

METAL CHLORINS -
A POSSIBLE ROUTE TO PETROPORPHYRINS

By
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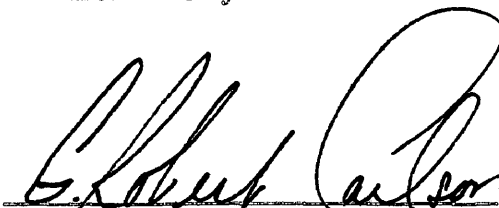
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A thesis submitted to the Faculty and the Board of Trustees of the Colorado School of Mines in partial fulfillment of the requirements for the degree of Master of Science in the combined option of Chemistry and Petroleum Refining.


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ABSTRACT

Petroleum porphyrins are apparently derived from chlorophyll, and probably contain only vanadium or nickel. It is important to establish a good understanding of the alteration of the original chlorophyll to the porphyrins of crude oil and therefore, a knowledge of the intermediates between the initial chlorin and the final metal porphyrin would be important in defining the conditions under which the associated petroleum was formed.

Two major ways of changing the chlorophyll molecule are apparent: either replacement of the magnesium of chlorophyll by a vanadyl, nickel, or other metal ion; or prior oxidation of chlorophyll, a dihydroporphyrin, to a porphyrin structure. The former route, in which the magnesium is released to form a free chlorin, that is subsequently recomplexed with another metal, could demonstrate the alteration of chlorophyll to a petroleum-type metal porphyrin.

There is little prior evidence to support the former route, but it is shown chemically in this thesis that it is possible to convert pheophytin, the first decomposition product of chlorophyll, to a metal complex of pheophytin.

The present study was an investigation of one possible reaction in a likely sequence of reactions leading to the formation of petroleum porphyrins. Mixtures containing pheophytin a along with vanadyl, nickel, and copper ions in various solvents were refluxed until the complex was formed. The formation and presence of the metal complex of pheophytin a was determined by observing the visible spectra of the metallation reaction mixture.

From the metallation reactions the following conclusions were drawn:

1. The rate of reaction for the complexing process is sufficiently rapid to indicate a ready formation of the metal complexes of pheophytin a.
2. The formation of the vanadyl complex is favored by a more acidic condition than those required for the formation of either the nickel or copper complex.

3. The complex formed, is possibly the sitting-atop type rather than a true metal chlorin in which the metal is inserted into the center of the chlorin molecule.

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INTRODUCTION

One of the more basic and intriguing questions of petroleum geochemistry is the origin of petroleum. The general consensus is that petroleum is of organic origin and formed by sedimentation and related processes. The obvious source of such a tremendous amount of organic matter necessary to form the vast amounts of petroleum and related deposits in existence is the living things on earth, both plant and animal. Plant material is estimated to outweigh the animal matter by a factor of as much as 100,000 to 1 (1), but the chance of finding a relatively large store of animal material in one spot might be possible; examples are known: guano beds and sulfur mines represent vast stores of animal-matter deposits resulting from specialized activity of living organisms. Therefore, it cannot be dismissed that materials found in petroleum might possibly have resulted from some specialized activity by animal organisms. Then, too, chlorophyll is known to be

formed in the plant organism by a biosynthetic chain in which protoporphyrin, the porphyrin from which the animal hemoglobins are derived, is an intermediate step (2). However, on the basis of quantity, the probability favors plant origins.

The occurrence of porphyrins in petroleum and related substances is evidence of its biological origin. Treibs, in 1934, published a series of papers dealing with the discovery and partial identification of a series of porphyrins in organic mineral deposits (3). These porphyrins, although simpler in structure than either heme or chlorophyll, most certainly appeared to be derived from chlorophyll. Here was a link between plants and the process of petroleum formation and a significant step in the elucidation of the origin and history of petroleum.

Porphyrin is a class term referring to compounds composed basically of four pyrrole units linked together by four methine bridges into a cyclic planar molecule. Because of the eight beta hydrogen atoms as well as the four hydrogen atoms on the methine bridges, there exists by substitution of these hydrogens a large number of possible structures for individual porphyrin compounds. The most common substituent groups are alkyl and alkyl-acid groups, with the alkyl unit being methyl or ethyl and the

acid being a propionic acid side-chain. When found at all, porphyrin carboxylic acids in petroleum are present only in small amounts (4).

A closely related group of pigments are the chlorins. Present nomenclature usage applies the term "chlorin" to both chlorins and phorbins as defined by Fischer (5). All metal-free derivatives of chlorophyll a with the carbocyclic ring intact and one of the resonating double bonds in the basic porphyrin structure saturated are known by the class name of phorbides or phorbins. If the ring is opened, they are known as chlorins, as illustrated in Figure 1. Both porphyrins and chlorins form metal complexes. The best-known porphyrin compound occurring in living organisms is the blood-pigment heme, which is a dicarboxylic acid porphyrin with an iron atom complexed in the center of the molecule. The best-known and probably the most widely distributed chlorin pigment is chlorophyll a, which is complexed with a magnesium atom in the center of the molecule. Porphyrins and chlorins are thus common to nearly all living organisms; not surprisingly they are found wherever life is present, either in an active form or in a fossil form. Recent sediments are well known for their chlorin contents (6), and as it has already been pointed out, ancient sediments including petroleum, oil shale, and coal have long been recognized

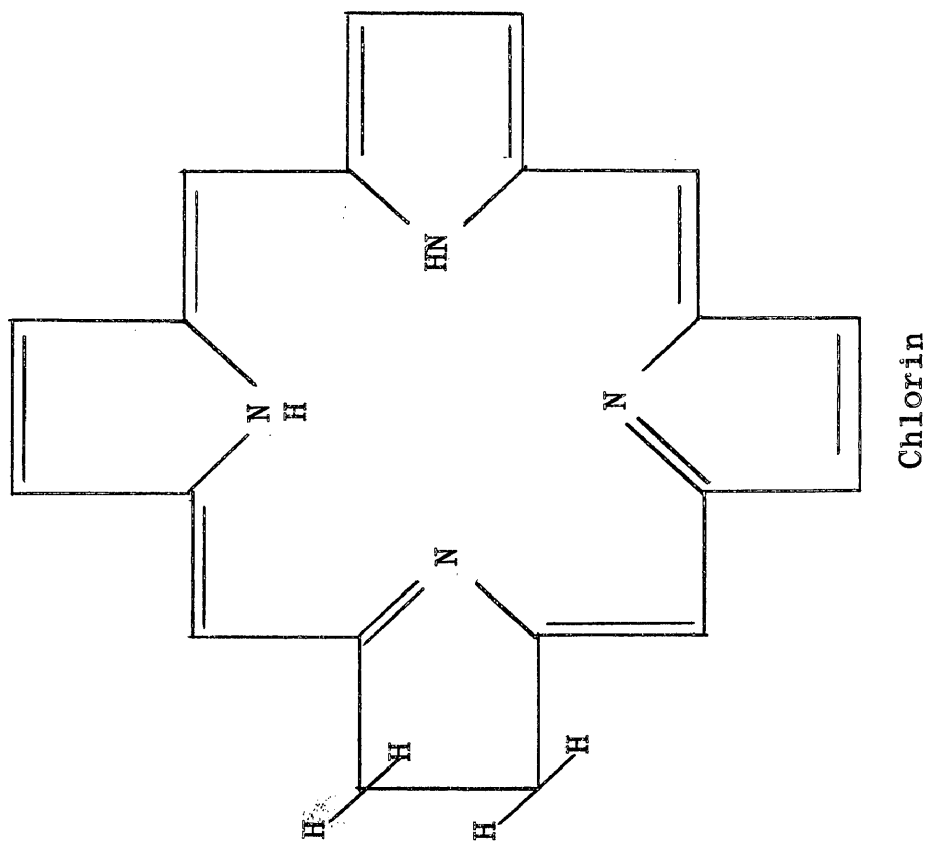
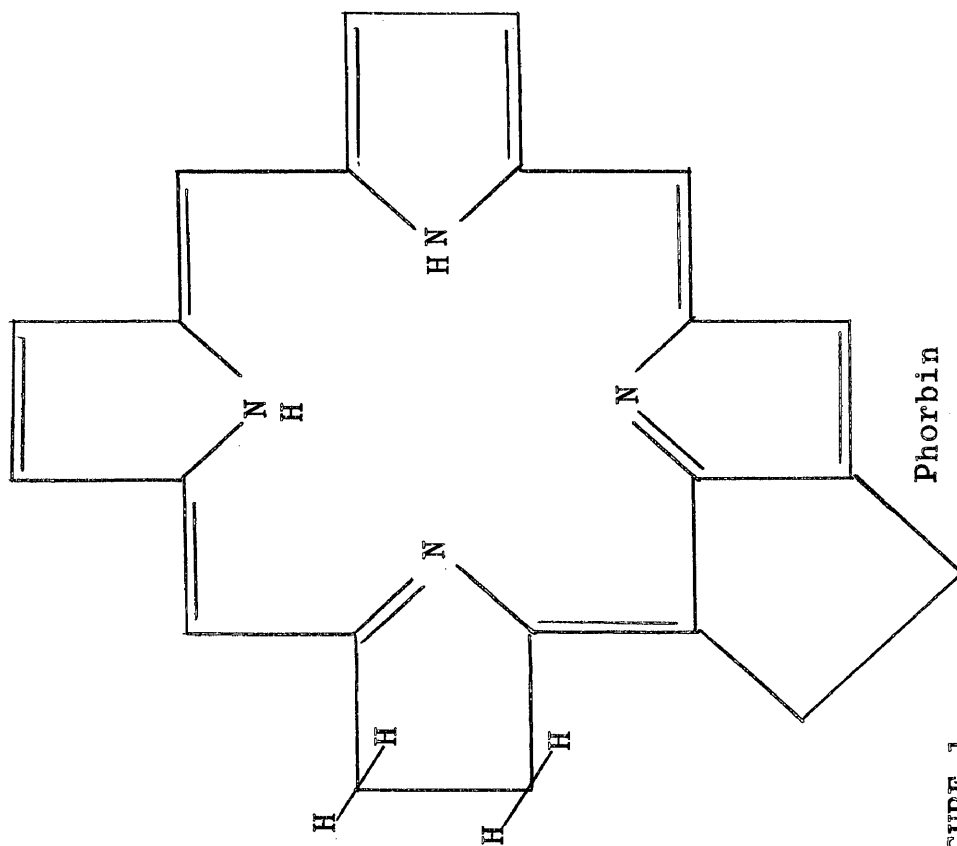


FIGURE 1

Molecular Structure of Chlorins

as containing porphyrin pigments.

