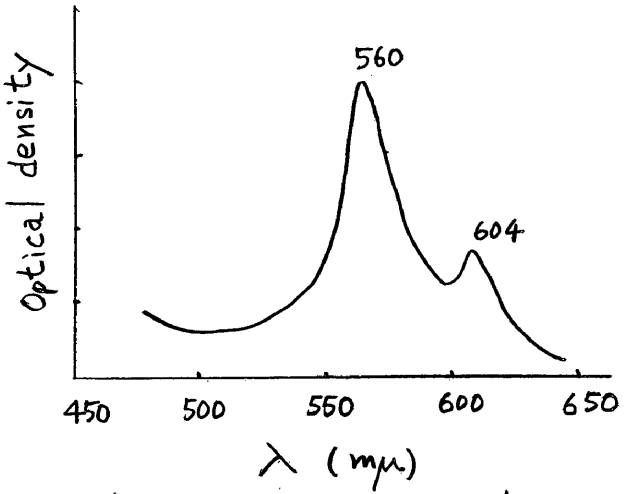


Figure 15.– Spectrum of PH_4^{++} in chloroform.

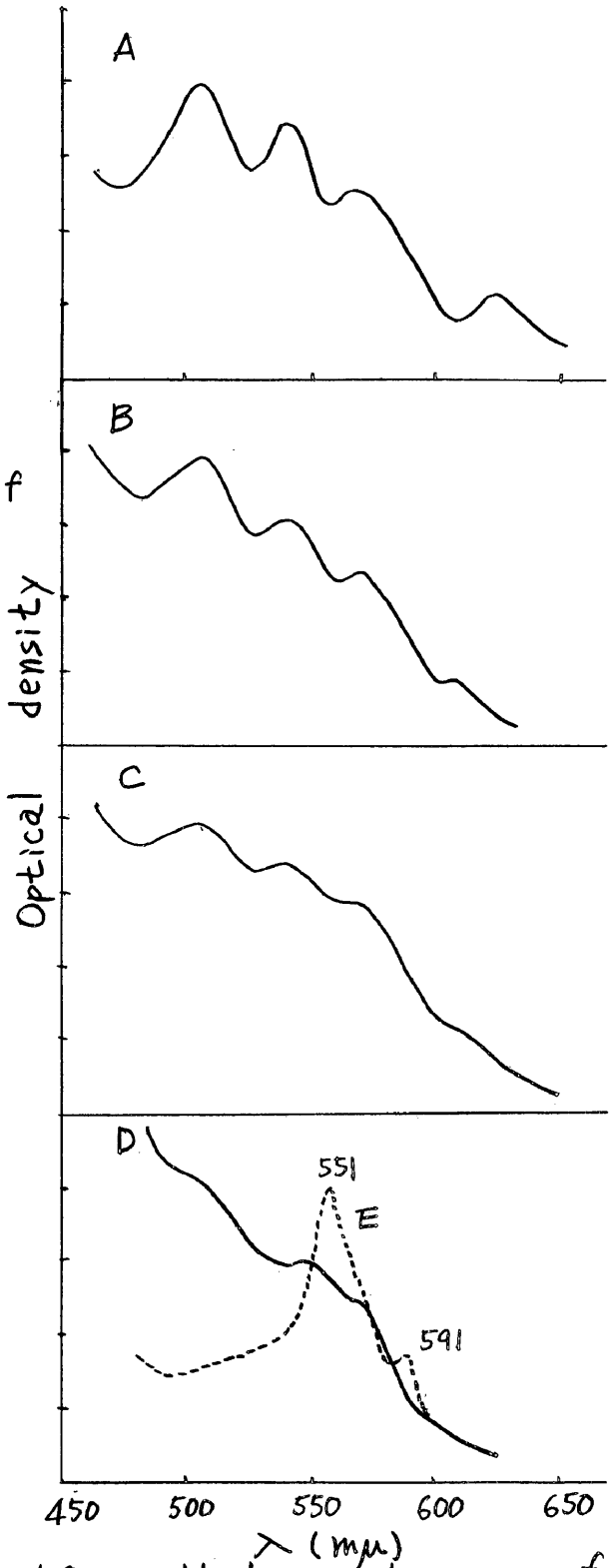


Figure 16.– pH dependence of PPA spectra: (A) pH 10.0; (B) 7.0; (C) 4.5; (D) 4.5 (+ $FeCl_3$); (E) 1.9.

