SOLUTION STUDIES OF MESO-TETRA(p-SULPHOPHENYL)PORPHINE: AGGREGATION, METALLATION AND POTENTIAL ANALYTICAL APPLICATIONS

By

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SOLUTION STUDIES OF A WATER-SOLUBLE PORPHYRIN

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ABSTRACT

Meso-tetra(p-sulphophenyl)porphine ($TPPS_4$), a synthetic water-soluble porphyrin, has been prepared in high purity and characterized in greater detail than previously reported. Several metal complexes of $TPPS_4$ have been prepared by a novel heterogeneous reaction. This simple reaction was also used in the synthesis of complexes of other porphyrins.

The solution behaviour of $TPPS_4$ was investigated by spectrophotometry and nuclear magnetic resonance methods, with a view toward ultimate analytical applications. In neutral or alkaline ionic media, $TPPS_4$ was found to dimerize, with $K_D = 1.58 \times 10^4\ M^{-1}$. At higher concentrations, $TPPS_4$ was shown to aggregate further to tetrameric species, with $K_{TT} \approx 4\text{-}15\ M^{-1}$. In acidic media, the behaviour of $TPPS_4$ was found to be complex and completely different from that observed in neutral media. A model involving micellization of $TPPS_4$ has been advanced to account for observations made in both the present work and that of other workers.

The kinetics of metal-ion incorporation, as exemplified by the reaction of Cu(II) with $TPPS_4$, was studied at pH 3.55. In general, the findings agreed with those of other workers, but an initial slow reaction, not previously reported, was found to occur under conditions favouring aggregation of $TPPS_4$.

The reactivity of $TPPS_4$ towards metal ions was examined in both neutral and acidic (pH 3.55) solutions. $TPPS_4$ showed kinetic and thermodynamic selectivity toward a relatively small group of metal ions, with the greatest selectivity towards Cu(II). The anionic metal complexes were found to be extractable as ion-associated species (with tetraphenylarsonium nitrate, for example) into organic solvents such as 1-pentanol.

Based on these findings, an extraction-spectrophotometric method was developed for the determination of Cu(II), with a detection limit of 1.1 ppb. This limit compares favourably with those of atomic absorption and inductively-coupled plasma emission. With further development, the use of $TPPS_4$ as a complexing and preconcentration reagent in trace metal analysis appears to be most promising.

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CHAPTER I

INTRODUCTION

I.1 GENERAL

Organic reagents have held a position of importance in chemical analysis for many years. Their use as precipitants, titrants, sequestering agents, spectrophotometric and fluorometric reagents and in separations has been well established. Increased demands for greater sensitivity in areas such as environmental and clinical chemistry and electronics technology have resulted, however, in a growing emphasis on the application of instrumental methods of analysis with a concomitant decrease in the prominence of organic reagents. Notwithstanding the foregoing, organic reagents continue to be important and the need for them in separation methods has, in fact, increased because the effects of interferring species become more significant as the detection limits of analytical techniques are approached. With regard to sensitivity, the importance of organic reagents has also appreciated because of their convenience in preconcentration procedures, i.e., procedures developed to augment the sensitivity of instrumental techniques.

Over the years, considerable effort has been expended to determine the factors that govern not only the sensitivity of organic reagents but also their selectivity. Many systematic studies have been made that relate the solution stability of metal complexes to such factors as donor-atom basicity, the nature of the donor atom and certain structural features of the ligand such as chelate-ring size and potential steric-hindering substituents. While these studies have greatly increased our knowledge of the solution chemistry of metal complexes and have led to exhaustive compilations of stability constants (e.g., 1, 2) which have proven to be invaluable to analytical chemists, they have not led to the design and synthesis of a significant number of highly selective reagents. More recently, the development and application of cavity-type ligands such as cyclic polyethers and cryptates, which discriminate on the basis of metal-ion size and charge, has renewed interest in the search for selective organic reagents (e.g., 3, 4).

Porphyrins and related compounds are other examples of cavity ligands. Because of their biological importance, exemplified by such compounds as the hemoproteins, the chlorophylls and vitamin B_{12}, the porphyrins have been extensively investigated over the years (5). These studies, concerning both the natural compounds and synthetic analogues prepared as model systems, have been largely fundamental in nature. Recently, however, there has been some interest in the application of porphyrins as antitumour and tumour-imaging agents (6-10), additives in laundry detergents to prevent colour transfer (11), coatings in photovoltaic cells to increase efficiency (12,13), catalysts (14-19), shift reagents in NMR spectroscopy (20-22), photographic reagents (23) and as analytical reagents (24-29). The analytical application of porphyrins has been restricted to their obvious use as spectrophotometric reagents based on their intense absorption ($\varepsilon = 1 \times 10^5 - 5 \times 10^5$ cm^{-1} M^{-1}) in the 350-450 nm region. For example, trisulphonated tetraphenylporphine has been used for the spectrophotometric determination of Cu(II) (25) and Pb(II) (26) at ppm levels. Similarly, meso-tetra (3-N-methylpyridyl)-

porphine has been used for the determination of Cu(II) (27). In these instances, the investigators took advantage of the high selectivity of the ligands in acid solution and of their high molar absorptivities. In an interesting study, Corsini and Mehdi (29) used the dipiperidyl complex of meso-tetraphenylporphinato iron(II) to determine the carbon monoxide content of synthetic atmospheres.

I.2 AIM OF THESIS

The aim of this investigation is to explore the analytical potential of porphyrins, in particular, of meso-tetra(p-sulphophenyl)porphine, $TPPS_4$, a synthetic water-soluble compound. The philosophy of this study is that it is more fruitful to initially examine certain fundamental solution properties in detail before attempting the direct development of specific analytical applications. This approach was vindicated by subsequent findings, reported herein, which illustrate the complex behaviour of $TPPS_4$ in solution in both the absence and presence of metal ions. The direct approach to the development of applications might yield results more rapidly but it can also lead to an erroneous or a restricted view of the system, as demonstrated by some of the analytical applications of porphyrins thus far developed.

The work described in this thesis is divided into four main sections:

(1) the synthesis and characterization of $TPPS_4$ (as its ammonium and sodium salts) and of a few of its metal complexes;

(2) aggregation of the ligand and metal complexes in aqueous media and in dimethylsulphoxide media;

(3) metal-ion incorporation into $TPPS_4$ as exemplified by the

insertion of Cu(II); and,

(4) initial investigations into possible analytical applications of $TPPS_4$.

In the following sections, a brief description of the chemistry of porphyrins and metalloporphyrins is given, with emphasis on those aspects which are of relevance to this thesis.

I.3 STRUCTURE AND STABILITY OF PORPHYRINS

Porphyrins are conjugated tetrapyrrole macrocyclic molecules and can be considered to be substituted derivatives of porphine (Figure I). Unfortunately, porphyrin nomenclature has always involved the use of trivial rather than systematic names for the substituted porphine moiety. The literature on porphyrins and related compounds is voluminous and can be found in monographs such as those by Falk (30), Marks (31) and Smith (5). In addition, several reviews exist (e.g., 32-37).

The structure of porphyrins was first proposed by Küster in 1912 (38), but gained acceptance only after the total synthesis of hemin by Fischer and Zeile in 1929 (39). Positions 1-8 and α-δ (meso positions) on porphine can be substituted by a variety of groups. Naturally-occurring porphyrins have no ~~meso-substituents~~. Substituted porphines can show positional isomerism although with naturally-occurring porphyrins, usually only one isomer is present in a given system.

Metal chelates, known as metalloporphyrins, are formed by replacement of the two central hydrogens of the porphyrin by a metal ion. Mono, di and trivalent metal ions form chelates of the type M_2P, MP and MPX (where X is an anion), respectively. Chelates have been formed with almost

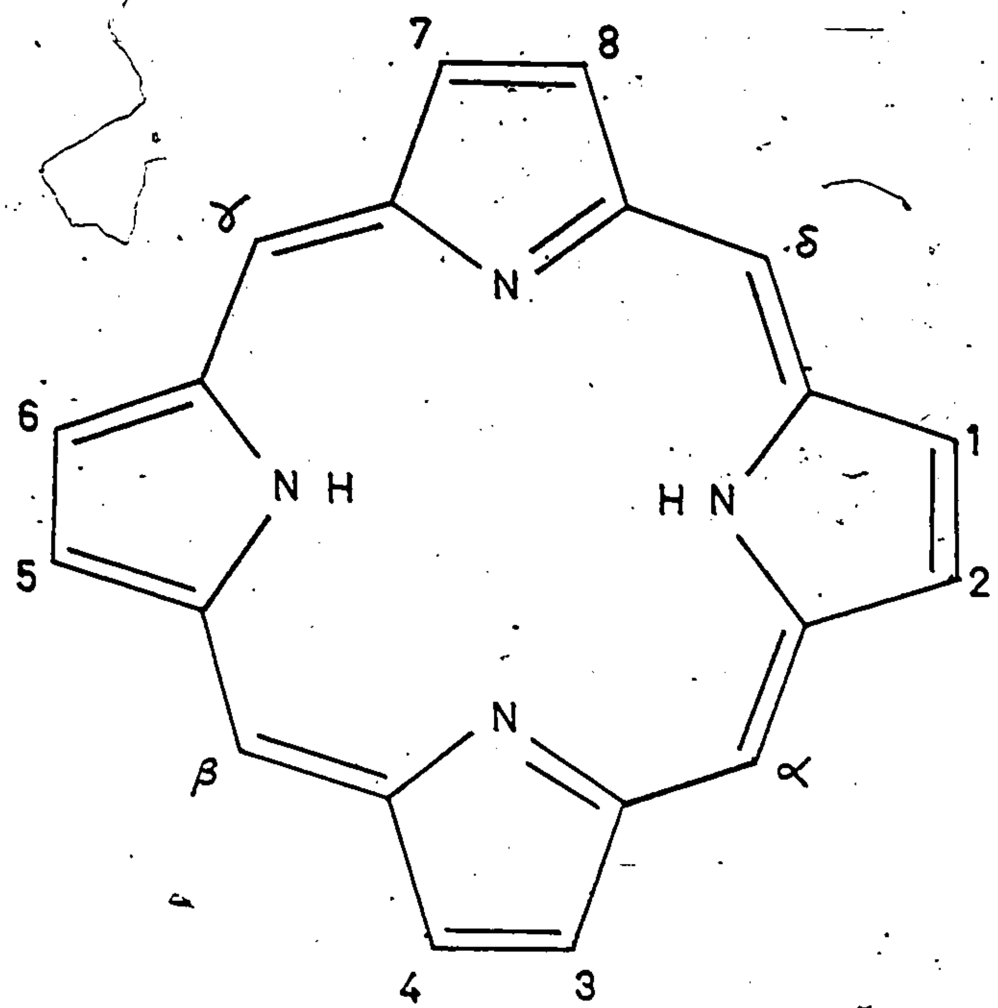

Figure I. Structure of porphine. Positions α-δ also known as meso positions.

every metallic and semi-metallic element in the Periodic Table (40-42).

It has been shown by X-ray studies that the basic porphyrin-ring structure is essentially planar (32,43,44), with a ring diameter of about 8.5 Å. In meso-tetraphenylporphine (TPP) and various derivatives with substituents on the phenyl rings, the plane of the phenyl rings is tilted with respect to the mean plane of the porphyrin ring. The angle between the phenyl and porphyrin planes has been found to range from 60-85° in the solid state (32) and theoretical calculations indicate it to be about 45° in solution (45). A number of studies (e.g., 46-48) suggest that the degree of coplanarity can be greater, thus allowing considerable resonance interaction to occur between the phenyl and the porphyrin π systems. Divalent metal-ion complexes are also planar (32) except when the metal ion is unable to fit into the central cavity (diameter about 4.2 Å). Metalloporphyrins of trivalent metal ions are regarded as square pyramidal because of the presence of the anion in the fifth coordination position. In many metalloporphyrins, the metal ion may be further coordinated to axial ligands, resulting in octahedral (often distorted) structures. Large metal ions often form metalloporphyrins with coordination numbers greater than six (e.g., 49-51).