Willstaetter (7) showed that magnesium is removed from chlorophyll by the action of carbon dioxide and other weak acids. Thus, assumedly, the removal of magnesium from samples of chlorophyll occurs within a few hours of the demise of the plant or a chlorophyll-bearing segment of it. The resultant pheophytin or pheophorbide could then take up any other metal which might be present in sufficient concentration to permit the reaction of chelation to proceed, Figure 2. Then over some period, the green pigments thus formed would gradually dehydrogenate to form the more stable porphyrins. Because metalloporphyrins are more stable than porphyrin free bases, resulting from their increased resonance possibilities, this chelation should provide appreciable survival value so that the pigments failing to chelate might be expected to be destroyed faster than those which did chelate. Evidently vanadyl and nickel ions form the most stable porphyrin complexes, because, since the discovery of metalloporphyrins in petroleum, no metal porphyrins other than nickel and vanadium have ever been detected in petroleum, although crudes do have other metals, but not as porphyrin complexes. The kinetic studies of Hobbs (8), the difference in the standard free energies of the formation of nickel and vanadyl porphyrins, and the

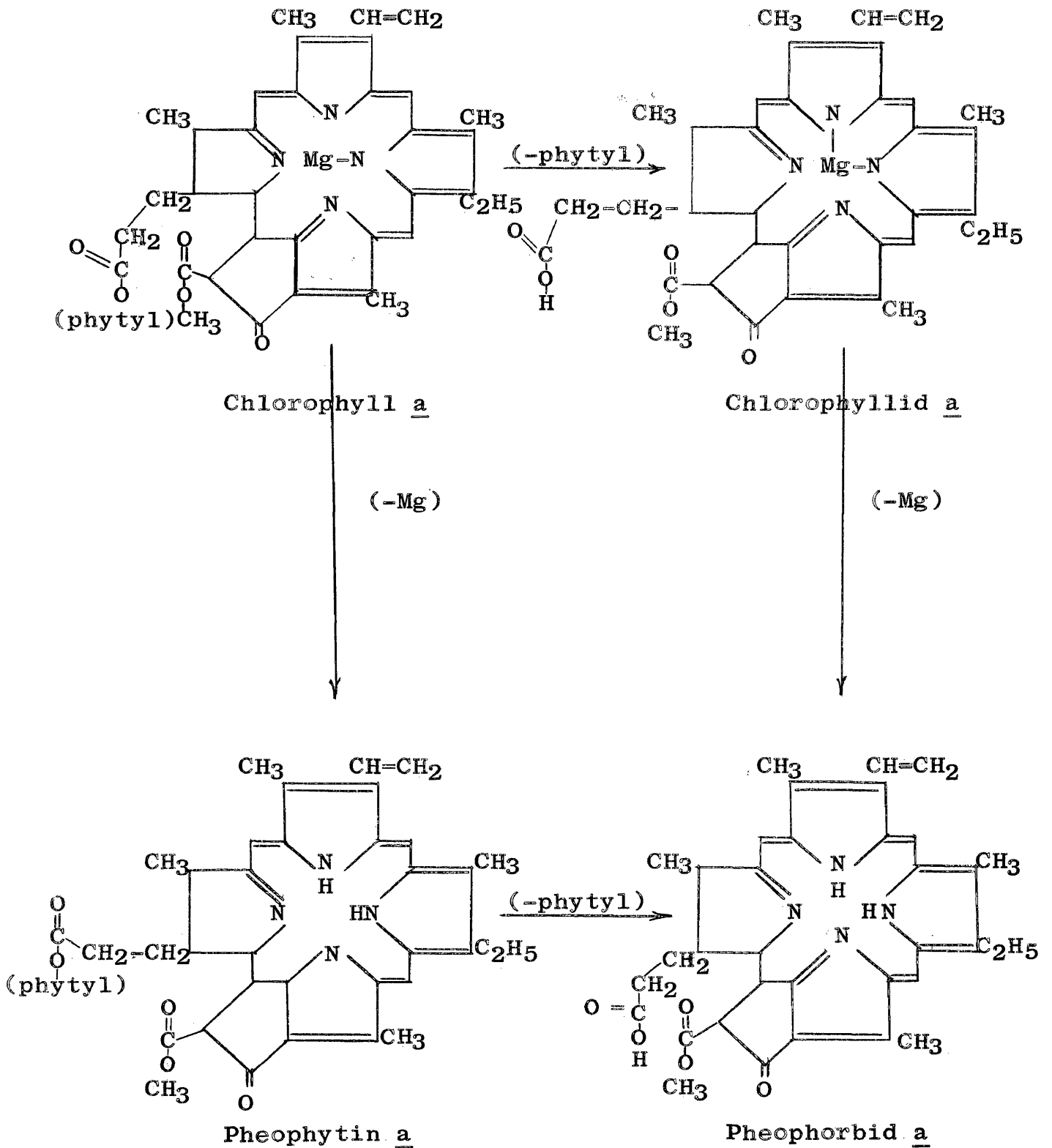


FIGURE 2

Chlorophyll Series

cleavage rates for the removal of the complexed metals (9), would indicate that nickel porphyrins should predominate over vanadyl porphyrins. Vanadyl porphyrins generally are found to be more abundant in petroleum than nickel porphyrins (Figure 3). However, this is not strictly true, in fact no data are available on the vanadyl-porphyrin to nickel-porphyrin ratio in crude oil. What is invariably obtained is the total V/Ni ratio in the crude, assumed to represent the vanadyl-porphyrin to nickel-porphyrin ratio. Because the evidence is strong that there is non-porphyrin vanadium and non-porphyrin nickel in crude oils, the assumption may be completely invalid (10).

Although the kinetic factor explains the common occurrence of nickel-porphyrin complexes in crude oils, it does not as such explain the wide variation in the V/Ni ratio exhibited by crudes of various ages and geographic origin (Figure 3). The simplest explanation is probably to be found in the relative proportions of vanadium and nickel present at the time the complexes were formed. Certain marine organisms, for example snails (11), selectively concentrate nickel, where others, for example the tunicates (12), concentrate vanadium. Because these metal concentrations are very probably the result of biological activity, their magnitudes may be expected to vary with the ecology.

FIGURE 3
Occurrence of Vanadium and Nickel in Crude Oil

Age	Location	Number of Samples	Vanadium (ppm)	Nickel (ppm)	V/Ni Ratio
Cenozoic	United States (California)	4	87 (34-223)*	53 (33-97)	1.4 (0.9-2.3)
	United States	1	0.16	3.0	0.05
	U.S.S.R.	97	---	---	0.2
	U.S.S.R.	16	46 (0.3-127)	17 (2.5-43)	2.6 (0.02-7.3)
	Venezuela	1	1160	110	10.5
Mesozoic	United States (Texas)	1	1.2	1.7	0.7
	United States	11	40 (0.03-106)	11 (0.03-29)	2.5 (0.1-6.1)
	Canada	37	62 (0.1-220)	25 (0.3-77)	2.0 (0.5-3.4)
	Kuwait	1	22.5	6.0	3.8
	U.S.S.R.	36	---	---	0.7
Paleozoic	United States	11	44 (0.8-106)	13 (0.2-27)	3.2 (1.5-4.8)
	United States (Texas)	4	2.8 (0.8-79)	2.4(1.0-4.8)	0.9 (0.5-1.6)
	United States (Texas)	1	---	---	0.6
	United States (Oklahoma)	23	0.44(0.1-5.2)	1.6(0.1-8.5)	0.3 (0.02-1.0)
	United States (Oklahoma)	1	---	---	2.5
	United States (Kansas)	1	---	---	0.5
	Canada	30	9.3 (0.04-84)	8.1(0.1-5.7)	0.8 (0.1-3.2)
	U.S.S.R.	28	---	---	8.0
	U.S.S.R.	--	---	---	---

* Indicates range of variation (after Hodgson and Baker, 1957 (31))

However, the vanadium and nickel-porphyrin complexes occurring in petroleums are probably not formed directly by the organisms providing the source material.

The stability of isolated metal porphyrin complexes varies greatly. Water removes sodium, potassium, and arsenic; dilute acetic acid removes magnesium and lead; hydrochloric acid removes zinc, tin (Sn II), and iron (Fe II); concentrated sulfuric acid removes copper, iron (Fe II), and cobalt. The work of Sweany (13), using various acid treatments, showed that trifluoroacetic acid demetallized the nickel porphyrins, but not the vanadyl porphyrins, and only polyphosphoric acid was successful in demetallizing both the vanadyl and nickel porphyrins. In respect to the synthesis of porphyrin metal complexes, the vanadium complex, despite its stability (9), forms slower than the nickel complex. This difference in rate has been attributed to the fact that the vanadyl ion, being unsymmetrical, must approach the coordinating site in the porphyrin ring with a particular orientation; thus, it would seem reasonable to expect that nickel should be taken up more rapidly. Taking into account the period over which these complexes are formed in petroleum, it is not necessary to think of these metal complexes as being kinetically or sterically controlled.

If it could be established that chlorin materials are present in petroleum, then a possible explanation of the varying V/Ni ratio is that metalloporphyrins in petroleum were formed by oxidation of metallochlorins instead of metallation of free porphyrins. Erdman and co-workers (14) helped to strengthen this premise with their synthesis of vanadyl porphyrin by showing that there is no equilibrium between porphyrin and vanadyl ion on one hand and the complex on the other. Howe (15) reported finding chlorin-like materials in crude oil, but Blumer (16) was unconvinced as to the interpretation of the data, saying that Howe's method of splitting the complexes of the very stable porphyrins, would, when applied to more sensitive compounds (chlorins), produce artifacts with a spectrum different from that of the original material. He also stated that at best, Howe's chlorin was impure and that it probably was something completely different. Fisher and Dunning (17) also indicated that the chlorin-like material in the crude-oil porphyrin aggregate was probably an oxidation product produced in handling the material. Whatever Howe's material was, and he still believes it to be a chlorin, he could not oxidize it to a porphyrin.

Porphyrins with absorption spectra similar to those of known plant chlorophylls have been detected in sediments

(6, 18). Hodgson and co-workers (6) stated that they found good indication of a chlorin metal complex in recent sediments, and Hodgson (19) also stated that the conversion of pheophytin to a nickel-complexed pheophytin has been demonstrated in his laboratory with the subsequent conversion of the nickel pheophytin complex to a porphyrin complex very similar to the nickel porphyrin of crude oil. Corwin (20) reports the reduction of tin porphyrins to tin chlorins. Orr and co-workers (18) did not report the presence of metal chlorins, but possibly their methods of analysis could have destroyed any that might have been present.