TABLE 10

Spectra of Copper Metallation Reaction in Chloroform

Porphyrin		Time	λ max (mμ) I	II	III	IV	Soret
Protoporphyrin dimethyl ester	$Cu(Ac)_2$	5 min.	627	575	535	506	---
		10 min.	627	572	535	506	---
		20 min.	627	572	535	506sh	---
		30 min.	627	572	535	506sh	---
		40 min.	627	572	535		---
		90 min.	660h	572	535		---
		24 hr.	660h	572	535		406
	$CuCl_2$	15 min.	627	571	538	506	406
		48 hr.		585	563	530	---
		6d		586	564	530	402 (412sh)

Temperature: room temperature

TABLE 11

Spectra of Copper Metallation Reaction in Acetone

	Time	λ max (mμ)					
		Ia	I	II	III	IV	Soret
	5 min.	666	629	574	537	502	---
	60 min.	666	628	572	535	502	---
$Cu(Ac)_2$	3 hr.	666	628	572	535	502	---
	4 hr.	667	629	568	534	502	---
	7.5 hr.	667h	630	567	530	502sh	---
	11 h	667		568	531		401
	3 d	670		567	529		401
	5 min.			566	526		
$CuCl_2$	3 d			566	526		

Temperature: room temperature

TABLE 12

Spectra of Copper Metallation Reaction in Methyl Alcohol

	Time	λ max (mμ)					
		Ia	I	II	III	IV	Soret
	2 min.	701	628	568	532	502sh	---
	5 min.	701		568	532	502sh	---
$Cu(Ac)_2$	10 min.	701		568	530		---
	2 hr.	704		568	530		---
	8 hr.	700		568	533		400

sh: shoulder

Temperature: room temperature

TABLE 13

Spectra of Iron Metallation Reaction in Chloroform

Solvent	Time	λ max (mμ) I	Ia	II	III	IV	Soret
	5 min.	600		556			---
	4 d	602		558			412
	11 d	603		558			411 (397sh)
Chloroform	13 d			562h	530h		---
	15 d		decomposed				
	4 d*	629		573	537	502	408

4 d*: By adding DMF, complex reverted to initial spectrum.

sh: shoulder

h: hump

Temperature: 50 - 55°

TABLE 14

Spectra of Iron Metallation Reaction in Acetone and Methyl Alcohol

Solvent	Time	λ max (mμ) I	II	III	IV	Soret
Acetone	5 min.	610	562	536		402
	2 d	608	560	535		363 (395sh)
	6 d	610	561	534		362 (395sh)
	16 d	610	561	534		---
Methyl alcohol	5 min.	608	566	534	502h	402
	3 d	609	564	533	501h	---
	7 d	607	564	533	500h	---
	17 d	607	564	533	500h	399

Temperature: room temperature

TABLE 15

Reversion of PPM - Fe (III) Complex

Added Solvent (50%)	λ max (mμ) I	II	III	IV	Type
Pyridine	630	574	538	503	etio
DMF	632	574	538	505	etio
DMS	631	575	539	506	etio
Isopropyl Alcohol	630 (608h)	572	539	505h	methyl alcohol
THF	632 (611h)	567	537	505h	methyl alcohol
Acetonitrile	608	561	538		acetone
Thiophene	607	563			chloroform
Ethyl Mercaptan	600	557			chloroform
Nitromethane	600	556			chloroform

TABLE 16

PPM - Fe (III) Complex by Anhydrous Iron (III) Chloride

Solvent	λ max (mμ) I	II	III	IV
Benzene	606	562		
Chloroform	601	557		
Acetone	609	564	534	
Methyl Alcohol	607	564	533	501h