No quantitative measurements of the solution thermodynamic stability of metalloporphyrins have been made, primarily for the following reasons: (1) the high thermodynamic stability of metalloporphyrins results in very low concentrations of unbound species, making measurements difficult; (2) the relatively high activation energy required for complex formation often results in a very slow attainment of equilibrium; (3) difficulties are often posed by limited solubility and interferring equilibria. An estimate of the stability of Zn(II) mesoporphyrin IX dimethyl ester in 0.25% cetyl tetramethylammonium bromide places log K_s at about 29 (52). Qualitative schemes that "order" the stability of complexes for a given ligand and group of metal ions have been developed. These are based on transmetallation reactions and the severity of ~~conditions~~ required for demetallation. For example, the following stability order for divalent metal ions has been reported (30,33,52): Pt(II) > Pd(II) > Ni(II) > Co(II) > Ag(II) > Cu(II) > Zn(II) > Mg(II) > Cd(II) > Sn(II) > Ba(II). Buchler (53) has challenged this order mainly on the basis that the relative order of Ni(II), Co(II), Ag(II) and Cu(II) had been taken from spectroscopic and kinetic rather than from thermodynamic measurements and that the Sn(II) porphyrins investigated were later shown to be Sn(IV) porphyrins. Attempts to correlate various spectral features such as the intensity ratio of absorption bands with stability have been successful only within the limited number of complexes originally considered. The scheme that considers perhaps the largest variety of metalloporphyrins is that developed by Buchler (54). His stability index is given by the equation

$$S_i = 100\ Z \cdot E_N / r_i$$

where Z is the charge number of the ion, E_N is the Pauling value for the electronegativity of the element irrespective of oxidation state and r_i is the effective ionic radius given by Shannon and Prewitt (55). This stability index gives a reasonable correlation with the findings of the transmetallation and demetallation schemes. Unfortunately, this stability index is based on metal complexes of octa-alkylporphyrins which are not water-soluble and hence is not applicable to $TPPS_4$ complexes.

I.4 ELECTRONIC SPECTRA

Porphyrins show a distinctive five-banded absorption spectrum in the near-ultraviolet to visible region (350-650 nm). The most intense band is the so-called Soret band, with its maximum usually found in the region 350-450 nm and with a molar absorptivity of about 10^5 cm^{-1} M^{-1}. Because of its intensity, this band is often used for the spectrophotometric determination of porphyrins themselves. The order of intensity of the other four bands depends upon the type of porphyrin. TPP and its derivatives, including $TPPS_4$ have spectra of the 'etio-type', in which the bands increase in intensity with energy (Figure II). Spectra of the other types, such as the rhodo- and phyllo-type, in which the band intensities are not as directly ordered with energy, may be found elsewhere (56,57). Falk (30) and Smith (5) provide extensive tables of absorption spectra. Protonation or metallation of the porphyrin leads to changes in molecular symmetry and thus in the absorption spectrum; namely, there is a reduction in the number of main bands in the visible region. Metalloporphyrins of the same metal in different oxidation states have different spectra. For example, a 'normal' spectrum with a Soret band and two bands in the visible region is obtained when the metal is present as M(II). Upon oxidation to M(III), the bands are usually shifted to higher or lower wavelengths and one or more additional bands are present. Theoretical interpretations of porphyrin absorption spectra are not within the scope of this thesis.

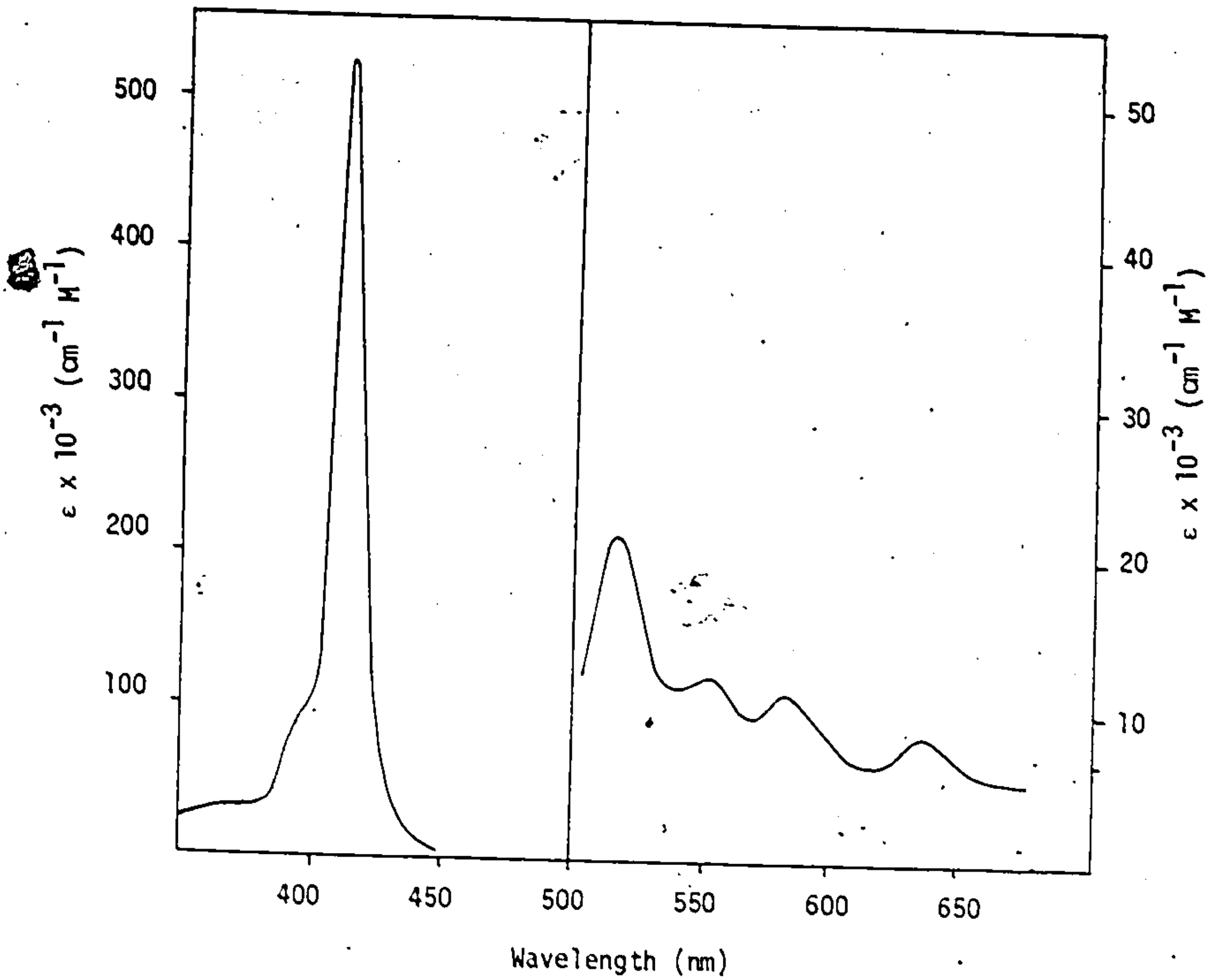

Figure II. Etio-type spectrum of $TPPS_4$

I.5 NUCLEAR MAGNETIC RESONANCE SPECTRA

In the following section, only those aspects of NMR spectroscopy which specifically relate to porphyrins are discussed. Since almost all published NMR work is with reference to the solvents $CDCl_3$ and trifluoroacetic acid (TFA), the use of these solvents in the studies discussed below is implied unless stated otherwise.

The long-range diamagnetic contribution of the aromatic macrocyclic system to the chemical shift is the most important factor that distinguishes the NMR spectra of porphyrins from those of similar non-aromatic compounds. According to the classical ring-current model, an external magnetic field induces a Larmor precession of the entire π-cloud of an aromatic system (58). This induced ring current gives rise to a secondary magnetic field which opposes the applied field. Since this effect is not averaged "to zero" by tumbling of the molecules within the solution, the ring current results in anisotropic shielding of protons within its range. The shielding is positive for nuclei within cone-shaped volumes of space located above and below the plane of the aromatic system, with the apices of the cones directed toward the center of the aromatic system (Figure III). The shielding is negative for nuclei outside these regions. Various refinements of the ring-current model have proven useful in the interpretation of chemical shifts in aromatic systems and have been widely used, although they give only qualitative agreement with experiment.

The sizeable ring-current effects associated with the porphyrin π system cause a spread in the ^{1}Hmr spectra of these compounds over a range of about 15 ppm. A number of studies have been concerned with

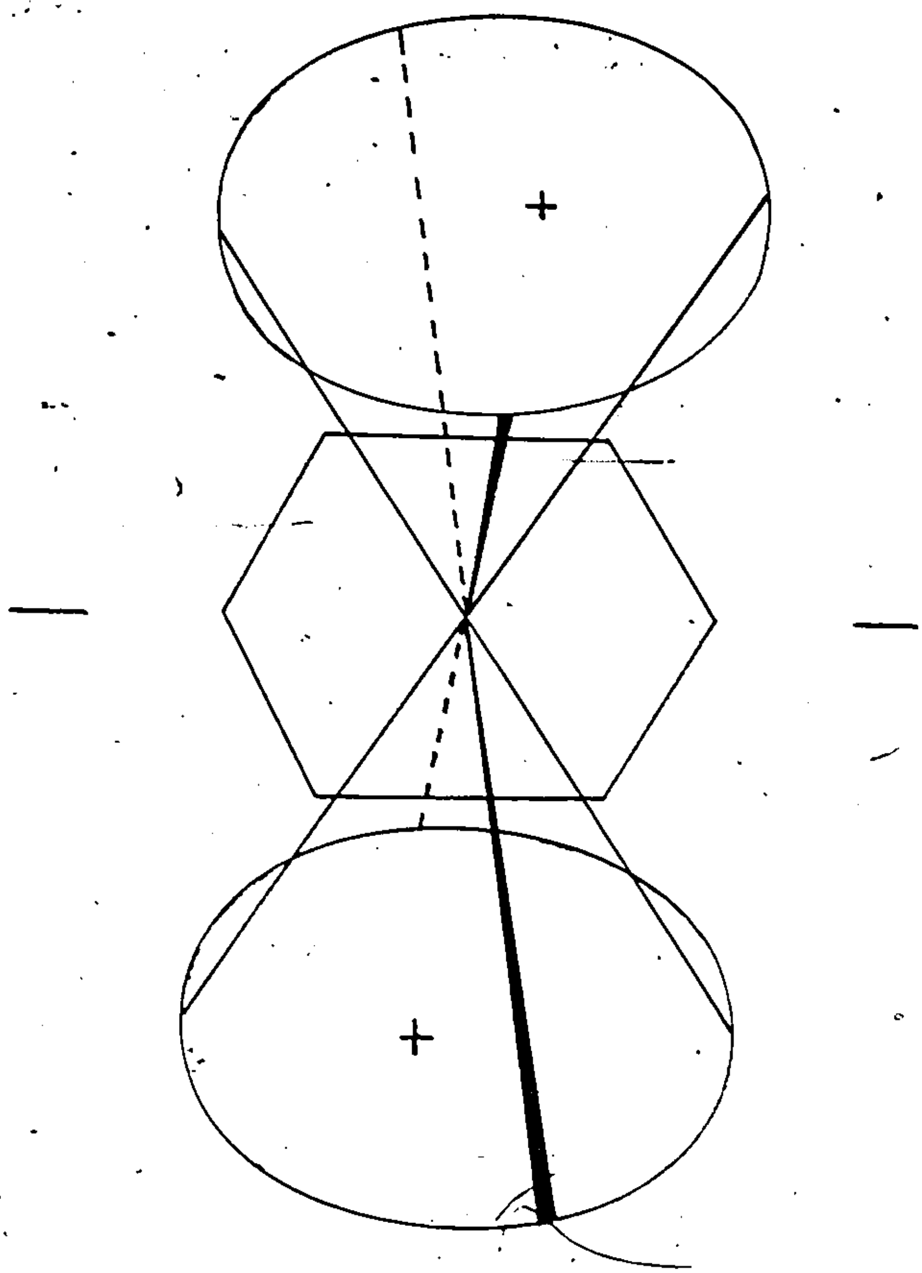

Figure III. Aromatic shielding regions of benzene.

this effect (59-65). The spectrum of porphine provides a classic example. The protons at the meso and β-pyrrole positions are deshielded by the ring current and their resonances are observed at 10.58 and 9.74 ppm,* respectively. Comparison of these values with those for tri-alkylsubstituted ethylene (5.17 ppm) and for the 3- and 4-protons of 2,5-dimethylpyrrole (5.57 ppm) shows the effect of the ring current. An even larger effect is observed on comparison of the N-H proton resonance of 2,5-dimethylpyrrole (7.1 ppm) and porphine (-3.76 ppm). In this case, the ring current has a strong shielding effect.