Elementary analysis can be used to establish the empirical formulation of an unknown, but it is obviously unsatisfactory as a method for distinguishing between isomers. Spectra, both visible and infra-red, are used extensively in examining porphyrins. Infra-red permits the detection and estimation of certain peripheral groups, but generally it does not permit distinction between isomers. Visible and ultraviolet spectra permit the identification of the metallo-component of the metalloporphyrin, but does not distinguish between isomers except in special cases. The visible spectra of porphyrins and chlorins in neutral solvents are illustrated in Figure 4; there are four bands, numbered I-IV as shown. The locations of these peaks vary somewhat

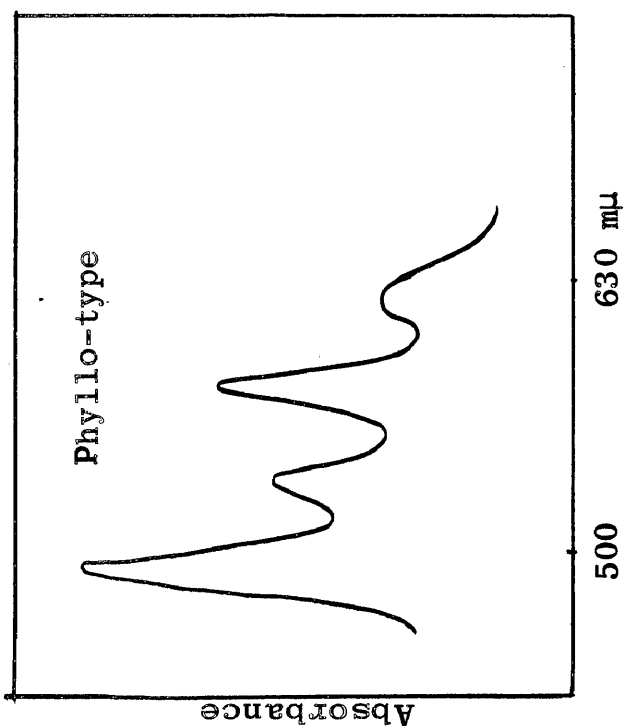
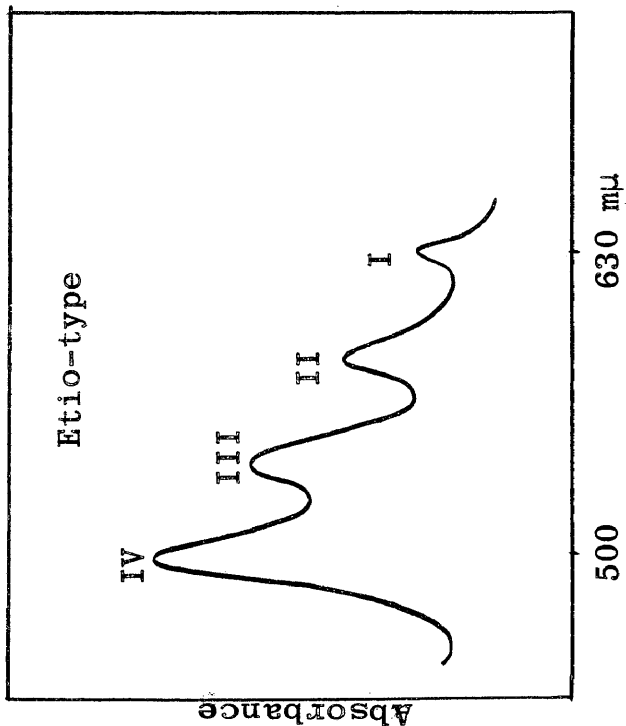
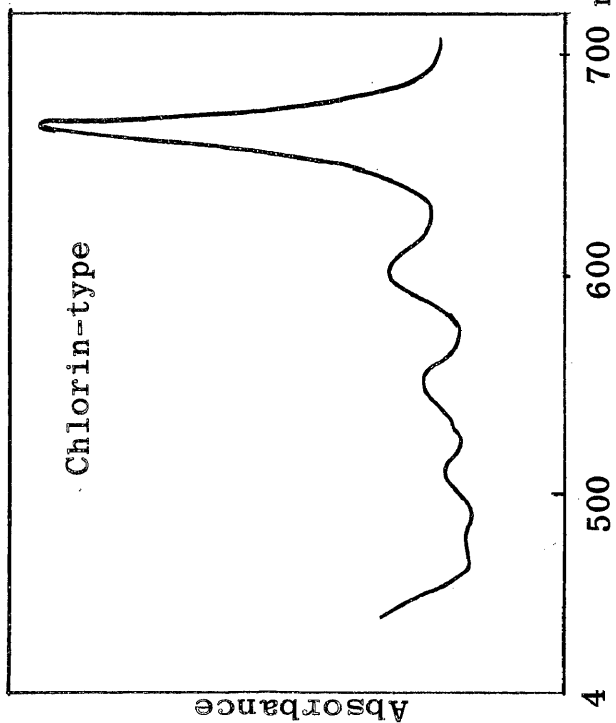
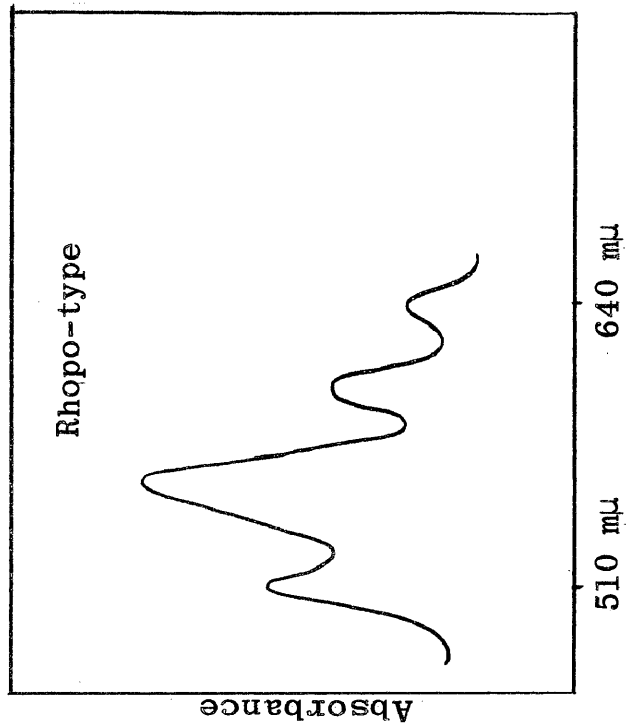
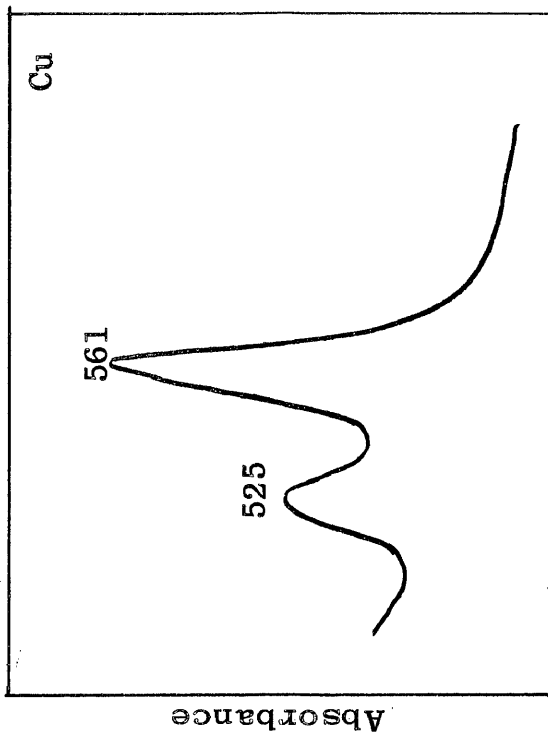
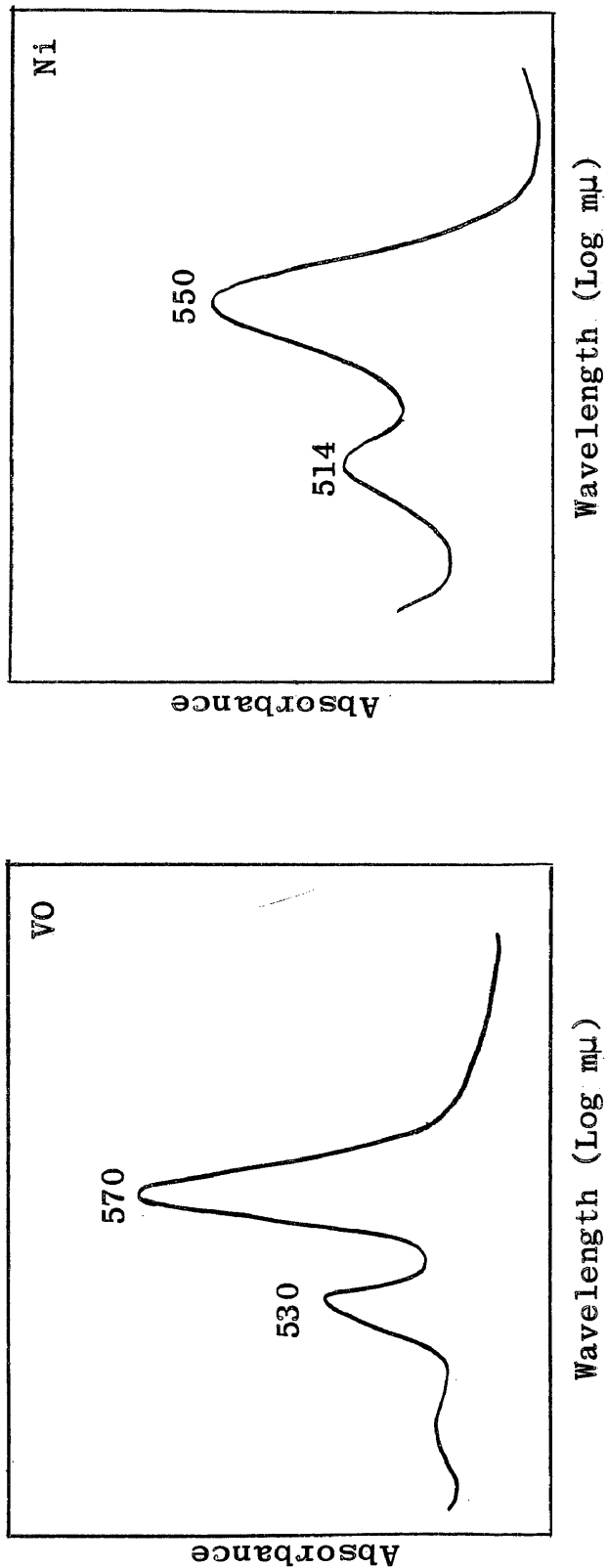


FIGURE 4
Visible Spectra of Metal-Free Porphyrins

with porphyrin type and solvent used, but generally are located at about 620, 565, 535, and 500 m μ , respectively. Another intense absorption band at about 400 m μ , the Soret band, is found in all tetrapyrroles in which the nucleus is fully conjugated -- including porphyrins, metalloporphyrins -- and is present, though weaker, in chlorins and metallochlorins. In chlorins, band I in the visible is very prominent (see Figure 4), and is about 25 m μ to longer wavelength than in porphyrins. The ratio Soret:Band I is only about 5, in chlorins, compared with about 50 in porphyrins (21).