CONCLUSION

Fleisher and Wang (26) claimed that the iron metallation reaction occurred in chloroform and acetone through an intermediate of PPM - Fe (III) complex; however, the preceding experimental data indicate that the iron metallation reaction does not occur in chloroform, acetone or methyl alcohol. But the formation of PPM - Fe (III) complex does occur, and three different and very stable PPM - Fe (III) complexes are produced, of which spectra are characteristic of each solvent in peak height and maximum absorbance; the chloroform type with two peaks, the acetone type with three peaks and the methyl alcohol type with three peaks and a small hump. All of these three complexes were quite stable. The chloroform type remained unchanged for 4 days at room temperature and further 11 days at 50 — 55°. After that, it decomposed within the next two days. Perhaps this decomposition is due to carbene produced in chloroform as follows:

$$CH\,Cl_3 \rightleftharpoons C\,Cl_3^- + H^+ \quad \text{(slow)}$$

$$C\,Cl_3^- \longrightarrow \cdot\overset{\cdot}{C}\,Cl_2 + Cl^- \quad \text{(fast)}.$$

This reaction occurs readily under basic condition, but Fe (III) may cause the formation of dichlorocarbene:

$$Fe^{+++} + CH\,Cl_3 \longrightarrow Fe\,(C\,Cl_3)^{++} + H^+$$

$$Fe^{++}\,(C\,Cl_3) \longrightarrow Fe\,Cl^{++} + {}^{\circ}\dot{C}\,Cl_2.$$

Both the acetone type and the methyl alcohol type remained unchanged for more than 2 weeks, and then finally decomposed.

Notwithstanding Fleisher and Wang's statement, no conversion occurred from PPM - Fe (III) complex to Fe (III) - PPM. Indeed there has been no previous experimental example of a direct synthesis of Fe (III) - PPM other than Fleisher and Wang's paper. The usual preparation is via Fe (II) - porphyrin followed by oxidation to Fe (III) - porphyrin (38). In the metallation reaction two protons must be removed from the porphyrin nucleus by pyridine or some other base. Therefore, it is doubtful that they were able to synthesize Fe (III) - PPM in chloroform or acetone without the presence of a proton acceptor.

This author would rather conclude that these PPM - Fe (III) complexes are all simple porphyrin cations from the preceding data. Such data are summarized for the sake of convenient comparison in Table 17.

The striking resemblance of the spectra of the chloroform type complex to PH_4^{++} and the acetone type complex to PH_3^{+} can be recognized in the preceding data. If we compare the chloroform type of spectra with the spectra of the dication, PH_4^{++}, in chloroform, the maximum absorbances are slightly shifted to longer wavelength by 2 mμ in benzene and shorter wavelength by 4 mμ in chloroform, but the separations between peaks are the same. The acetone type gave almost the same spectra as the monocation, PH_3^{+}, in water. The methyl alcohol type has an extra small hump at 501 mμ in comparison with PH_3^{+}. The author calls this the quasi-monocation type.

TABLE 17

Spectra of Porphyrin Cations

Type	Solvent	Porphyrin	λ max (mμ)				Ref.
			I	II	III	IV	
Dication (PH_4^{++})	$CH\ Cl_3$	PPM	604	560			Fig. 15A
	H_2O	PPA	591	551			Fig. 16E
Chloroform	$CH\ Cl_3$	PPM	600	556			Table 13 Fig. 9A
	benzene	PPM	606	562			Table 16
Monocation (PH_3^+)	H_2O (2.5% SDS)	PPM	609	568	535		(37)
Acetone	acetone	PPM	610	562	536		Table 14 Fig. 9B
Quasi-monocation							
Methyl alcohol	CH_3OH	PPM	609	564	533	501h	Fig. 9C Table 14

The resemblances in absorption spectra mean that, in the visible range of wavelength, the chloroform type and the acetone type must have very nearly the same electronic transitions and symmetry as PH_4^{++} and PH_3^{+} do, respectively, because the same absorption spectra are caused by the same electron transition and the same symmetry. If the chloroform type of complex or the acetone type of complex is composed of PPM and Fe (III) ion, the bond and the symmetry between PPM and Fe (III) will cause different spectra from PH_4^{++} and PH_3^{+}. Because in the process $PH_4^{++} \longrightarrow (PH_4^{++})^*$ and PPM - Fe (III) complex $\longrightarrow$ PPM - Fe (III) complex* (where the * represents an exited state), the bonding between PPM and proton, and the bonding between PPM and Fe (III) are different in both symmetry and energy. Therefore the chloroform type and the acetone type are probably not PPM - Fe (III) complex but no more than simple dication and monocation of porphyrin, respectively.