The effects of substitution on the spectra of porphyrins are often significant. For example, the effect of meso substitution is threefold: (1) the ring current is reduced by about 3% if the neighbouring β-pyrrole position is unsubstituted and to a greater extent (because of steric hindrance) if it is substituted; (2) protons in the vicinity of the substituent, i.e., β-pyrrole protons, experience additional shielding effects which are partly steric in origin but which can result mainly from the magnetic anisotropies of substituents such as phenyl or cyano groups; (3) the proton in the opposite unsubstituted meso position is more strongly shifted to higher field than protons at the neighbouring meso positions, because of folding of the macrocycle. (This last effect is, of course, not relevant for TPP and other tetra meso-substituted porphyrins.) The effect of substitution is illustrated by comparison of the spectrum of

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* Chemical shift values given in this thesis are based on the δ system which assigns a value of 0 ppm to the tetramethylsilane (TMS) resonance.

porphine (β-pyrrole, 9.92 ppm; N-H, -4.40 ppm) to that of meso-tetramethylporphine (β-pyrrole, 9.55 ppm; N-H, -3.01 ppm) in TFA.

The choice of solvent can have a significant effect on the spectrum. In TFA, the porphyrin is protonated to form the dication; the C-H resonances are shifted by 0.8-1.0 ppm to lower field and the N-H resonances by 0.4-1.0 ppm to higher field, compared to those for the free-base porphyrin (in $CDCl_3$). Abraham (61) proposed that the increased molecular symmetry of the dication resulted in an enhanced ring current because of a larger resonance energy. For the N-H resonance, this effect would be partly compensated by deshielding from the positive charges. Expansion of the π system in the dication to the periphery of the macrocycle (66) and changes in hydrogen bonding (67) have also been suggested as contributing to the chemical shift changes.

TPP and its derivatives show a number of interesting effects in addition to those discussed above. The spectrum of TPP in CS_2 is given in Figure IV. Only one signal is observed for the β-pyrrole protons because tautomeric exchange of the N-H protons among the nitrogen sites is rapid at ambient temperatures. When this exchange is "frozen out" at lower temperatures (e.g., -80°C), a doublet is observed for the β-pyrrole protons. This phenomenon is common among free-base porphyrins (68-71). Another interesting effect is observed for metalloporphyrins in which the metal ion is out of the plane of the central nitrogen atoms. In the free ligand, the two ortho (and two meta) protons of the phenyl substituents are chemically equivalent because of the symmetry of the molecule. In the above complexes, the chemical equivalence is removed

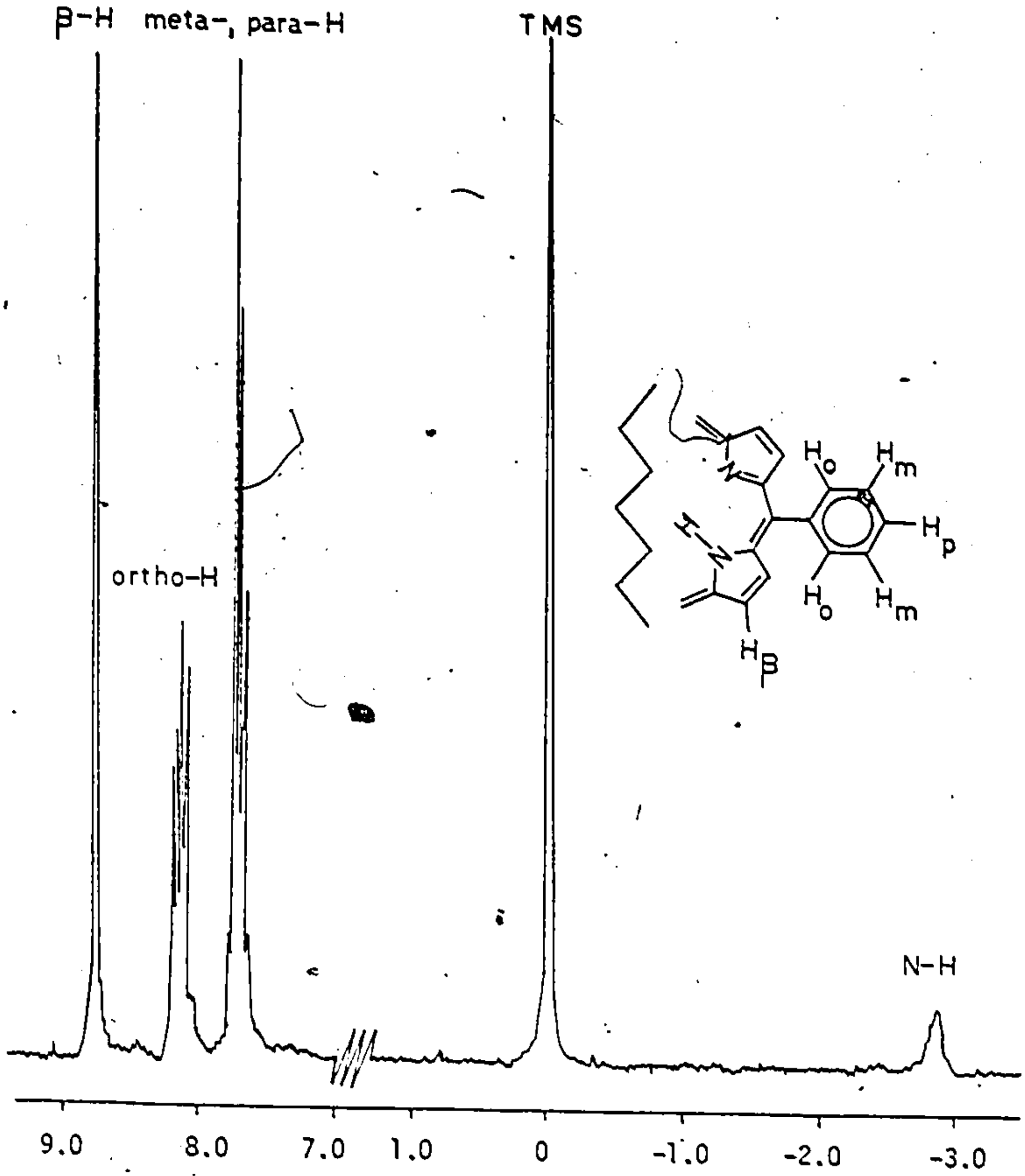

Figure IV. ^{1}Hmr spectrum of TPP (0.012 molal) in CS_2 at 28°C because the molecule is no longer symmetrical with respect to its mean plane and because the hindered rotation of the phenyl groups prevents averaging of the environments of the ortho (and meta) protons. As a result, increased multiplicity of the phenyl-proton resonances is observed. This effect is useful for determining the position of the metal ion relative to the porphyrin plane (e.g., 65).

NMR spectroscopy has proven to be a valuable technique for the study of molecular complexes formed between foreign molecules and porphyrins (and metalloporphyrins). Two interesting examples are the metalloporphyrin complexes with 1,3,5-trinitrobenzene (72,73) and with caffeine (74). There have been several investigations of molecular complexes based on the use of metalloporphyrins as shift reagents. These studies have shown non-lanthanide metalloporphyrins to be more stable and selective shift reagents than the β-diketonates but, unfortunately, they produce a smaller chemical shift (20,65,75). Recently, lanthanide-porphyrin complexes have been prepared and evaluated relative to their β-diketonate counterparts (21,22).

NMR spectroscopy is also an important technique for the study of self-aggregation of porphyrins in solution. Self-aggregation of porphyrins and metalloporphyrins is indicated by the strong dependence of the chemical shifts on concentration, solvent and temperature. This dependence arises from the molecular interactions combined with the magnetic anisotropy of porphyrins. In porphyrins, aggregation is due to π-π interactions and to intermolecular interactions between substituents. Aggregation of metalloporphyrins is caused by π-π and metal-ligand interactions.

In general, the π-π forces are weak and produce an upfield shift. This upfield shift depends on the positioning of protons above and below the plane of the macrocycle. Since the surface defining zero shielding is not perpendicular to the macrocycle plane, an associated molecule must be substantially larger in area than the porphyrin ring in order to protrude into the deshielding area. This situation can occur, of course, in the aggregation of porphyrins with large substituents.

In ^{1}Hmr and ^{13}Cmr studies with coproporphyrin tetramethyl esters, Abraham et al. (76-78) examined the dependence of chemical shifts on concentration. Quantitative analysis of the concentration dependence led these workers to draw the following conclusions: (1) the data could be accounted for in terms of a monomer-dimer equilibrium (higher aggregates did not need to be considered); (2) the molecules are parallel and about 8 Å apart in the dimer; and, (3) the dimer components are staggered due to steric interaction of the substituents. Similar observations were made with the meso-, proto- and deuteroporphyrin IX dimethyl esters (79,80), except that the dimer components of mesoporphyrin IX dimethyl ester were found to be about 10 Å apart. In metalloporphyrins, the metal-ligand interactions are often much stronger than the π-π interactions. Abraham et al. (81) have recently shown a specific interaction between the central metal ion of one metalloporphyrin molecule and the side chain of another molecule.

Although the chemical shifts caused by aggregation are a useful means by which to study molecular interactions, they present a problem with regard to the assignment of the resonances. The most rigorous approach involves concentration-dependence studies and extrapolation of the data to infinite dilution. Other approaches have involved attempts to disrupt the aggregates. Often, bases such as tetrahydrofuran, methanol, and pyrrolidine are added in small amounts to compete for metal-coordination sites to destroy the aggregation of the metalloporphrin. Also, solvents such as TFA can be used since most dications are not extensively aggregated. Furthermore, TFA will often preferentially ligate the axial coordination sites and thus reduce aggregation.

The sensitivity of the NMR spectra to so many factors requires that caution be exercised in the comparison of spectra and in the drawing of conclusions. The concentration and solvent system (among other conditions) at which spectra are recorded must always be noted.

Since the advent of pulse Fourier transform NMR, an increasing number of studies have involved the use of ^{13}C and other nuclei. In the ^{13}C studies, the spectra encompass a range of 200 ppm and hence the relative contribution of the ring current to the chemical shift is less, although its magnitude is the same as in ^{1}Hmr. At the present time, ambiguities in the interpretation of ^{13}C shifts do not allow complete separation of the ring-current contributions. The enhanced resolution and the ability to distinguish totally-substituted* carbon atoms give structural information not available from ^{1}Hmr. The meso-carbon resonances occur at 90-100 ppm relative to TMS. The spacing of these resonances is affected by β-substitution. Alkyl substitution on the β-pyrrole carbon

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* The terms "totally substituted" and "fully substituted" are used herein to refer to carbon atoms which bear no protons. The literature term "quaternary" to indicate a fully-substituted carbon is unfortunate.

shifts the neighbouring meso-carbon resonance upfield by about 3.5 ppm compared to that of the unsubstituted compound (82). Resonances for fully substituted α- and β-pyrrole carbon atoms occur in the 130-170 ppm region, with the latter at higher field. The two sets are closely spaced in porphyrins in which the pyrroles are completely alkyl-substituted and more spread in less-substituted porphyrins (78,82,83). In ^{13}Cmr, N-H tautomerism is readily observed as it is in ^{1}Hmr. For example, in an interesting study, the resonances of the α-pyrrole carbons in coproporphyrin isomers were found to be close to coalescence at ambient temperature, indicating only moderately rapid exchange (82). When the N-H exchange is slower, as in chlorins, two distinct types of rings are observed, pyrrole- and pyridine-like (84). Abraham et al. (82) studied the effect of nitrogen protonation on the ^{13}C spectra. At low concentrations of TFA, the α-carbons are shielded and the β- and meso-carbons are deshielded. At higher concentrations, all the resonances are shifted to lower field.

In addition to the studies discussed above, there is considerable work in the literature which involves the use of other nuclei and paramagnetic complexes. As this work is not relevant to this thesis, it is not discussed here.