When a porphyrin chelates a metal ion, the four principal non-Soret absorption peaks are generally reduced to two which are different in magnitude and position from the original four. The position and magnitude of these two bands are different for the various metal ions. A few examples of these spectra are shown in Figure 5. Chlorin-mono- and di-cations have spectra similar to those of the neutral compounds, band I and the Soret band being displaced to shorter wavelengths in the di-cations. The Soret band in chlorins has a tendency to split, this condition being more noticeable where there is considerable distortion of the resonance pathway, as in pheophytin b (21).



Wavelength (Log mμ)

FIGURE 5

Visible Spectra of Metalloporphyrins

Another property of importance in analyzing for porphyrins is their strong fluorescence under ultraviolet light. Porphyrins typically have a strong fluorescence band at about 625 m μ and weaker bands at other wavelengths. This strong red fluorescence allows the detection of porphyrins at levels far below those detectable by absorbance studies. The fluorescence is best observed in dilute HCl solutions (21). The orange to red fluorescence can be detected by eye at concentrations of the order of 10^{-8} molar, and photoelectrically down to 10^{-10} . No instance is known of fluorescence in a porphyrin complexed with a transition metal ion. The fluorescence spectra of chlorophylls and chlorophyllids are no doubt due to the presence of water molecules coordinated to the magnesium atoms (21). Fluorescence is, therefore, a valuable tool for identification in studying these pigments.

This study was conducted to prepare and identify a nickel and/or a vanadium metal complex of pheophytin or pheophorbide. A complete treatment of this problem would require an unlimited amount of time and the investigation of numerous works already reported, as well as checking of avenues opened by each investigation. The structure of the plant pigments must undergo chemical changes before the pigments found in petroleum are formed. Because the number

of classes and subclasses of compounds which might be involved is numerous, the individual number of compounds which might be involved could be very large. The nature of these changes for chlorophyll a (see Figure 6) shows a few of the possible intermediates. Likewise, chlorophylls b, c, and d could fit into this scheme and lead to the same or very similar final products. There is experimental basis for proposing an order in which the changes occur in pathway A, but no experimental basis for the changes in pathway B, except for the change of chlorophyll to pheophorbide a. With this metallization accomplished, the spectra of the metallochlorins should be indicative of the spectral type which had chelated the metal ion. Therefore, this thesis can be classified primarily as an experimental effort, because the conclusions are based primarily on the laboratory methods and results.

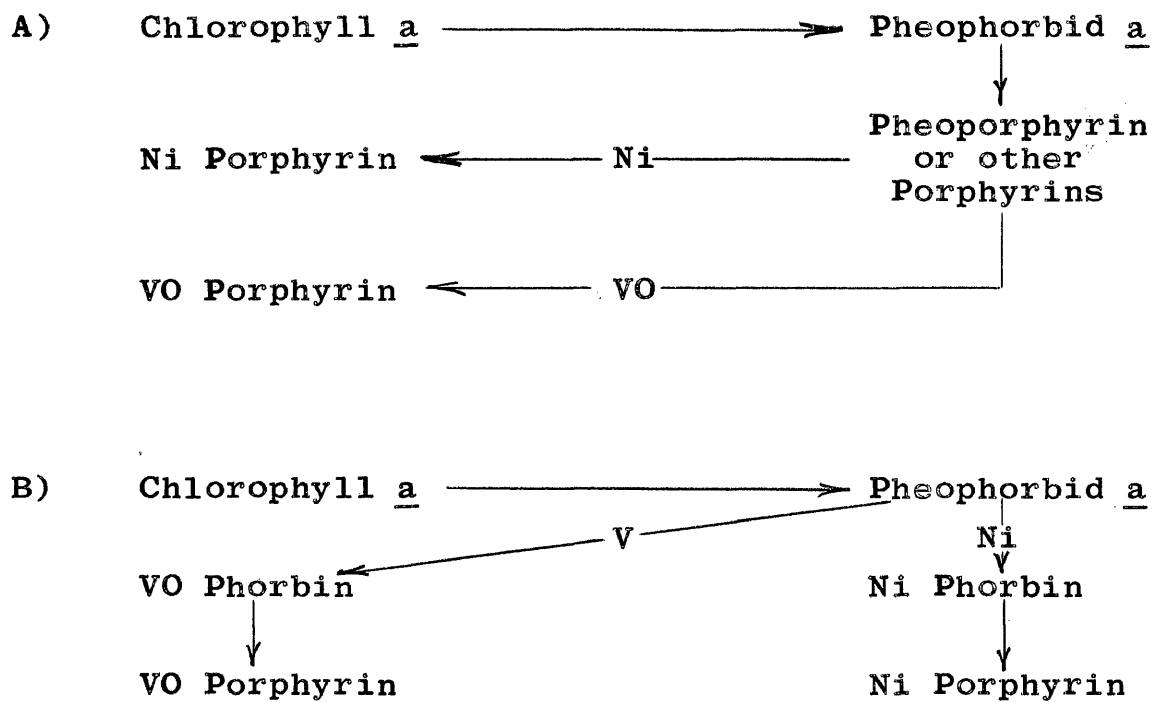


FIGURE 6

Two Possible Pathways to
VO- and Ni-Porphyrin Formation

EXPERIMENTAL WORK

The main body of the experimental work discussed in this thesis was undertaken to prepare reagents and to perform the metallation reactions.

Reagents

Reagents which did not require laboratory preparation are listed in this section along with available miscellaneous information.

Solvents: The following solvents were prepared by simple distillation to avoid contamination or degradation of the chlorins.

Acetonitrile (technical grade)

Benzene (technical grade)

N, N-dimethylformamide (technical grade)

Glacial acetic acid (reagent grade)

n-hexane (technical grade)

Methyl alcohol (technical grade)

a Chlorophyll, 5 Percent in Oil, 6930 Without Copper
5403108: This reagent was obtained from Matheson, Coleman,
and Bell.

Nickel Acetylacetonate: This reagent was donated by
Dr. George Lucas.

Vanadyl Acetylacetonate: This reagent was donated
by Dr. George Lucas.

Vanadyl Sulfate: The vanadyl sulfate was obtained
from Rocky Mountain Research Inc., Denver, Colorado.

Nickel Carbonate: The nickel carbonate used was a
C. P. reagent.

Copper Acetate: The copper acetate used was a
C. P. reagent.

Sugar: The sugar used was fine granulated and
powdered, available in any food store.

Illite, Kaolinite, and Montmorillonite: These
A. P. I. Reference Clay Minerals were donated by Dr. S. W.
Bailey of the University of Wisconsin (see Appendix I).

Analysis

The ultraviolet and visible absorption spectra, simply referred to as "spectra" in the text, were recorded on a Beckman DK-2a ratio-recording spectrophotometer with the following settings:

Scan speed:	5
Scale expansion:	1x
Function:	absorbance
Time constant:	0.10
Range:	0-1 absorbance
Sensitivity:	0.020

A tungsten lamp was used for spectra over a range of 350- to 700-m μ wavelength. The photomultiplier detector used in this range was adjusted for zero with no sample or reference cells in place, and when the spectra were being recorded, the sample was placed in the instrument in 1-cm quartz cuvettes.

There were some instances in which a Beckman DU spectrophotometer was used to prepare a point-by-point spectrum using the blue-sensitive phototube. The dark current and slit adjustment were checked between each reading and adjusted as necessary. The spectra which were determined on the DU model spectrophotometer are indicated to

distinguish from the spectra which were determined on the DK-2a model spectrophotometer. Any deviation from the above procedure is indicated in the individual discussion to follow.

Determination of Vanadium and Nickel: The purpose of these determinations was to find the metal concentrations of the metallation reactions. These determinations were developed by Milner, Glass, Kirchner, and Yurick (22).

The electrolysis part of the vanadium determination was omitted because vanadium was the only metal present in the analysis sample. The vanadium determination was carried out as follows: One ml of the standard VOSO_4 reagent was diluted to 200 ml with water. One ml of the resulting solution was added to about 10 ml of water in a 50-ml beaker. One percent KMnO_4 solution was added by drop until a purple color persisted. By drop, 1:1 HCl was added until the purple color disappeared. The solution was heated to boiling and cooled. The following reagents were added in order: 2.5 ml 2.5 M H_2SO_4 , 0.8 ml 85 percent H_3PO_4 , and 1.2 ml NaWO_4 . The resulting mixture was heated to boiling, and cooled, then diluted to exactly 25 ml. The percent transmission was obtained at 420 μ on the DU, and the ppm vanadium calculated by a calibration chart previously prepared by Dr. Howe (Figure 7).