The results of reversion test of the chloroform type of PPM - Fe (III) complex second the conclusion mentioned above. Basic organic solvents such as pyridine, dimethyl sulfoxide or dimethyl formamide reverted the chloroform type of spectrum to the free porphyrin spectrum. It is believed that these solvents are basic enough to abstract two protons from porphyrin and neutralize the dipositive charges of the chloroform type complex. When less basic solvents such as isopropyl alcohol or tetrahydrofuran were used, the chloroform type spectra changed to the methyl alcohol type spectra. This is believed to mean that such solvents caused incomplete dissociation of PH_4^{++}. Very weakly basic solvents such as acetonitrile converted the chloroform type spectra into the acetone type spectra, which suggests that the basicity of acetonitrile is just strong enough to produce the unipositive porphyrin ion,

PH_3^+, from the dipositive ion, PH_4^{++}. Thiophene, nitromethane and ethyl mercaptan are so weakly basic that they are unable to abstract a proton; hence they did not give any change of the chloroform type spectra. These solvents are arranged below in the order that they produced each of the following change: the methyl alcohol type, the acetone type and no change.
Pyridine, dimethyl formamide, dimethyl sulfoxide $>$ tetrahydrofuran, isopropyl alcohol $>$ acetonitrile $>$ thiophene, ethyl mercaptan, nitromethane.
This relative order is quite suggestive of the relative order of basicity.

Judging from these data, the chloroform type complex is probably the porphyrin dication and may readily transfer a proton to any added basic solvent molecule to an extent to produce free porphyrin, quasi-monocation or monocation of porphyrin. The reason why the methyl alcohol type is called quasi-monocation is that this type is considered to have less positive charge than acetone type, monocation type.

Concerning the rate of the complex formation, it should be emphasized that this reaction, whatever it may be, is very rapid. Equilibrium or completion occurs immediately upon mixing. Therefore this reaction is undoubtedly ionic in nature.

However, before the final conclusion is made, the simple question about the source of proton which forms PH_4^{++} or PH_3^+ must be explained. When iron (III) chloride with water of crystallization ($Fe\ Cl_3 \cdot 6H_2O$) was used, in organic solvents, the following dissociation of water molecules is quite possible:

$$Fe^{+++} + H_2O \rightleftarrows Fe\ (OH)^{++} + H^+$$

and this proton forms PH_4^{++} and PH_3^+. However, it was found that anhydrous iron (III) chloride gave identical results as the hydrated salt. The source

of proton from anhydrous iron (III) chloride must be studied further.

Considering all of these results, the spectral similarity, reversion test of the chloroform type and $_{p}H$ dependence of PPA, it seems likely that the PPM - Fe (III) complexes do not exist but are just porphyrin cations. The determination of structural formula must be pursued by further study, for these porphyrin cations are intimately associated with solvent properties.

These conclusions are contrary to those of Fleisher and Wang. The author would like to point out some differences between his and their results.

Firstly, they referred to the spectrum shown in Fig. 2B as the standard spectrum of Fe (III) - complex. However, this does not look like the spectrum of Fe (III) - PPM, which is shown in Fig. 5A. The standard spectrum of Fe (III) - PPM has two discernable absorptions at 642 mμ and 508 mμ, and two broad humps approximately at 587 mμ and 540 mμ. The spectrum shown in Fig. 2B has no discernable peak but shows a broad absorption in which it is difficult to distinguish the maximum. As they did not state the solvent used for the measurement, a straight comparison of Fig. 2B with Fig. 5A, measured in dioxane, may not be made; however, from the fact the pure Fe (III) - PPM gives almost the same spectra (Fig. 8)in ether and chloroform, it is quite doubtful that their spectrum is that of Fe (III) - PPM.

Secondly, they did not recognize the acetone type of spectrum, and mistook the chloroform type of spectrum for the new type of PPM - Fe (III) complex.

Concerning the structure of a sitting-atop type of complex (Fig. 3), the author believes that this complex does not involve Fe (III) as stated

by them.

The results of the copper metallation reaction give several suggestive informations. The fact is that in any three solvents the reaction advanced readily when copper (II) acetate was used, and not so when copper chloride was used. Further studies are necessary for convincing conclusions, but the following suggestions can be made:

1) The copper metallation reaction may be dependent upon the anion of the copper salt.