I.6 AGGREGATION

With regard to porphyrin aggregation, a curious dichotomy has arisen insofar as there have been two distinct kinds of investigations. One kind has dealt exclusively with the relationship between NMR spectra and aggregation phenomena. The spectral effects have been discussed above. The porphyrins considered are those soluble in organic solvents, e.g., chloroform. The results have been interpreted in terms of a monomer-dimer equilibrium, with small amounts of higher aggregates postulated at the upper concentration levels. The main thrust of these studies has been to attain structural information and chemical shifts free of aggregation effects (e.g., 76,79,80,84b). A few dimerization constants (K_D) have been determined. For example, the K_D value for coproporphyrin tetramethyl esters is 3.55 M^{-1} (76) and for mesoporphyrin IX dimethyl ester, 30.9 M^{-1} (79). A value of 60.1 M^{-1} has been reported (79) for the Ni(II) chelate of mesoporphyrin IX dimethyl ester.

The second kind of investigation has dealt with the study of aggregation behaviour by spectrophotometric and temperature-jump techniques. The porphyrins considered are water soluble. These studies have been done at low concentrations (10^{-7} to 10^{-4} M) whereas the NMR studies (of necessity) have been carried out at higher concentrations ($\geq 5 \times 10^{-4}$ M). There is almost no overlap in the kind of porphyrins used between the two groups except in one study (spectrophotometric) which involved deutero-, copro-, haemato- and protoporphyrins (all previously studied by NMR spectroscopy). This study (85) was so qualitative that the results of the two techniques could not be compared. In other studies in aqueous systems, NMR spectra have been reported in the course of characterization but one or more of concentration, solvent or reference were not specified (86-88).

Some general trends have been established with regard to aggregation in aqueous systems but specific values reported should be used with caution. Porphyrins with negatively charged peripheral substituents such as carboxylate or sulphonate groups have been found to aggregate while those with positively charged meso substituents such as protonated pyridines and N-alkylated pyridines remain monomeric (89). Metal complexes of these positively-charged porphyrins also do not aggregate (90). The degree of aggregation of negatively charged metalloporphyrins appears to depend on the coordination number of the metal. Those in which the metal ions are four-coordinate aggregate whereas those in which the metal ions are five- or six-coordinate do not (91), unless the axial ligand is bridging or is part of another porphyrin molecule. Temperature-jump relaxation studies have shown that aggregation occurs at a rate approaching diffusion and that the main difference between individual porphyrins arises from the rates of dissociation of the aggregates (89-92). Dimerization constants reported are for the most part in the range of 10^4 to 10^6 M^{-1} (e.g., 88-92). A number of unfortunate practices have been perpetrated in these studies. One of these is the lack of estimates of precision, making it difficult to judge the degree of reliability of the values reported. Also, molar absorptivities for monomers in one medium (no added electrolytes, mixed solvents) have been determined and applied in calculations involving data obtained in a different medium. In other studies, values of ΔH and ΔS for dimerization have been obtained by determination of K_D over very limited temperature ranges, such that the values for these thermodynamic properties are based on as few as three data points.

The sulphonated derivatives of TPP have proven especially interesting. The derivatives most used have either three or four of the phenyl groups sulphonated ($TPPS_3$ or $TPPS_4$). Except in acetate or chloroacetate buffer solutions (25), $TPPS_3$ is considered to be readily aggregated. A K_D value of 4.82×10^4 M^{-1} has been reported (92). Opinion is varied with regard to $TPPS_4$. In three separate studies, the compound is said to be monomeric over a wide concentration range (86), readily dimerized with $K_D = 9.6 \times 10^4$ (91), and in existence as a mixture of polymer and monomer with little or no evidence for dimer (93). Both $TPPS_3$ and $TPPS_4$ exhibit complicated behaviour upon protonation. In dilute solutions ($< 10^{-6}$ M), where the compounds are monomeric, protonation to form the dication results in a shift of the Soret band to longer wavelength (from 413 nm to 435 nm). At the same time, there is a reduction from four to two bands in the remaining spectrum. At higher concentrations, these same three bands are present, but in addition, two new bands appear at 490 and 710 nm. Both the rate of appearance and the intensity of these new bands are strongly dependent on ionic strength and the nature of the electrolyte (25,86,89,90,92). The species responsible for these bands have not been identified, although aggregates of undetermined structure and colloidal suspensions have been proposed. The aggregation behaviour of the sulphonated tetraphenylporphines continues to present a challenge.

I.7 KINETICS AND MECHANISM OF METALLOPORPHYRIN FORMATION

The reactivity of the porphyrin molecule with protons must be considered in a kinetic study of metal-ion incorporation since the metal ion must compete with protons for the available binding sites. The following protonic equilibria pertain where PH_2 represents the neutral free base:

$$PH_4^{+2} \xrightleftharpoons{K_4} PH_3^{+} \xrightleftharpoons{K_3} PH_2 \xrightleftharpoons{K_2} PH^{-} \xrightleftharpoons{K_1} P^{-2}$$

These equilibria* can be conveniently studied by noting changes in the absorption spectrum as a function of pH but caution must be exercised. For example, meso-tetra(p-pyridyl)porphine, TPyP (96), and meso-tetra-(4-N-methylpyridyl)porphine, TMPyP (97), apparently display single isosbestic points (based on the Soret band) in acid solution, suggesting that PH_2 and PH_4^{+2} are the only major species present. Consideration of the entire spectrum, however, shows that an appreciable concentration of the monocation is present (98). In this study, the values obtained for pK_4 and pK_3 for TMPyP were 0.4 and 1.5, respectively, at an ionic strength of 0.2 and 22°C. For some water-soluble porphyrins, the first (K_4) and second (K_3) dissociations overlap extensively and the individual constants have not been extracted from the experimental data. Thus, for $TPPS_4$, $TPPS_3$ and meso-tetra(p-carboxyphenyl)porphine ($TPPC_4$), pK_4 and pK_3 values of about 5 have been reported (86,92,99). In a more recent study (25), the values pK_4 = 4.86 and pK_3 = 4.95 were obtained for $TPPS_3$. The effect of aggregation has not been considered in most protonation studies and when it has been, gross assumptions have been made (88). The anions HP^- and P^{-2} are so basic that values of K_2 and K_1 cannot be obtained in aqueous solution. An exception is TMPyP for which pK_2 = 12.9 (97). For porphyrins that are not water-soluble, detergents have been added to both increase aqueous solubility and to decrease the extent of aggregation. However, the addition of detergents can affect the pK

* The designation of the first to last dissociations by the constants K_4 to K_1, respectively, is the practice (94,95) in porphyrin chemistry. This reversal of nomenclature from standard procedure is regrettable.

values.

Numerous attempts have been made to elucidate the mechanism by which metal ions are incorporated into porphyrins in solution (e.g., 33, 35,100,101). Despite this considerable effort, the situation is still far from clear. It is difficult to evaluate the results and to propose a common mechanism because of the many variations in the reaction conditions used. Two basic mechanisms may, however, be envisaged, namely, the S_E1 (dissociative) and the S_E2 (associative) mechanisms.

In the S_E1 mechanism, the porphyrin is considered to dissociate to form an anion which then reacts with the metal ion:

$$PH_2 \rightleftharpoons P^{-2} + 2H^+$$

$$P^{-2} + M^{+2} \rightleftharpoons MP$$

This is, in fact, the process postulated for the reaction of many of the more familiar ligands such as EDTA and oxine with metal ions. Unfortunately, for porphyrins no evidence exists for such a mechanism, undoubtedly because of the very high values for pK_2 and pK_1. A mechanism involving only the monoanion is possible:

$$PH_2 \rightleftharpoons PH^- + H^+$$

$$PH^- + M^{+2} \rightleftharpoons MP + H^+$$

This mechanism could be operative in the reaction of $Zn(NH_3)_3^{2+}$ with TMPyP (102). ($Zn(NH_3)_4^{2+}$ is apparently non-reactive.) Similarly, the reaction of Mg^{2+} with deuteroporphyrin IX dimethyl ester in MeOH could be another example (103), although alternative explanations may exist for the observed kinetic data (104).

In the $S_{E}2$ mechanism, the metal ion reacts with the porphyrin and the protons are displaced from it, perhaps through an intermediate species.

$$PH_2 + M^{+2} \rightarrow [MPH_2^{+2}]$$

$$[MPH_2^{+2}] \rightarrow MP + 2H^+$$

Most of the proposed mechanisms for porphyrin metallation fall into this category, with variations arising from efforts to explain the finer details of the kinetic data.

An outstanding example of this mechanism and one which is in dispute involves the "sitting-atop" (SAT) intermediate. In this species, the two central protons are retained and the metal ion sits above the plane of the central nitrogen atoms prior to its insertion. Fleischer and Wang (105) reported that in non-aqueous solvents, a spectrally distinct species resembling the dication is formed which subsequently proceeds to form the regular metalloporphyrin. Similar observations have been reported in aqueous solutions of TPyP (106). Additional detail was provided in a study (107) in which the concentration term ($[Li^+][Cu^{+2}]$) in the relevant kinetic expression suggests that the SAT metal ion is not the ion inserted but serves only to distort the porphyrin to facilitate the insertion of a second metal ion from the distal side. While confirming these observations, Zuckerman and Burnham (108) noted the possibility that interaction between the solvent and metal ion could labilize solvent protons which could then protonate the porphyrin. The SAT complex model has been challenged by Longo et al. (100) who found no evidence for any type of reaction intermediate in an extensive study. Hambright (98,102) has also criticized the SAT model. He explained previous observations by proposing the formation of unreactive porphyrin mono- and dications and the involvement of the background electrolyte. His explanations, in turn, have been questioned by Rau and Longo (109).

To conclude, some relevant observations that have been made in kinetic studies are noted:

(1) Protonation of the porphyrin leads to a decrease in the rate of metalloporphyrin formation (110). (2) Correlation of incorporation rates with the lability of water coordinated to the metal ion has been noted (99,102). (3) Whereas open-chain ligands favour stepwise solvent replacement around the metal ion, some degree of simultaneous desolvation is likely to occur in the complexation of macrocyclic ligands (111,112). (4) Deformation of the porphyrin nucleus might be necessary for metal-ion incorporation. This concept is supported by the observation that N-alkyl porphyrins show faster incorporation rates than the corresponding non-alkylated compounds (113). (5) The rate of metallation increases with the number of negative peripheral substituents on the porphyrin (102,111). (6) The rate of metallation is affected by detergent molecules used to solubilize porphyrins. The rates are greater in anionic than in cationic detergents (114,115). (7) Auxilliary complexing agents affect the rate of metal-ion incorporation. For example, changes in rate have been noted as a function of the concentration of carboxylate anions (116-118). Also, some chelating agents have been found to enhance the rate of metallation (37,115).

I.8 SUMMARY

As noted in Section I.2, the aim of this thesis is to elucidate some fundamental solution properties of $TPPS_4$ with respect to self-aggregation, kinetics of metallation and preparation of complexes. These studies were held to be prerequisite prior to making knowledgeable attempts to exploit $TPPS_4$ analytically. A further purpose of this work is to make preliminary investigations with regard to the analytical possibilities of $TPPS_4$.

The information of the preceding sections provides the background in the context of which the work which follows may be interpreted.

II. EXPERIMENTAL SECTION

II.1 General

All chemicals were of reagent-grade quality and unless otherwise specified, were used without further purification. All aqueous solutions were prepared with distilled deionized water.

For quantitative work, Class A volumetric glassware was used. Small volumes (0.0500 to 2.5000 ml) of solutions were dispensed with a Gilmont microsyringe burette. The microburette was essential for the preparation of porphyrin solutions because the costly synthesis of $TPPS_4$ limited the amount of porphyrin available. The microburette was calibrated as described by Vogel (119). The correction factor was found to be insignificant for this work as the accuracy and precision were limited by the remaining equipment. Details of the calibration are provided in Appendix I.

Microanalytical data reported for the salts of $TPPS_4$ and for certain metalloporphyrins were obtained by Alfred Bernhardt,* A.B. Gygli** or by the author.

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II.2 Syntheses and Characterization

(a) Meso-tetraphenylporphine (TPP)

Tetraphenylporphine was synthesized from benzaldehyde and freshly distilled pyrrole by the method of Adler *et al.* (120). The yield of crude TPP was 19%.

The TPP was purified by chromatography on a column of Brockman Activity I neutral alumina (Fisher Scientific) with chloroform as the eluant. In the early stages of this work, slurry-packing of the column was used but was later discontinued because a large decrease in the efficiency of the column was observed after prolonged contact between the alumina and the chloroform. Dry-packing, advocated by Finarelli (121), resulted in severe chanelling, which also led to decreased column efficiency. An effective compromise was achieved by a modified dry-packing technique. The column was filled to a height of about 10 cm with chloroform (freshly passed through an alumina bed beforehand) and dry alumina was slowly added while the column was tapped vigorously. In this way, the column was packed in stages to the desired height (40-50 cm). The process required less than 5 minutes.