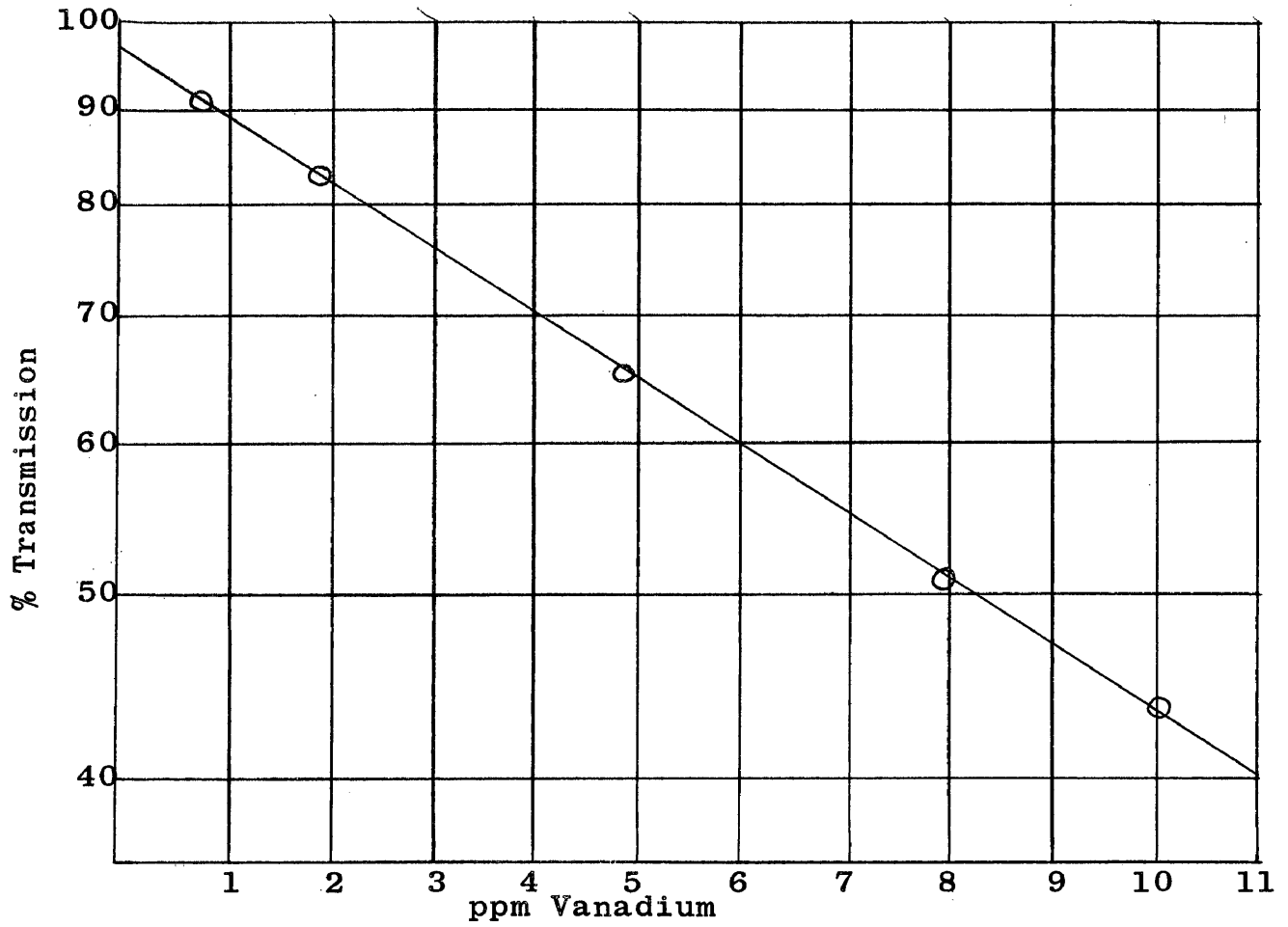


FIGURE 7

Standard Curve for Vanadium Determination

The nickel-determination procedure was as follows:

One ml of the standard nickel reagent was diluted to 2,500 ml. One ml of the resulting solution was diluted to about 15 ml. The following reagents were added in order: 0.5 gram citric acid, 0.5 ml I₂-KI reagent (64 g I₂ and 12.5 g KI in 500 ml water), 3 ml concentrated NH₄OH, and 0.5 ml 1 percent dimethylglyoxime solution. The volume was adjusted to exactly 25 ml and the percent transmission measured at 540 mμ on the DU with a water blank. The ppm nickel was calculated from a calibration chart previously prepared by Dr. Howe (Figure 8).

Preparations

Procedures used in preparing of various reagents are described below.

Pheophytin: The pheophytin samples used in this thesis were prepared as follows: Equal parts (by weight) of granulated and powdered sugar was mixed thoroughly and then placed in a 1½-in.-diam chromatographic column, and washed down with n-hexane. After the n-hexane had drained through the column, and all air had been removed, six drops of a chlorophyll, 5 percent in oil was put on the column. The various components quickly separate into bands and can be seen by looking through the column. With an ultraviolet

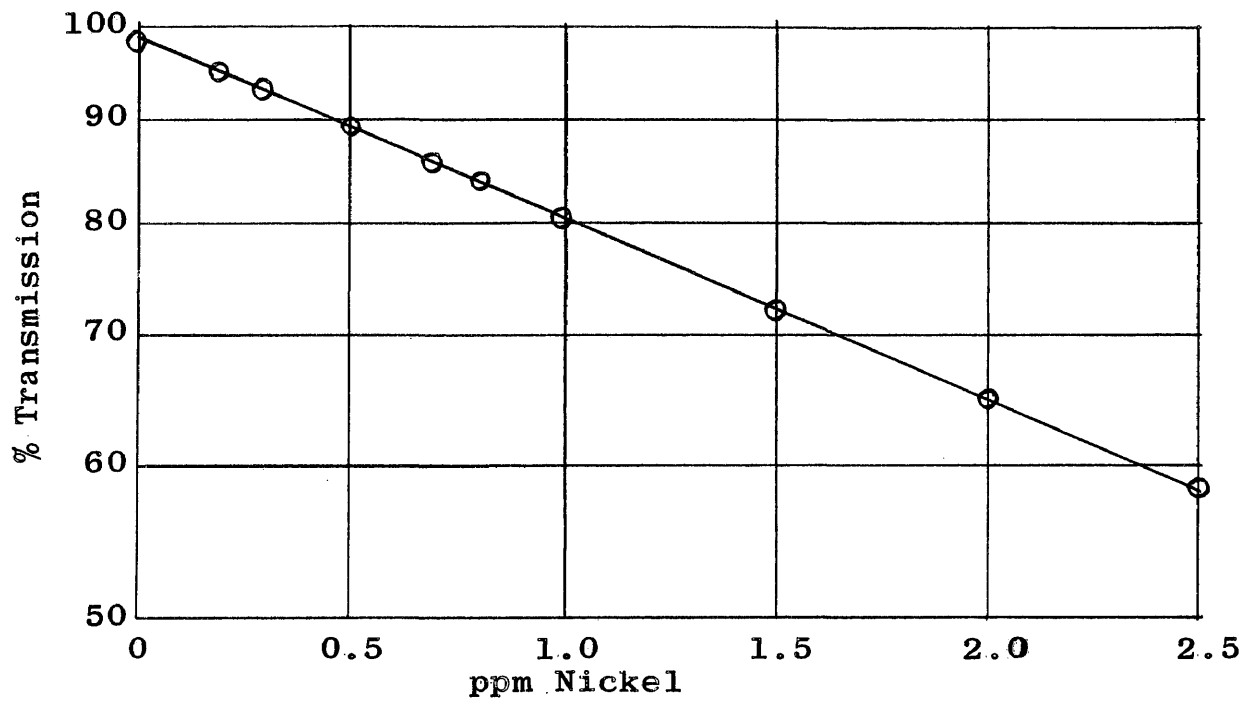


FIGURE 8

Standard Curve for Nickel Determination

light these bands can more easily be distinguished along with some minor bands not detectable without the aid of the ultraviolet light. The materials proceed down the column at a rapid rate, and each fraction is identified by its spectra. The first fraction off is the yellow solution of the carotenes, followed by the first green fraction (Figure 9) which is the pheophytins. This solution of pheophytin in n-hexane is kept in a tightly stoppered flask and stored in the dark.

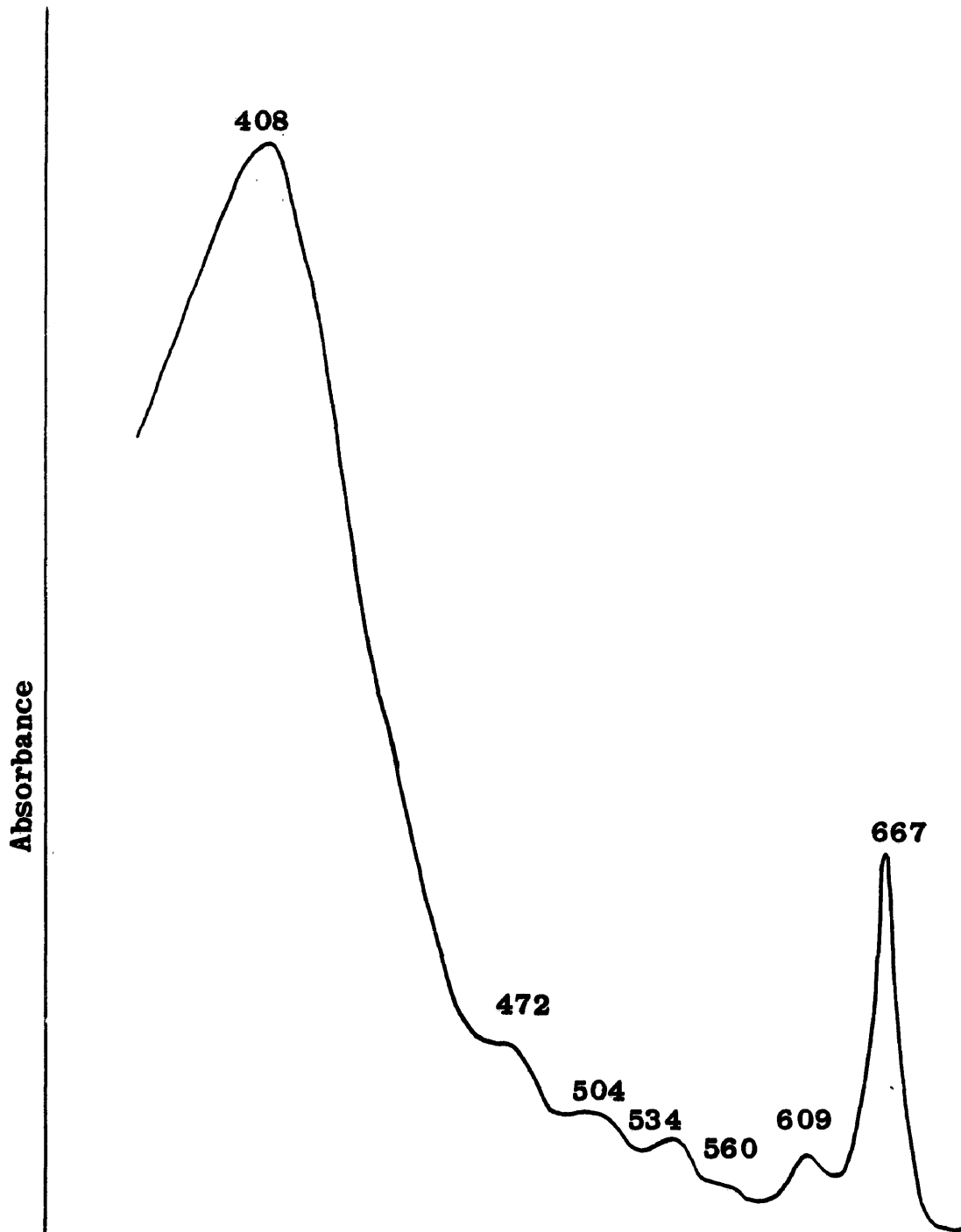


FIGURE 9

Spectra of Pheophytin a in n-Hexane
as Used for Metallation-Reaction Experiments

METALLATION REACTIONS

The topics to be discussed as experimental results of this thesis are the types of metallation reaction experiments, the results of each type of metallation reaction, and the attempts to produce the metallation reaction under more natural conditions.