2) If another kind of iron salt (e. g. iron (III) acetate) were used, the iron metallation reaction might be possible in chloroform, acetone or methyl alcohol.

3) The combination of the kind of metal salt and solvent may be influencial for the metallation reaction.

4) The decrease in the intensity of the peak I and IV, and the increase in the intensity of the peak II and III as the metallation reaction advances may give a hint to the mechanism of the metallation reaction.

5) The reaction rate of the copper metallation in chloroform or acetone is fast enough to monitor its change; thus the rate determining step may be determined.

As to the further development of this research, there are some interesting aspects. They are more detailed study of solvent field effect as well as ligant field effect for the metallation reaction of porphyrin, in which the solvent molecule as well as the porphyrin itself are ligands (octahedral) and produce ligand field effects upon the metal ion. Thus the effect of solvent

should be emphasized. Corwin and co-workers interpreted that the gradual change of spectrum with dilectric constant of solvent in the preparation of iron (III) mesoporphyrin is due to increasing amount of high spin iron (III) complex (39). The author believes that current advancements of MO theory or ligand field theory are powerful tools in helping to explain these phenomena (40, 41).

APPENDIX

NOMENCLATURE AND DEFINITIONS

APPENDIX

As a supplementary aid to reading this thesis more readily, the nomenclature and the structural formulas of porphyrin compounds, and the definitions and the symbols that the author of this thesis used are presented in this section.

The Nomenclature and the Structural Formulas of Porphyrin Compounds (7, 12)

The prefix to "porphyrin" indicates color or derivation of each porphyrin:

Aetio - applied to porphyrins derived by decarboxylation of a substituent

Meso - intermediate reduction state (vinyl to ethyl)

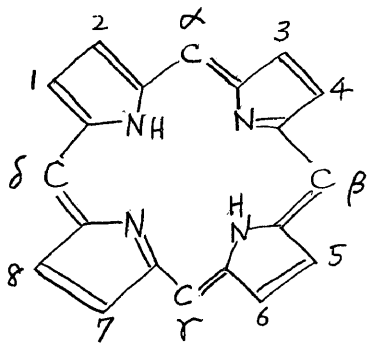
Proto - first of a series

uro - derived from urine

rhodo - means red

Aetioporphyrin implies that it is a porphyrin which resulted from decarboxylation.

Protoporphyrin was the first porphyrin isolated from haemin (ferriprotoporphyrin chloride), so it is designated "proto".



Porphine

Porphyrins	Substituents on position							
	1	2	3	4	5	6	7	8
Porphine	H	H	H	H	H	H	H	H
Aetioporphyrin I	M	E	M	E	M	E	M	E
Mesoporphyrin IX	M	E	M	E	M	P	P	M
Protoporphyrin IX	M	V	M	V	M	P	P	M
Uroporphyrin III	A	P	A	P	A	P	P	A
Rhodoporphyrin XV	M	E	M	E	M	W	P	M
N_1N^1-bis - (2 - aminoethyl) Protoporphyrin Diamide	M	V	M	V	M	AE	AE	M

Side-chain abbreviations: A, - CH_2COOH;

E, - CH_2CH_3; H, - H; M, - CH_3; P, - CH_2CH_2COOH;

V, - $CH = CH_2$; W, - COOH; AE, - $(CH_2)_2CONH\ (CH_2)_2\ NH_2$

Definitions

Metalloporphyrin: a porphyrin which is chelated with a metal in the central

hole of the porphyrin nucleus.

Porphyrin metal complex: a porphyrin which is loosely bonded to a metal.

Free porphyrin: a porphyrin which is not chelated with a metal.

Metallation: a reaction of metal incorporation into the porphyrin nucleus to form the metalloporphyrin.

Spectrum: visible absorption spectrum.

Symbols

PPM: protoporphyrin dimethyl ester

PPA: N_1N^1-bis - (2-aminoethyl) protoporphyrin diamide

Fe (III) - PPM: iron (III) - protoporphyrin (haemin)

PPM - Fe (III) complex: Protoporphyrin-iron complex (not haemin)

Cu (II) - PPM: copper (II) - protoporphyrin

PH_2: free porphyrin

PH_4^{++}: porphyrin dication

PH_3^{+}: porphyrin monocation

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