A concentrated solution of crude TPP in chloroform was continuously added to the column until the coloured front had progressed to half of the column length. As the eluant was added, a narrow green front was observed to precede the main dark purple band. This green portion was discarded and the purified TPP, represented by the purple band, was collected. At some distance above this band was a very slowly moving dark brownish-green band arising from the main impurity, tetraphenylchlorin.

Between these two was another green band which "tailed" the length of the column. The green substance was not identified. The recovery of purified TPP was approximately 65% based on the weight of crude material taken.

(b) Meso-tetra(p-sulphophenyl)porphine ($TPPS_4$)

The ammonium and sodium salts of tetra(p-sulphophenyl)porphine, $(NH_4)_4TPPS_4$ and Na_4TPPS_4, were prepared from the purified TPP by modification of the procedure described by Fleischer et al. (86). A mixture of 2 g (0.0033 moles) of TPP and 70 ml of concentrated sulphuric acid was heated on a steam bath with stirring for 18 to 24 hours. The mixture was cooled to room temperature and unreacted TPP was removed by filtration through a sintered-glass Büchner funnel (porosity M). The filtrate was transferred to a large beaker cooled in an ice bath. A methanolic ammonia solution (6 volumes of absolute methanol, 1 volume of concentrated aqueous ammonia) was added very slowly, with continuous stirring, until the mixture changed colour from green to purple. The ammonium sulphate produced was removed by filtration and washed free of porphyrin with absolute methanol. The washings were combined with the filtrate. (An alternative method in which calcium oxide was used for the neutralization (87) was rejected since the calcium sulphate formed was difficult to filter and adsorbed large quantities of porphyrin.) The volume of the filtrate was reduced to about 100 ml by rotary evaporation (Büchi Rotavapor R) under reduced pressure (about 20 torr) and elevated temperature (the water-bath temperature was increased to 100°C slowly to prevent "bumping"). Then, to the filtrate was added 5- to 6-fold its volume of absolute methanol and the resulting solution was filtered to remove a further portion of ammonium sulphate. The volume was again reduced until a saturated solution of porphyrin was obtained. The product, $(NH_4)_4TPPS_4$, was precipitated by the addition of two volumes of acetone and filtered. The porphyrin was reprecipitated three times from absolute methanol with acetone. It was then dissolved in water and passed through a column (2.5 cm x 30 cm) of a strong anion-exchange resin (Dowex 1, 100-200 mesh) in the hydroxide-ion form. This procedure converted residual ammonium sulphate to aqueous ammonia which was then removed by boiling. The $(NH_4)_4TPPS_4$ was recovered on evaporation of the solvent. Its yield was about 50% based on the amount of TPP taken. Based on the amount of pyrrole, the yield was about 5%. A portion of the purified ammonium salt was converted to the sodium salt without significant loss by passage through a column (2.5 cm x 30 cm) of a strong cation exchanger (Amberlite CG 120, 100-200 mesh) in the sodium-ion form. Both salts were dried at 120°C in vacuo overnight (GCA/Precision Scientific heated vacuum desiccator), to form the anhydrous salts. The ammonium salt slowly lost ammonia on prolonged storage in vacuo but the sodium salt could be safely stored in the vacuum desiccator for extended periods at 120°C. Acetone from the purification process was strongly retained by these salts. It required approximately a month at 120°C in vacuo to remove the acetone (as shown by NMR spectroscopy). For the ammonium salt, the use of such long drying periods necessitated replacement of lost ammonia by recrystallization from aqueous (or methanolic) ammonia or passage through a column of a cation-exchange resin in the ammonium-ion form. The salt could then be redried at 120°C for a shorter interval (e.g., 24 hours) to produce the anhydrous salt.

Elemental analyses for C, H, N, S and Na are given in Table 1, RESULTS AND DISCUSSION.

The hygroscopicity and thermal stability of the salts were examined thermogravimetrically (Stanton-Redcroft TG-750 thermobalance). Approximately 10-mg samples were used and the rate of temperature increase was 20°/minute.

Several NMR experiments which were qualitative in nature were carried out as part of the characterization in order to provide the necessary background for the quantitative studies described in part 3(b) of this section. In these experiments, it was convenient to use molality as a concentration measure and hence solutions were prepared by addition of known weights of the components to the NMR sample tubes. These experiments included: (1) temperature effects over the range 0 to 100°C at various concentrations of $(NH_4)_4TPPS_4$; (2) electrolyte effects caused by the addition of (weighed) quantities of sodium chloride or ammonium nitrate; and, (3) solvent effects as shown by repetition of several experiments in DMSO-d_6 and by the use of D_2O/DMSO-d_6 binary solvent systems containing a fixed quantity of $(NH_4)_4TPPS_4$.

(c) Deuterated meso-tetra(p-sulphophenyl)porphine

Toluene was deuterated by the high-temperature, high-pressure, acid-catalyzed process of Werstiuk and Kadai (122). Deuterium oxide (100 ml), toluene (20 ml) and deuterium chloride (2 ml) were sealed in a

Pyrex glass tube (4 cm x 30 cm, 2 mm thick) after degassing the mixture under vacuum. The tube was next placed into a bomb (21 Parr Pressure Apparatus, Model 4914) containing 100 ml of a water-toluene mixture in the same ratio as the reaction mixture and heated at 200°C for 48 hours. The toluene was then decanted and distilled and ^{1}Hmr spectroscopy showed it to be partially deuterated at the ortho and para positions. It was converted to benzyl chloride by chlorination with 5% sodium hypochlorite solution (commercial bleach) and sulphuric acid (123), and was then oxidized to benzaldehyde by refluxing with an aqueous solution of cupric nitrate (124). The benzaldehyde was extracted into ether, distilled and used in the synthesis of $TPPS_4$ as described above. ^{1}Hmr spectroscopy showed that about 60% of the phenyl protons ortho to the porphyrin ring were replaced by deuterium.

(d) Metalloporphyrins

Several metal complexes were synthesized, all by heterogeneous reaction. The desired quantity of Na_4TPPS_4 was weighed and dissolved in water. A greater than 50-fold molar excess of the metallic element or the oxide was added and the heterogeneous mixture refluxed. The metal oxides were usually in the form of finely divided powders; the free metals were in the form of powders, lumps, turnings or pellets. In general, since the metals were not as finely divided as the oxides, larger quantities of the former were used. Before the metals were used, their surfaces were etched with dilute hydrochloric acid to dissolve any oxide layer and then washed thoroughly with water. Metals which form insoluble chlorides (e.g., silver) or which form oxides resistant to hydrochloric acid were etched with dilute nitric acid.

The refluxing mixture was kept well stirred for up to 3 days, during which time small aliquots were withdrawn to monitor the progress of the reaction spectrophotometrically. Most reactions, however, were complete within one hour as judged by the change from the four-band spectrum of the porphyrin in the visible region to a two-band spectrum of the metalloporphyrin, and also by the non-appearance of the intense absorption peak at 434 nm corresponding to the porphyrin diacid when the reaction mixture was acidified. The latter test was not applicable to the less stable calcium and magnesium complexes. The slowest of the successful metallation reactions was with SnO and took about 48 hours to complete. After refluxing, the residual metal or oxide was collected on a Millipore filter (0.22 μ) and the filtrate evaporated to dryness. The metalloporphyrin obtained was dried at 120°C _in vacuo_. In the preparation of the $AgTPPS_4$ complex, the mixture of Ag_2O and $TPPS_4$ in water was simply stirred for 2 hours at room temperature since refluxing caused the oxide to peptize, thus rendering it difficult to filter.

The heterogeneous preparative technique was tested with other porphyrins, including hematoporphyrin (HP), protoporphyrin IX dimethyl ester (PPDME), octaethylporphine (OEP) and meso-tetraphenylporphine. For HP, suitable solvents for the metallation reaction were methanol, DMF or an aqueous alkaline solution, although the reaction in methanol took somewhat longer (e.g., one day for CuO) to reach completion, presumably because of the lower reflux temperature.. DMF was used as a solvent for the PPDME reactions and mixtures of DMF and chloroform containing 50-75% v/v DMF were found to be suitable for TPP and OEP.

Elemental analyses for C, H, N and S for a number of metalloporphyrins are given in Table 5, RESULTS AND DISCUSSION. The metal contents were determined only for the Cu(II) and Zn(II)$TPPS_4$ complexes. The procedure was as follows. Approximately 10 mg of the complex was weighed (to ± 0.03 mg) on the thermobalance at about 250°C. The weighing at high temperature was necessitated by the extreme hygroscopicity of the complexes. The complex was then transferred to a 100-ml beaker and wet-ashed by slowly heating after the addition of 2 ml of a mixture of sulphuric, nitric and perchloric acids (3:1:1 by volume) until a clear, colourless solution which barely fumed was obtained. The solution was cooled, diluted to approximately 30 ml and neutralized by the dropwise addition of sodium hydroxide solutions (10 M and 1 M). The Zn(II) and Cu(II) were determined by EDTA titration (125,126), using the Gilmont microburette. Since the end points were difficult to detect, the determinations were also done using the method of standard additions.

The hygroscopicity and thermal stability of several metalloporphyrins were examined thermogravimetrically. Approximately 10 mg of each complex was used and the rate of temperature increase was 20°C/minute.

II.3 Studies on Aggregation

(a) Spectrophotometry

Spectra in the uv-visible region were recorded on a Cary 14 double-beam recording instrument (Applied Physics Corporation). A Bausch and Lomb Spectronic 600 double-beam instrument with a Sargent SRL recorder was used for qualitative work. Absorbance measurements at a fixed wavelength were made on a single-beam Hitachi Perkin-Elmer 139 spectrophotometer with the cell compartment thermostatted at 25.0 $\pm$ 0.5°C. Quartz cells (Hellma Cells, Inc.) and Spectrosil cells (Canadian Laboratory Supplies) with path lengths ranging from 100 mm to 0.10 mm were used. The cells were cleaned after each experiment by soaking in a 25% (v/v) aqueous solution of Decon 75 detergent (British Drug Houses). This procedure was found to be effective in removing trace amounts of porphyrin from the cell walls.

The degree of aggregation of porphyrin was determined from the extent of deviation from the Beer-Lambert Law. The deviations were measured as a function of both pH and the nature of the electrolyte. All salts used as electrolytes were first recrystallized from water and dried. Appropriate amounts were then weighed to make final solutions with ionic strengths of 0.100. The following aqueous electrolyte solutions were prepared:

(1) formate buffer (9.92×10^{-3} M total formate) containing 9.50×10^{-2} M potassium nitrate; pH adjusted to 3.55 with nitric acid and potassium hydroxide;

(2) phosphate buffer containing 0.0170 M potassium dihydrogen phosphate, 0.0110 M disodium hydrogen phosphate and 5.00×10^{-2} M potassium nitrate, pH adjusted to 7.00;

(3) 0.100 M potassium nitrate; and,

(4) 0.100 M sodium acetate.

Stock solutions of Na_4TPPS_4 (10^{-3} M) were prepared in the appropriate electrolyte solution as solvent. The stock solutions were stored in the dark and found to be stable for several weeks. (A 12% decrease in absorbance at 412 nm was noted after 8 months for a 6×10^{-5} M porphyrin solution in the phosphate buffer.)

Test solutions were prepared by dispensing appropriate volumes of the stock solutions into 10-ml and 50-ml volumetric flasks with a microburette, followed by dilution with the appropriate electrolyte solution. About 10 test solutions were prepared within each decade of concentration, ranging from 10^{-7} M to 10^{-4} M Na_4TPPS_4. These solutions were stored in the dark and were used within 2 to 24 hours after preparation. The absorbance at 412 nm was measured as a function of the concentration of Na_4TPPS_4 in each electrolyte medium. Two to five runs were made for each medium, fresh stock solutions being prepared for each run.

Absorbance measurements of the Zn(II) and Cu(II) complexes of $TPPS_4$ were also made as a function of concentration in phosphate and/or formate buffers. For the Zn(II) complex, measurements were made at 422, 556 and 596 nm and for the Cu(II) complex, at 412 nm. The metalloporphyrin solutions appeared to be more photosensitive than solutions of the free porphyrin; consequently, stock solutions were wrapped in aluminum foil and stored in the dark. The barrel of the microburette was also wrapped in foil.