Types of Metallation Reactions

According to the solvent used, four types of metallation reactions were examined:

Type I	N, N-dimethylformamide
Type II	Acetonitrile
Type III	Glacial acetic acid
Type IV	Glacial acetic acid-pyridine

The basic steps in a metallation-reaction experiment consisted of preparing a solution of pheophytin a in the appropriate solvent and adding the metal. The mixture was then allowed to react, and then the spectra were analyzed for indication of the presence of the metal complex.

A generalized procedure for the experiments is given below.

Fifteen ml of the pheophytin a in n-hexane solution was evaporated to dryness on a rotary vacuum evaporator. Fifteen ml of the solvent to be used and then 0.1 gram of the metal were added to the residue in the flask. This mixture was then refluxed in a nitrogen atmosphere until no further change could be detected in the spectra. The solvent used in the spectral determination was the same as that used in the reaction. See Figures 10, 11 and 12. The spectra were observed on mixtures or otherwise impure fractions where the relative band positions and intensities were altered by the presence of impurities. Results of the metallation-reaction experiments are expressed in Figures 13, 14 and 15.

Results of Metallation Reactions

Inspection of the spectral data of the Type I metallation reactions would, by shift of band I and the Soret band to specific locations for the particular metal used, indicate the formation and presence of nickel and copper complexes, but no vanadyl complex. By an analogous relationship, the spectral data of the Type II reactions would indicate the formation and presence of nickel and

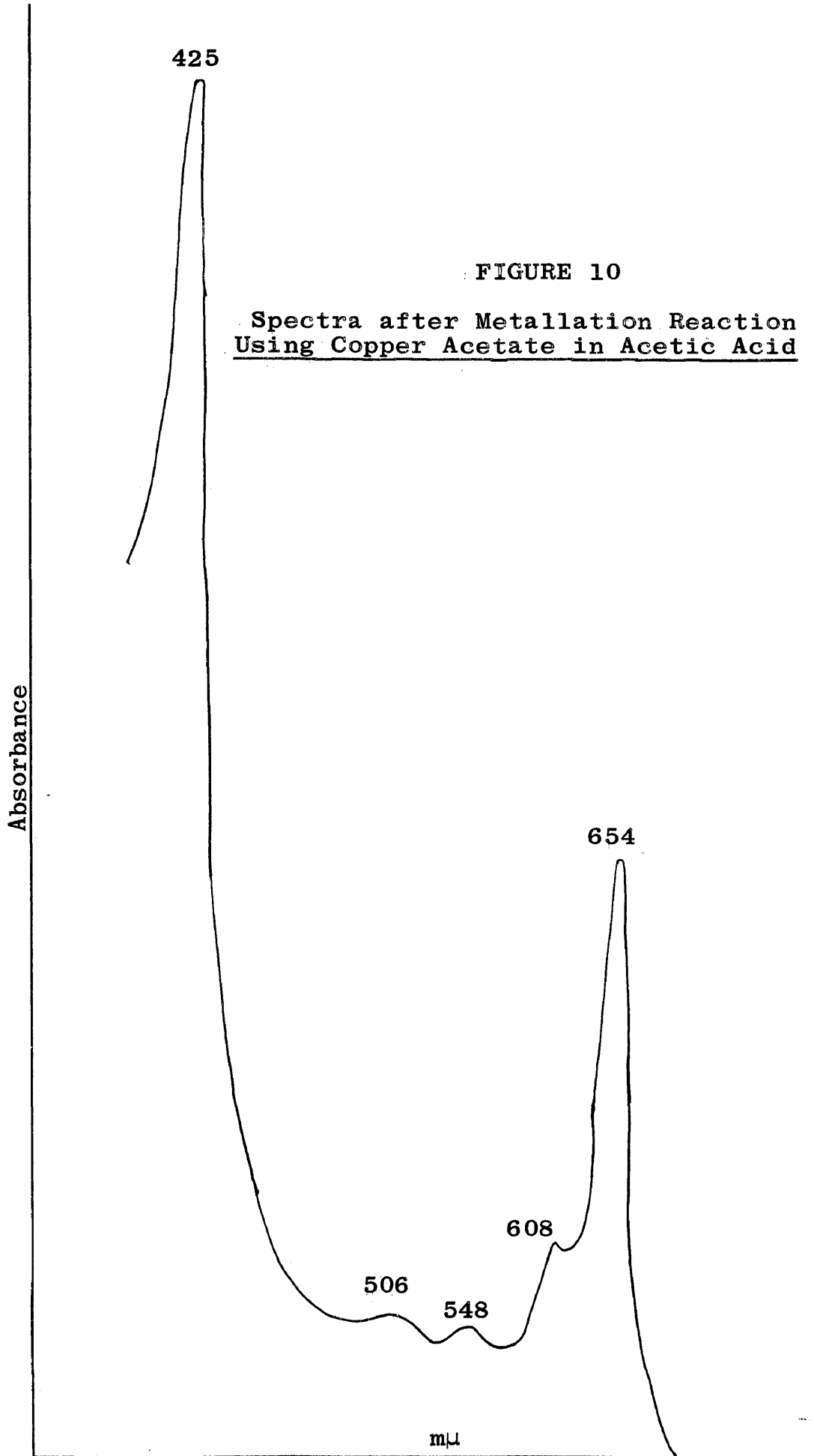


FIGURE 10

Spectra after Metallation Reaction
Using Copper Acetate in Acetic Acid

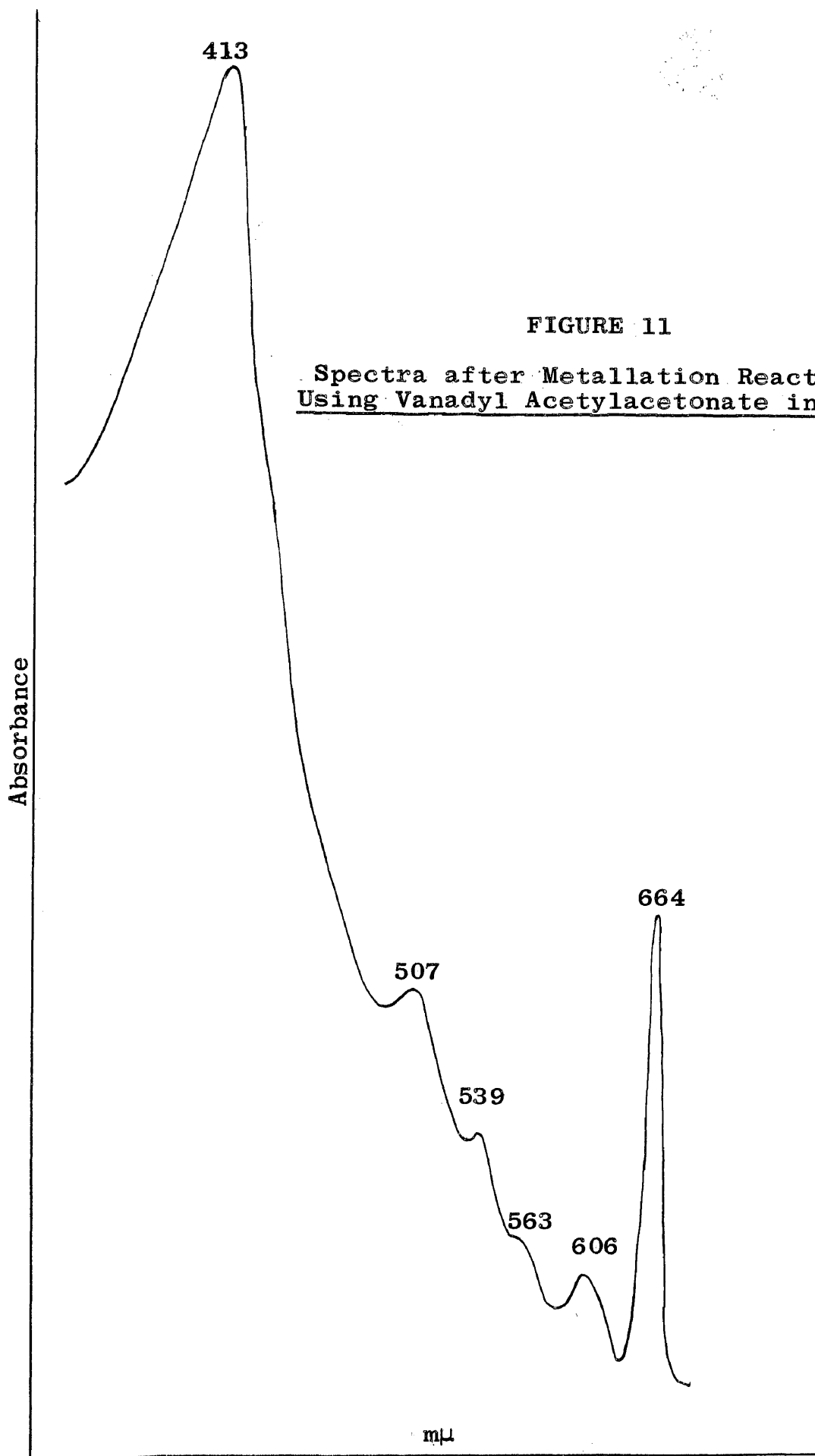


FIGURE 11
Spectra after Metallation Reaction
Using Vanadyl Acetylacetonate in DMF