The aggregation behaviour of $TPPS_4$ was further examined in acidic media. Each medium consisted of an aqueous solution of a given acid and its sodium or potassium salt. The pH of each solution was adjusted to 3.46 $\pm$ 0.02 with the acid under study and sodium or potassium hydroxide. The ionic strength was adjusted to 0.100 M. The variation in absorbance with concentration was determined at 435 and 491 nm. Similar studies were made on solutions at pH 3.55 (formate buffer). This medium was used in studies relating to the kinetics of Cu(II) incorporation into $TPPS_4$.

(b) Nuclear Magnetic Resonance Spectroscopy

The formation of aggregates was also examined by ^{1}Hmr. Spectra were recorded on a Varian HA-100 or a Bruker WH-90 Fourier Transform spectrometer. Thin-walled (5 mm o.d., 4 mm i.d.) NMR tubes were used. Paramagnetic impurities were removed by soaking the tubes in an alkaline EDTA solution followed by washing in distilled and deionized water.

The ^{1}Hmr aggregation studies as a function of porphyrin concentration paralleled those by spectrophotometry. Potassium nitrate and sodium acetate solutions in D_2O were prepared as before. Phosphate buffer solutions were prepared by adjusting the pD of a 0.0295 M trisodium phosphate solution with DCl. Stock solutions of Na_4TPPS_4 were 0.1 M. Less concentrated stock solutions were prepared by dilution. Individual test solutions (5×10^{-5} – 5×10^{-2} M) were made by addition of stock and electrolyte solutions to the NMR tubes with a microburette. The additivity of solution volumes was found to be a suitable approximation. The extent of aggregation was indicated by the difference in the chemical shifts of the phenyl protons ortho and meta to the porphyrin ring.

II.4 Kinetics of Metallation

The rate of Cu(II) incorporation into $TPPS_4$ was studied spectrophotometrically. The reaction was followed at 434 or 491 nm on a Hitachi Perkin-Elmer 139 uv-visible spectrophotometer. The thermostatted cell compartment, capable of holding four cells, was maintained at 25.0 ± 0.2°C.

The pH of the test solution was measured with an Accumet Model 520 Digital pH/Ion meter (Fisher Scientific Co.). The solution was placed into a jacketed 250-ml Pyrex beaker fitted with a Lucite cover with holes for electrodes and a burette. The temperature was maintained at 25.0 ± 0.2°C and the solution was stirred magnetically while the pH was adjusted to the desired value. The glass electrode was calibrated daily with standard buffer solutions.

Stock solutions of cupric nitrate (0.5 and 0.1 M) were standardized by EDTA titration (126). Stock solutions of $TPPS_4$ (10^{-3} and 10^{-5} M) were prepared in distilled and deionized water and stored in the dark. Formate buffer (pH 3.5, 1M total formate) was prepared from standard formic acid solution.

Test solutions were prepared by the addition of the appropriate volumes of cupric nitrate, formate buffer and potassium nitrate solutions to a 50-ml volumetric flask to yield the desired concentrations and ionic strength. The concentration of Cu(II) was always at least 20-fold greater than that of $TPPS_4$. The solution was then transferred to the jacketed beaker and the pH was adjusted to 3.550 ± 0.005 with potassium hydroxide (1M and 0.1 M) and nitric acid (1 M and 0.1 M). In no case did these final pH adjustments alter the total volume by more than 0.3 ml. The pH of the test solutions was carefully controlled in order to ensure a uniform degree of protonation of $TPPS_4$ since protonation of the porphyrin leads to a decrease in the rate of metalloporphyrin formation (110). Reaction was initiated by the addition of the porphyrin. An aliquot of the solution was transferred to a spectrophotometric cell and the absorbance of the solution was measured at time intervals (determined by the rate of absorbance change). The sample cell was moved out of the light path between measurements to minimize photochemical reaction. Measurements were recorded for a minimum of three half-lives and a final reading (corresponding to $t = \infty$) was taken after a minimum of ten half-lives.

The kinetic studies were divided into two parts. In the first part, the porphyrin concentration was maintained below the critical micelle concentration (about 3×10^{-6} M; see Section III.2(a), RESULTS AND DISCUSSION). The rate of Cu(II) incorporation was obtained as a function of $TPPS_4$, Cu(II), and total formate concentration and of ionic strength. In the second part, the porphyrin concentration was greater than 3×10^{-6} M.

II.5 Applications

(a) Reactivity towards Metal Ions

The general reactivity of $TPPS_4$ towards metal ions was tested in neutral or slightly alkaline aqueous solution. Evidence for reaction was obtained spectrophotometrically. Because there are only small differences between the spectra of a number of metalloporphyrins and that of non-protonated $TPPS_4$ in the Soret region, the less intense absorption bands above 500 nm were used. This necessitated the use of relatively concentrated solutions. Test solutions containing $TPPS_4$ (5×10^{-5} M) and metal ion (5×10^{-3}) were prepared in distilled, deionized water. The pH was adjusted to about 7 with NaOH (0.1 or 0.01 M) whenever hydrolysis of the metal ion resulted in a decrease in pH low enough to cause formation of the green protonated $TPPS_4$. If hydrolysis resulted in extensive precipitation, the test was repeated with lower concentrations of the metal ion (5×10^{-4} or 5×10^{-5} M). The solution was allowed to stand at room temperature for four hours and the spectrum of an aliquot was then examined for any change. If no evidence of reaction was observed, the solution was heated on a steam bath for up to 16 hours, with aliquots taken at intervals for spectral examination.

Similar tests were done in a formate buffer solution (pH 3.55) using only those metal ions which had been shown to react under neutral conditions. A much lower concentration of $TPPS_4$ (1×10^{-6} M) was used because not only is the solubility of $TPPS_4$ considerably reduced in acidic solution but also aggregation is severe. Fortunately, the Soret band of protonated $TPPS_4$ (435 nm) is usually well separated from those of the various metalloporphyrins (at least 15 nm lower), so that the reactions could be readily followed at this low concentration.

(b) Extraction of $TPPS_4$ and Metalloporphyrins

Tetraphenylarsonium chloride (Ph_4AsCl) was chosen as a reagent for the extraction of $TPPS_4$ because of its ability to form extractable ion pairs with a number of anions. Qualitative tests were done to establish suitable conditions for extraction of $TPPS_4$ and $CuTPPS_4$ (both 1×10^{-5} M). Consideration of the literature led to the testing of chloroform and 1-pentanol as organic phases. The effect of HCl, HNO_3, $HClO_4$ (all 0.01 M), KNO_3 and $NaClO_4$ (both 2.5 M, added incrementally) was noted on the extraction. The pH dependence of extraction from formate buffer was examined spectrophotometrically (418 nm, $TPPS_4$; 415.5 nm, $CuTPPS_4$, both in 1-pentanol) at two concentrations of Ph_4AsCl in the aqueous phase (2×10^{-4} and 1×10^{-2} M). To eliminate the effects of aggregation, the concentration of porphyrin in the formate aqueous phase was 1.8×10^{-6} M.

(c) Extraction-Spectrophotometric Determination of Cu(II) at Trace Levels

Studies were carried out on samples of distilled, deionized water spiked with Cu(II) (final concentration, 2 to 15 ppb). Refinements of the extraction procedure were required to adapt it to quantitative analytical use. A ten-fold concentration factor was adopted for convenience in these studies (i.e., 100 ml of aqueous sample, 10 ml of 1-pentanol). Because of difficulties with quantitative phase separation, the 1-pentanol was added to the aqueous phase in three portions, each portion being removed separately. The separatory funnel was drained after the third extraction and its walls were rinsed with a fourth portion of 1-pentanol. The portions were combined in a 10-ml volumetric flask and the solution was made up to the mark. The absorbance of an aliquot was measured at 415.5 nm.

Initial determinations showed the presence of significant memory effects of the glassware and new cleaning procedures, as follow, were required. The glassware was soaked in a 1% (v/v) solution of laboratory detergent (Decon 75) overnight, then washed with distilled water and with a hot (100°C) alkaline solution of EDTA (0.1 M in EDTA and NaOH). Finally, the glassware was rinsed ten times with distilled, deionized water.

In foreign-ion interference studies, the Ph_4AsCl was converted to the nitrate to avoid precipitation of ions such as Ag(I) and Pb(II). An aqueous phase (200 ml) containing 0.05 M Ph_4AsCl and 0.2 M KNO_3 was shaken with 200 ml of 1-pentanol to extract Ph_4AsNO_3. The organic phase was back-washed twice with an equal volume of aqueous KNO_3 (0.2 M) and then was reduced in volume on a hot plate until crystallization began. The mixture was cooled to room temperature and five volumes of diethyl ether were added. The precipitate was removed by filtration and further purified by two precipitations with diethyl ether from a saturated absolute ethanol solution. A 0.04 M aqueous solution of the final product gave no precipitate on addition to an equal volume of 0.1 M $AgNO_3$.

The reaction of $TPPS_4$ with Cu(II) is very slow at the low levels of Cu(II) used; therefore, the aqueous formate solution containing the Cu(II) was heated to 100°C. The $TPPS_4$ was added to the aqueous phase after the solution had been heated. Reaction times tried (after addition of the $TPPS_4$) were 2, 5, 10 and 20 minutes for a solution of 2 ppb Cu(II). The solution was then cooled in an ice bath, the pH was lowered to 1.5 by the addition of 1 M HNO_3 and KNO_3 was added. Extraction with 1-pentanol followed. Little difference was noted among solutions with reaction times ≥ 5 minutes but a safe lower time limit of 10 minutes was selected for later experiments.

After cooling and acidification of the test solutions, the addition of KNO_3 increased the rate of phase separation with no noticeable contamination of the test solutions. Since no improvements in separation were noted above a concentration of 0.1 M KNO_3 (final concentration in aqueous phase) this concentration was used in subsequent work.

The stability of the complex formed towards acid was tested by addition of HNO_3 while the reaction mixture was still hot. The extraction was then proceeded with immediately or after a 5-minute delay. Decomposition occurred in both cases but was especially severe after 5 minutes. No adverse effect was observed if the solution was first cooled and then acid added, up to 0.1 M, unless the solution was left overnight.

The final procedure adopted in light of the above experiments was as follows. A measured volume of the test solution (in general, 100 ml) was placed in a 150-ml beaker. To this, 5.00 ml of formate buffer (0.386 M formic acid; sodium formate approximately 0.195 M, added until pH 3.3) was added by pipette. The solution (covered) was heated to boiling and 1.00 ml of 4×10^{-5} M $TPPS_4$ added with the microburette. For samples containing more than 30 ppb Cu(II), the volume of $TPPS_4$ was scaled up proportionately. Heating was continued for 10 minutes. The beaker was then cooled in an ice bath for 30 minutes, following which 10.00 ml of 1 M HNO_3 was added. The solution was transferred to a separatory funnel (250 ml), the beaker was rinsed twice with distilled, deionized water (about 15 ml total; rinsings added to the test solution) and then 5.00 ml of Ph_4AsNO_3 (0.04 M) and 5.00 ml of KNO_3 (2.5 M) were added. The solution was extracted with about 12 ml of 1-pentanol added in 3 portions. The first extraction was carried out with about 6 ml since the solubility of 1-pentanol in water is approximately 3 ml/100 ml H_2O. The separatory funnel was drained and the walls rinsed with a small volume of 1-pentanol (~ 1 ml) which was then combined with the other portions in a 10-ml volumetric flask. The absorbance of an aliquot was measured at 415.5 nm.

This procedure was used in the preparation of a calibration curve, the test solutions for which contained known amounts of Cu(II) (up to 15 ppb) added to distilled, deionized water.

Potential interference from other ions was tested by the addition of known amounts of selected metal ions (Co(II), Ni(II), Zn(II), Ag(I), Cd(II)) to solutions containing 2 ppb Cu(II). A metal ion was considered to interfere if the absorbance of the extract differed from that obtained with Cu(II) alone by more than two standard deviations.

An estimate was obtained for the distribution ratio (D) under the experimental conditions above using radioactive copper. A standard solution of Cu(II) (0.15 ml; 1.574×10^{-3} M) was evaporated to dryness in a quartz vial then irradiated in the McMaster University nuclear reactor at a flux of 1×10^{13} neutrons cm^{-2} for 12 hours and cooled for 2 hours. The ^{64}Cu produced was dissolved in 100 ml of 0.01 M HNO_3. A 10-ml aliquot of this solution was then treated in the same manner as the standard Cu(II) solutions. Equal volumes of the aqueous and organic phases were counted using a NaI well-detector (511 keV gamma ray associated with the annihilation of positrons).