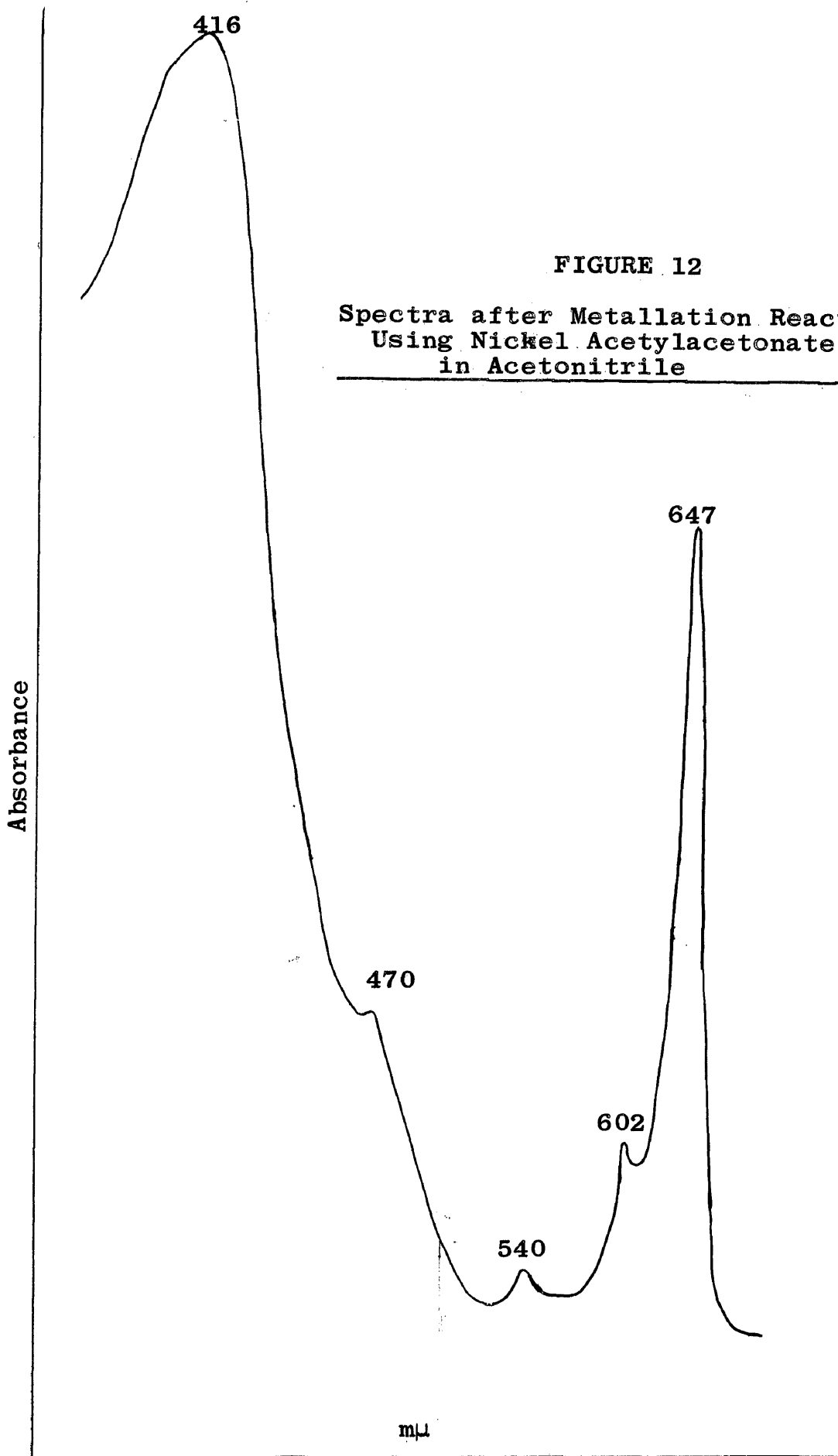


FIGURE 12
Spectra after Metallation Reaction
Using Nickel Acetylacetonate
in Acetonitrile

FIGURE 13

Metallation-Reaction Results

Trial Number	Metal	Solvent	Spectra				
			Soret	Band IV	Band III	Band II	Band I
I	Ni A.A.*	D.M.F.**	417	486	540	600	645
II	Ni A.A.	Acetonitrile	418	470	540	602	647
I	Ni A.A.	D.M.F.	412	484	540	590	645
I	Ni A.A.	D.M.F.	418	481	540	600	648
I	Ni A.A.	D.M.F.	418		545	595	646
I	Ni A.A.	D.M.F.	414	485	538	600	644
I	Ni A.A.	D.M.F.	414		540	595	645
I	Ni A.A.	D.M.F.	418	476	538	601	649
III	Ni(Ac) ₂	HAC***	408		540	598	645

* Nickel acetylacetonate

** N, N-dimethylformamide

*** Glacial acetic acid

FIGURE 14

Metallation-Reaction Results

Type	Metal	Solvent	Spectra					
			Soret	Band V	Band IV	Band III	Band II	Band I
III	VOSO ₄	HAc ***	417		530	562	600	650
I	VO A.A.*	D.M.F.**	412	506	536	562	604	663
IV	VOSO ₄	HAc-Pyridine	413	507	539	563	606	664
IV	VOSO ₄	HAc-Pyridine	413	506	539	562	606	664
III	Cu(Ac) ₂	HAc	425	506	548		608	654
II	Cu(Ac) ₂	Acetonitrile	411	500	548		600	650
I	Cu(Ac) ₂	D.M.F.	413	484	549		600	649

* VO A.A. - Vanadyl acetylacetonate

** N, N-dimethylformamide

*** Glacial acetic acid

FIGURE 15

Spectra of Pheophytin a in Various Solvents

	Soret	Band VI	Band V	Band IV	Band III	Band II	Band I
Pheophytin a n-hexane	408	472	504	534	560	609	667
N, N-dimethylformamide	410	480	492	534	560	608	665
Acetonitrile	409	473	504	533	560	607	663
Acetic acid	410	471	507	534	561	606	665
Benzene	409	470	505	536	562	608	665
80 percent acetone	409	472	505	536	558	610	666
Ether	408	471	505	534	560	609	667

copper complexes. For Type III reactions, it is clearly indicated that a vanadyl complex is present, with at least the indication of some interaction in the case of the nickel and copper.

The Type IV reaction, even after several attempts, produced no indication of a vanadyl complex with the pheophytin. This circumstance seems to raise a question, because Howe (23) stated that he metallated a chlorin with this procedure, and Blumer (24) stated that this procedure oxidized chlorins to porphyrins.

Metallation Reactions Under More Natural Conditions

Two procedures were studied in this area as follows:

Direct Metallation: A solution was prepared, consisting of 100 ml of water, three drops of "a chlorophyll in oil," 0.1 gram of nickel chloride, and 0.1 gram of vanadyl sulfate. This solution was stirred continually in an open flask for 18 days. During this period water was added to replace that which was lost by evaporation, and the spectra of the solution was checked periodically. During the course of this experiment, only a general deterioration of the spectra occurred.

Indirect Metallation: According to Hodgson (19) it is possible for chlorin pigments adsorbed onto clay minerals, to abstract copper from these clays. Six individual experiments were carried out using this idea. Samples were prepared as follows:

A) Mixtures of: One gram each of kaolinite, illite, and montmorillonite, 15 ml of pheophytin a in benzene, and 10 ml of 20 percent methanol in benzene was stirred continuously for two weeks in a stoppered, 50-ml erlenmeyer flask.

B) These three samples were prepared the same as in A), with the exception of the addition of 0.1 gram of copper acetate to each flask.

At various intervals, the spectra were determined as a means of distinguishing any change of the pheophytin to a pheophytin-metal complex. The spectra in all cases showed no change.

INTERPRETATION OF RESULTS

When Treibs (3) first noted the presence of green pigments in some ancient sediments, he found that the plant porphyrins generally constituted the major part of the total porphyrin aggregate. The major porphyrins were regarded as being related to chlorophyll. He assumed that they stood in a close genetic relationship to the chlorophylls, but with the isolation and structural identification in the case of etio-chlorin III, a hemin derivative, obviously the chlorin structure must be of secondary origin.

The chlorophyll-starting material contains magnesium, and the petroleum porphyrins contain nickel or vanadyl ions. The possibility that the original biocatalysts were nickel- and vanadyl-containing porphyrins similar in structure to chlorophyll, except for the metal, has been mentioned (25); however, such pigments are not known to exist in biological systems. The most reasonable conjecture seems to be that

the porphyrin was separate from these metals originally. The tightness of binding of these metals in combination with porphyrins is extraordinary.

Chlorophyll does not appear to require extensive chemical changes to produce nickel or vanadium derivatives. If these reactions (Figure 16) are assumed to take place for their formation, it is likely that only minor changes would be required on the chlorophyll molecule and that these changes would not require severe chemical conditions. If it is possible that these petroleum pigments may arise from these trace pigments, possibly the only conversions required are simple hydrogen transfer to convert chlorins into porphyrins, and the process of decarboxylation.

Probably the major compounds in this process are pheophytin and/or pheophorbide from the simple loss of magnesium of the source chlorophylls. A sufficient time, temperature, and a favorable acidity could combine with available metal atoms to permit the formation to the complexes. Metal-pigment complexes are formed in displacement reactions in which the complexing cation displaces two protons from the nitrogens of the basic pigment structure (26). Fleischer and Wang (28) have shown that the combination of metal ions with porphyrins takes place with the metal ion first combining with the porphyrin to

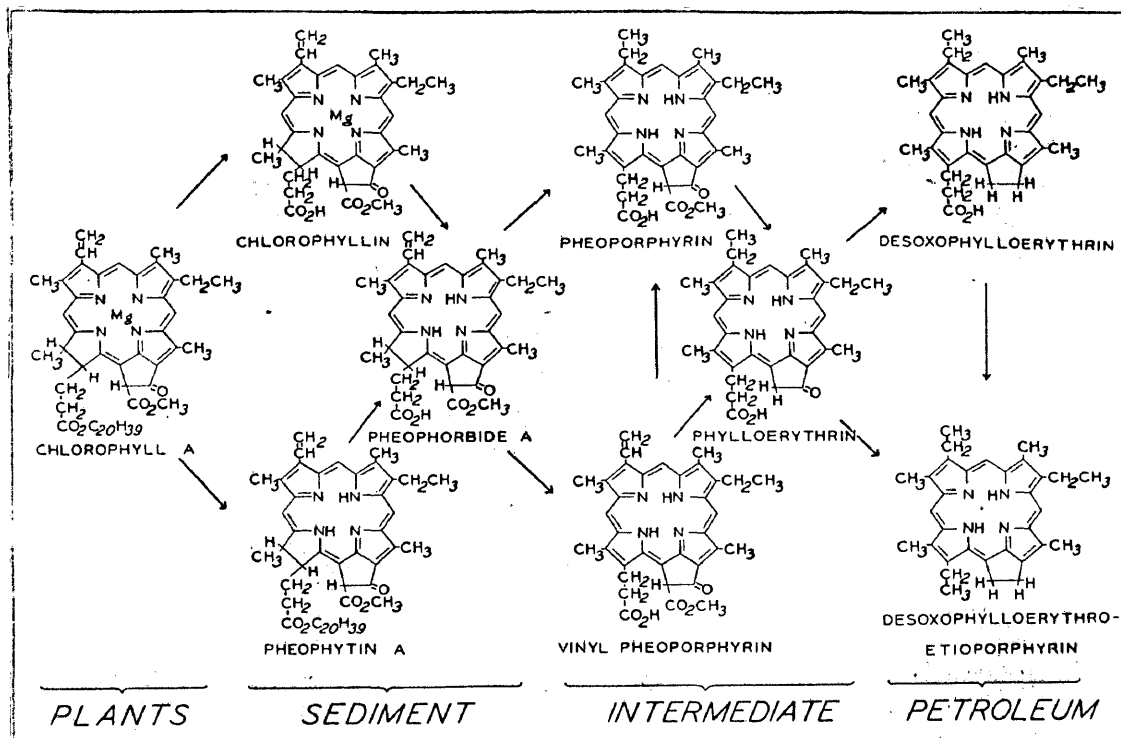


FIGURE 16

Nature of Chemical Changes Involved
in Forming Porphyrins Found in Petroleum
From Chlorophyll a

(after Orr, Emery and Grady, 1958 (18))

form a reaction intermediate which then decomposes to the normal metalloporphyrin complex and two hydrogen ions. Therefore, it is reasonable to suggest that the metal complexing of phorbins such as pheophytin a, and pheophorbide a takes place in a similar manner. The nature of the medium (8, 18) in which the reaction occurs and the relative concentrations of metallic ions are possibly equally important. The selective concentration of vanadium and nickel by the pigments is not easily understood, but once these ions enter the nucleus, they will not exchange later.