III. RESULTS AND DISCUSSION

III.1 Syntheses and Characterization

(a) Meso-tetraphenylporphine

The synthesis of porphyrins by the reaction of pyrrole with aldehydes was established by Rothemund (127,128). This method is also useful for the synthesis of porphyrins with identical substituents at the 3- and 4-positions of the pyrrole rings and has been widely used in the synthesis of various meso-substituted porphyrins. The optimum conditions for the synthesis of TPP by this method were determined by Adler et al. (120). It has long been recognized (129) that the main impurity in TPP thus produced is the 7,8-dihydro-derivative, tetraphenylchlorin (TPC). The most frequently used methods of purification are: (i) chromatography of the free base (120,130); (ii) chromatography of the zinc complex followed by demetallation with acid (121,131); and (iii) oxidation of the chlorin with quinones followed by chromatography (e.g., 132, 133). In the present work, chromatography of the free base was chosen for the purification of TPP because this method involved the least number of steps and avoided introduction of possible new sources of impurities, e.g., Zn(II) or quinones. The chromatographic conditions were optimized as described in the EXPERIMENTAL SECTION. A single passage through the alumina column gave a 65% yield based on the weight of crude TPP taken. The criterion of Rousseau and Dolphin (134) was used to monitor the purity of TPP, i.e., the ratio of the absorbance at 645 nm to that at 590 nm is 0.75 for pure TPP (in methylene chloride) and is greater in the presence of TPC.

The widespread use of TPP in porphyrin studies has resulted in its thorough characterization. The spectral characteristics of TPP in this work were in good agreement with uv-visible (e.g., 135), infrared (e.g., 136) and ^{1}Hmr (70,135) spectral characteristics reported in the literature.

(b) Meso-tetra(p-sulphophenyl)porphine

The basic procedure for the sulphonation of TPP was established by Menotti (137) and further developed by Winkelman (138,139) and Fleischer (86). Whereas Winkelman carried out the sulphonation at room temperature for 36 hours, to obtain primarily the trisulphonate, Fleischer allowed the reaction to proceed at 100°C for 4 hours and then 48 hours at room temperature, to produce the tetrasulphonate. Since TPP is relatively stable, more severe conditions (18-24 hours at 100°C) were used in the present work to promote complete sulphonation. However, the use of higher temperatures ($\sim$ 110°C) resulted in some decomposition. In the previous methods, the sulphonated product was precipitated (as the hydrogen sulphate salt of the diacid) by the addition of ice or water to the sulphuric acid mixture and the semi-colloidal precipitate separated by centrifugation (e.g., 4000 g's, 2 hours (138,139)). Since centrifugation was considered to be too lengthy and tedious, the procedure described in the EXPERIMENTAL SECTION was adopted.

Because the water content (determined by ^{1}Hmr) of the ammonium and sodium salts of $TPPS_4$ was found to be variable, characterization of the porphyrin was accomplished through the anhydrous salts, prepared as described in the EXPERIMENTAL SECTION. Elemental analyses for these compounds are presented in Table 1.

TABLE 1

Elemental Analysis for Salts of $TPPS_4$

		%C	H	N	S	Na
Calculated for $(NH_4)_4TPPS_4$ $[C_{44}H_{42}N_8S_4O_{12}]$:		52.68	4.23	11.17	12.78	
Found:	Batch 1	51.67	4.33	11.64	12.73	
	Batch 2	52.32	5.09	10.94	12.48	
Calculated for Na_4TPPS_4 $[C_{44}H_{26}N_4S_4O_{12}Na_4]$		51.66	2.56	5.48	12.54	8.99
Found:		51.61	2.54	5.51	12.37	8.83

The results of this work represent the first characterization of the anhydrous salts. Characterization of the hydrates is not reliable because of the variable water content. The results of Fleischer et al. (86) for the hydrate are not usable because the percentages calculated are incorrect (curiously, however, the experimental values are in agreement with these). Thus, it is essential that the anhydrous salts be used for quantitative work despite the tedious and difficult preparation of these compounds in pure form. Further studies designed to find a more efficient purification scheme are highly desirable.

The anhydrous salts are strongly hygroscopic and care must be taken in weighing samples. Thermal analysis, combined with ^{1}Hmr spectroscopy, of the ammonium salt showed that there was a slow loss of both water and ammonia gas below 320°C. Above this temperature, rapid decomposition occurred. For the sodium salt, there was a gradual loss of water up to 200°C. Between 200°C and 400°C, a very slow weight loss was observed. Above 400°C, rapid decomposition to Na_2SO_4 occurred.

The infrared spectrum of $TPPS_4$ (KBr pellet) showed the main features expected. Strong bands at 1225, 1195, 1130, 1043 and 635 cm^{-1} are characteristic of aromatic sulphonate salts (140). Bands of medium intensity at 1110 cm^{-1} (shoulder on the 1130 cm^{-1} band) and 800 cm^{-1} are indicative of para-substituted benzene. The strongly hygroscopic nature of this compound resulted in an intense broad absorption band due to H_2O which obscured the C-H and N-H bands.

The uv-visible absorption spectra of the protonated and unprotonated porphyrin are shown in Figure V. The positions of the absorption maxima agree with those given by Fleischer (86), although the observed apparent molar absorptivities (Absorbance/[C analytical x path length]) are, in general, greater than reported. These differences are the result of the purer compounds used in the present work.

The ^{1}Hmr spectrum of $TPPS_4$ in D_2O is shown in Figure VI. Direct comparisons with the spectra of TPP are difficult to make because of the different solvent used and because these compounds aggregate to different extents in solution. The ^{1}Hmr spectrum of $TPPS_4$ in D_2O is extremely de-

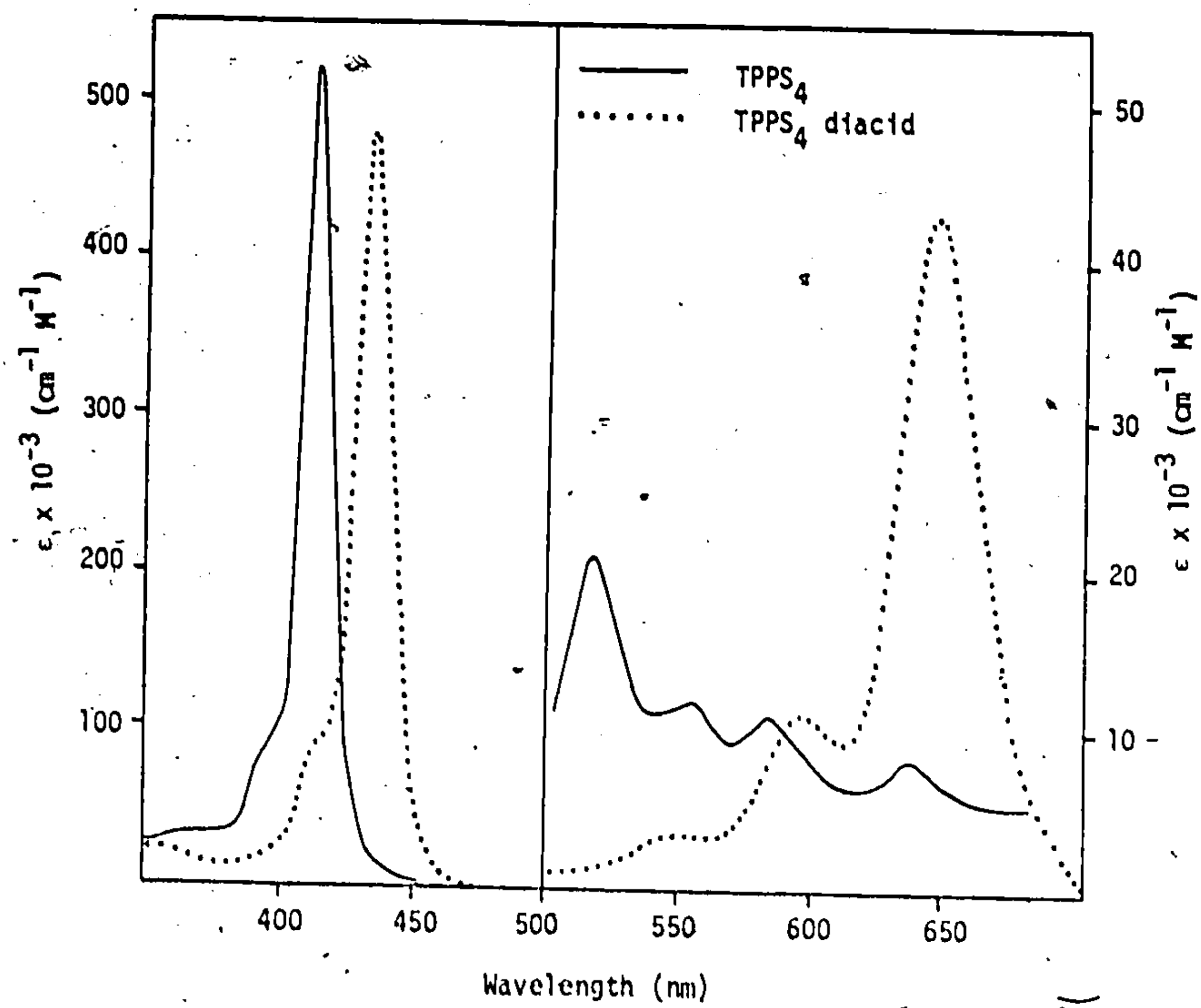

Figure V. Electronic absorption spectrum of monomeric $TPPS_4$ and its diacid (both 1×10^{-6} M).

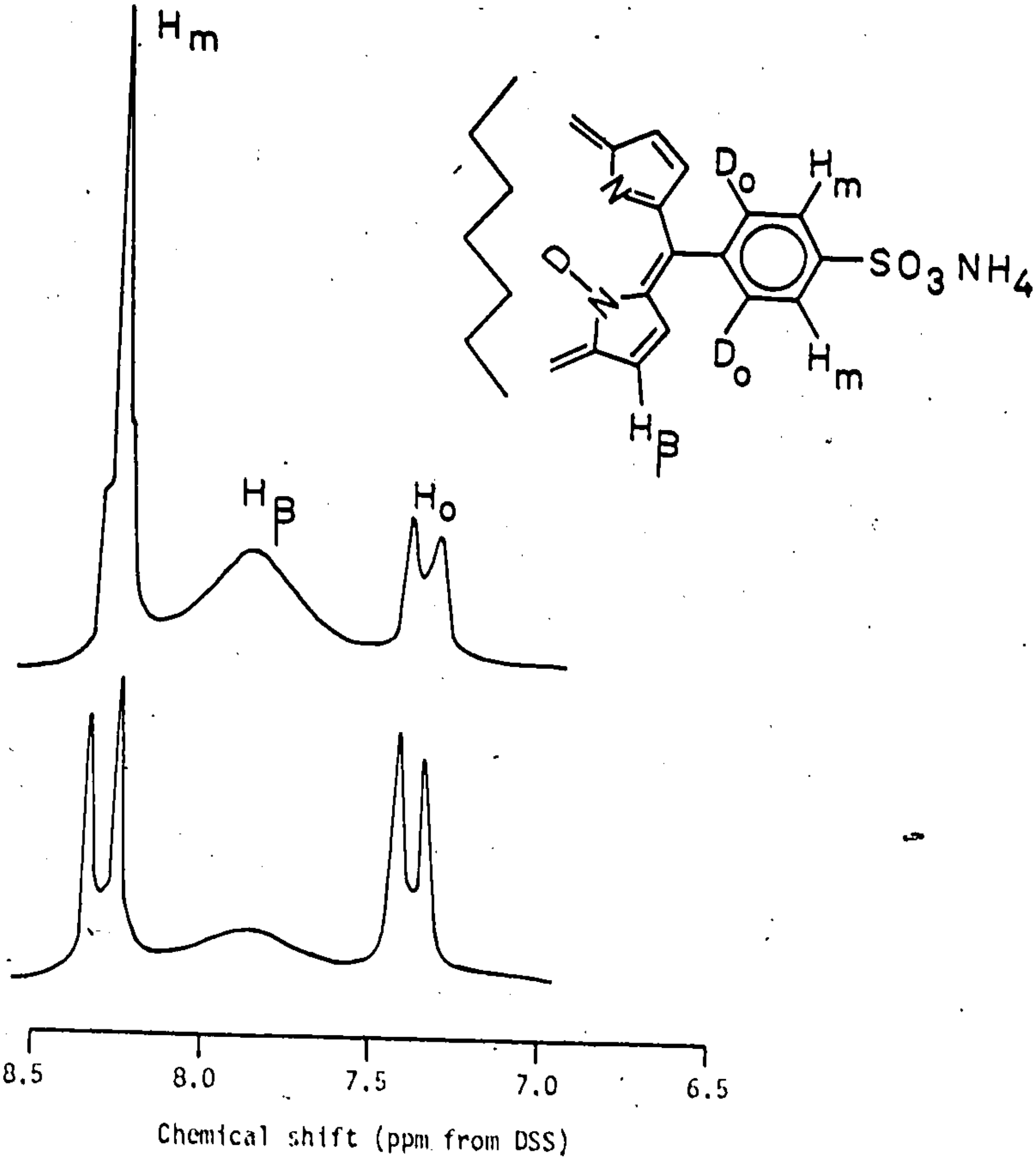

Figure VI. [1]Hmr spectra of $(NH_4)_4TPPS_4$ (0.0776 molal; bottom) and of its partially deuterated analogue (0.0750 molal; top) in D_2O at 35°C. Amplification for top figure was twice that for the bottom one.

pendent on experimental conditions, such as concentration, temperature and electrolyte concentration. An AA'BB' to AA'XX' spectrum is expected for the para-substituted phenyl group but the coupling constants other than J_{AB} (8 Hz) are small, giving rise to an AB to AX spectrum (depending on experimental conditions). Comparison of these spectra with those of $TPPS_4$ which had been partially deuterated in the phenyl position ortho to the porphyrin ring allowed unambiguous assignment of the phenyl signals.