The exploratory phase of this investigation covers the various attempts to prepare nickel, vanadyl, and copper complexes of pheophytin a. In light of the ensuing discussions concerning the formation and presence of nickel, vanadyl, and copper complexes, the spectra were observed on mixtures or otherwise impure fractions where the relative band intensities were altered by the presence of impurities.

The spectral data -- Figures 13, 14 and 15 -- indicate the presence of metal complexes. There is definite evidence of their presence by the shift in band I to the vicinity of 650 m μ from that of 667 m μ , and the shift of the Soret band to the vicinity of 416 m μ . The specific locations of these bands for the particular metal used is evidence that these

pheophytin-metal complexes were prepared. The resulting change in the spectra, the shift of band I and the Soret band, along with changes in the position of other minor bands, were the criteria used for stating that these three metal complexes had been prepared.

Specific wavelengths have not been assigned to the major bands of the metal-chlorin complexes and the assignments made to date are based on the premise that the band positions of the chlorin-metal complexes should be similar to those of the analogous metal porphyrins. Few specific locations are mentioned, but Howe (15) reports the following locations for his vanadyl chlorin: 411 m μ , 536 m μ , 568 m μ , and 638 m μ . Generally, the bands are described in this manner: the chlorins show a very strong absorption peak near 660 to 680 m μ , or, the spectra of the copper complexes of chlorophyll derivatives are characterized by Soret bands at about 400 m μ and two maxima in the range of 510 to 640 m μ . For nickel complexes, they were marked by significant shifts to lower wavelengths, and for vanadium, less significant shifts to higher wavelengths.

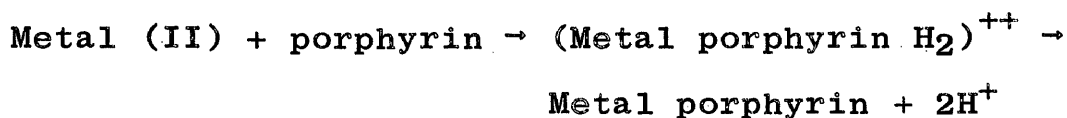
The spectra of chlorin metal complexes are typical of those listed below (27).

Cu	400 m μ	510 m μ	640 m μ
Ni	400 m μ	480 m μ	640 m μ
VO	400 m μ	530 m μ	650 m μ

These are only approximate locations for the various bands, and these bands are subject to additional changes by various solvents.

The regularity of the positioning of band I, generally varying only three or four $m\mu$, along with the same general regularities in the minor bands is not shown by the Soret band. A possible explanation for this anomaly could lie in the fact that the Soret band is generally a very broad band. This could be an indication of a mixture of the uncomplexed pheophytin and the metal complexed pheophytin.

An obvious question posed by the nature of this discussion is, what is the nature of this complex? Is this a true metal chlorin, with the metal inserted into the chlorin molecule, or is this a complex of the type discussed by Fleischer and Wang (28)? Fleischer and Wang have proposed a displacement mechanism for the metallation reaction in their study of porphyrin metallation by Fe (III). The reaction would proceed as follows:



They have detected the intermediate as a stable entity, which they call the sitting-atop complex in order to emphasize the structure they propose. The work of

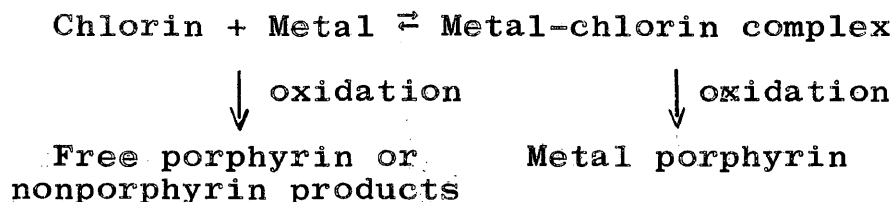
Murata (29) indicates that this complex is not an intermediate, but a stable, though nonisolatable product.

Although the spectral data preclude that the product is a true metal chlorin, other facts present would indicate that this might well be a complex of the type of Fleischer and Wang. The two facts substantiating the latter are that the rate of reaction for the complexing process is sufficiently rapid to indicate a ready formation of the metal complexes and that although it was possible to prepare these metal complexes, it was not possible to isolate them in any instance.

Porphyrin-metal complexes found in crude oils apparently are derived from chlorophyll. Recent sediment studies dealing with the origin of crude oil have identified metal-porphyrin complexes similar to, if not identical with, petroleum pigments; but no clear-cut path of development of the pigments has been apparent from the results of these examinations (6).

Once again the question is, did nickel and vanadium enter the porphyrin nucleus by forming chlorin-metal complexes which were subsequently oxidized to the metal porphyrins, or were the chlorins first oxidized to porphyrins, and these porphyrins metallated? See Figure 6. The purpose of this thesis was to investigate the former route, and the

results of the metallation reactions show that there are chlorin-metal interactions even though they are weak. The possibility is that there is an equilibrium existing between free chlorin and the metal. If the equilibrium theory is taken, and the metal-chlorin complex is considered to be even slightly more stable to oxidation than the free chlorin, it then becomes possible for the metal to be trapped to form the metal porphyrin. This might be visualized as follows:



The metal-porphyrin content of petroleum can be accounted for in this way even if only one percent of the chlorophyll derivatives can be considered as potential source-bed deposits by surviving oxidation as proposed (30). Even though it can be shown to be possible from the evidence presented above, the metal-chlorin to metal-porphyrin route is possible only if the metal-chlorin complex is the true metal chlorin with the metal inserted into the chlorin molecule. If the metal complex is the sitting-atop type, then there is still no explanation as to how the metal gets inserted into the porphyrin nucleus.

Because a vanadyl chlorin seemed to form only under acid conditions, whereas nickel formed under other conditions, a possible answer to the unexplained change of the V/Ni ratio for various oils lies not in the thermodynamic stability, but in the nature of the media that existed when the complex formed.

A more complete understanding of the metal complex formed in the metallation reactions would help to determine whether metal porphyrins are formed through metal chlorin intermediates. The proposed future work following this thesis would be to use larger quantities of pheophytin a so that the metal complex might be isolated and its true nature investigated.

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APPENDIX IReference Clay Minerals From
American Petroleum Institute
Research Project 49Illite

A name proposed as a general term (not a specific mineral name); widely distributed clay mineral constituent of argillaceous sediments apparently related to the mica group with the general formula (Grim, 1939):

$(\text{OH})_4\text{Ky}(\text{Al}_4\text{Fe}_4\text{Mg}_4\text{Mg}_6)(\text{Si}_{8-y}\text{Al}_y)\text{O}_{20}$. This name (illite) was proposed by Grim, Bray, and Bradley (1937) while working on clays, soils, and shales from Illinois, and was intended as recognition of a state in which clay study has received much encouragement.

Kaolinite

The mineral characteristic of kaolin, and have the general formula $\text{Al}_2\text{O}_3 \cdot 2\text{SiO}_2 \cdot 2\text{H}_2\text{O}$ is ordinarily recognized as kaolinite. The Al-Si ratio may vary from 2:1 to 3:1,

the major structural characteristics as indicated by x-ray diffraction patterns remaining the same. When the Al-Si ratio approaches 3:1, the mineral is ordinarily classed as anauxite.

Montmorillonite

The general formula being; $5Al_2O_3 \cdot 2MgO \cdot 24SiO_2 \cdot 6H_2O$ ($Na_2O \cdot CaO$). The three minerals montmorillonite, beidellite, and nontronite are members of an apparently continuous series of solid solutions, in which Fe^{3+} and Al^{3+} proxy for one another in octahedral coordination in all proportions, and Al^{3+} can proxy for Si^{4+} apparently not to exceed one ion in four.

Analytical Data on Reference Clay Materials

H-4 Kaolinite, O'Neal Pit, Macon, Georgia

	<u>%</u>
SiO ₂	44.82
Al ₂ O ₃	37.20
Fe ₂ O ₃	00.41
FeO	0.07
MgO	0.25
CaO	0.58
Na ₂ O	0.40
K ₂ O	0.43
H ₂ O	12.92
H ₂ O	1.76
TiO ₂	1.26
MnO	---
SO ₃	---
C	---
92.48% kaolinite	
7.52% impurities	100.10

H-19 Montmorillonite, Bentonite from Polkville Mine,
Polkville, Mississippi

	<u>%</u>
SiO ₂	50.95
Al ₂ O ₃	16.54
Fe ₂ O ₃	1.36
FeO	0.26
MgO	4.65
CaO	2.26
Na ₂ O	0.17
K ₂ O	0.47
H ₂ O	8.28
H ₂ O	15.01
TiO ₂	0.32
MnO ₂	0.01
C	0.01
	100.28

H-36 Illite, Morris, Illinois

	<u>%</u>
SiO ₂	57.41
Al ₂ O ₃	17.96
Fe ₂ O ₃	4.99
FeO	0.26
MgO	2.25
CaO	0.64
Na ₂ O	0.15
K ₂ O	5.75
H ₂ O	6.70
H ₂ O	2.97
TiO ₂	0.82
	99.90

Further details as to purity, analysis, x-ray data, infrared, etc., available in American Petroleum Institute publications.