The concentration dependence of the ^{1}Hmr spectra of $TPPS_4$ in D_2O is illustrated in Figure VII. All signals were shifted upfield with increasing concentration; but to different extents, resulting in increasing separation between the H(ortho) and H(meta) doublets. This observation can be satisfactorily accounted for by porphyrin aggregation in solution, in which the molecules are stacked vertically with their planes approximately parallel (76,79,80). In this orientation, intermolecular interactions due to the ring current (see INTRODUCTION) result in a greater upfield shift for protons H(ortho) than for H(meta). Quantitative studies of this phenomenon are discussed in Section III.2(b).

The effect of the addition of inert electrolytes such as NaCl or NH_4NO_3 could also be explained by aggregation of the $TPPS_4$. At constant concentration of $TPPS_4$, increasing amounts of these and other salts caused upfield shifts in the same manner as increasing concentrations of the porphyrin itself (Figure VIII). These electrolytes promote aggregation by the salting-out effect.

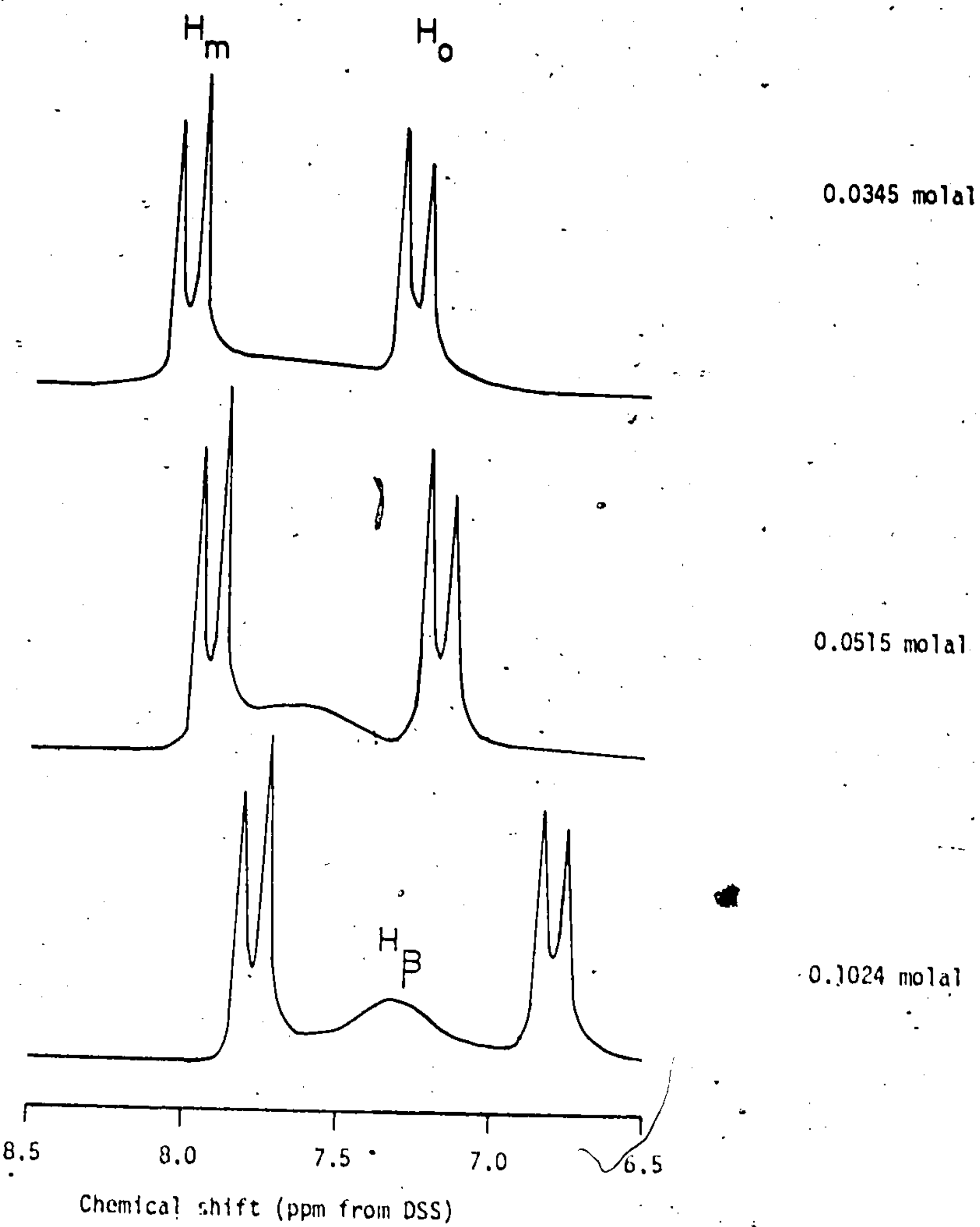

Figure VII. ^{1}Hmr spectra of $(NH_4)_4TPPS_4$ in D_2O at 35°C at different concentrations. See Figure VI for assignments. The amplification for each spectrum was adjusted to compensate for the difference in concentration between samples.

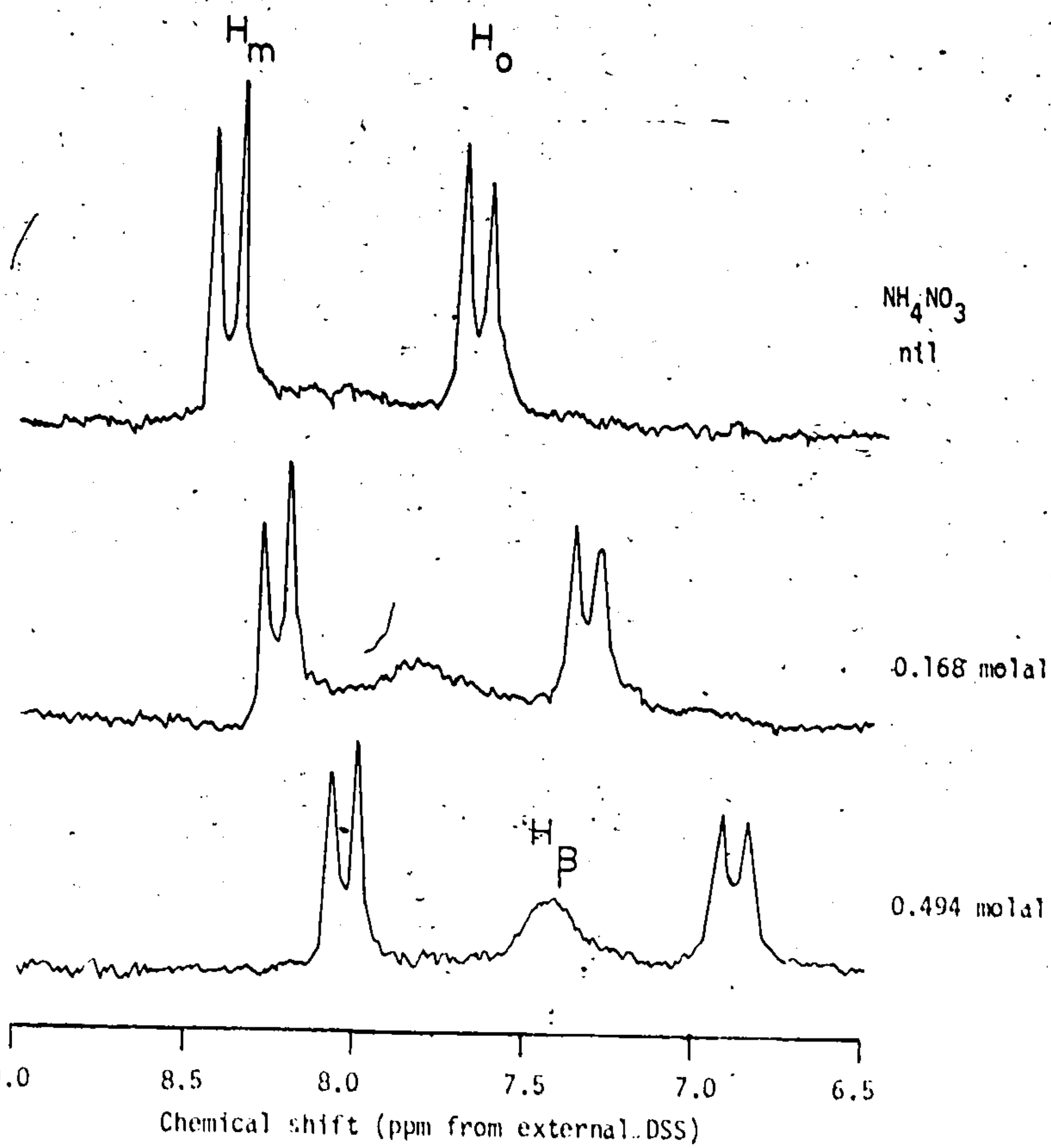

Figure VIII. The effect of added salt (NH_4NO_3) on the ^{1}Hmr spectrum of $(NH_4)_4TPPS_4$ (0.0345 molal) in D_2O at 35°C. See Figure VI for assignments.

At a fixed concentration of $TPPS_4$, shifts to lower field occurred with increasing temperature, resulting in decreasing separation of the H(ortho) and H(meta) doublets (Figure IX). This observation could be explained by the fact that at the higher temperatures, a reduction in aggregation would be expected. In fact, dilute solutions of $TPPS_4$ at elevated temperatures (e.g., 100°C) showed coalescence of the H(ortho) and H(meta) signals and even crossover of the signals (Figure X).

Figure IX also demonstrates the effect on the ^{1}Hmr spectrum of tautomeric exchange of the central protons between the pyrrole nitrogens. It is now generally accepted that such an exchange occurs with a simultaneous rearrangement of the delocalization pathway in the porphyrin π system (82). At low temperatures (-80°C to +20°C depending on the identities of the porphyrin and the solvent), this tautomeric exchange can be frozen out and two signals are observed for the β-pyrrole protons (69-71,82). In D_2O solutions, the central protons are replaced by deuterium, thereby decreasing the rate of tautomerism so that freezing out occurs at relatively high temperatures ($\leq$ 20°C), as observed in the present work. The downfield signal was tentatively assigned to the β-pyrrole protons of the protonated pyrrole rings since the delocalization pathway is thought to include these pyrrole rings (leading to a deshielding of the β-pyrrole protons) and to exclude the periphery of the non-protonated pyrroles. Definite assignment of these signals is difficult because of the simultaneous effect of aggregation on the chemical shifts. As the temperature is increased, thereby increasing the rate of tautomerism, the two β-pyrrole-proton signals approach each other and finally coalesce to form a single broad signal which then sharpens with further temperature increases.

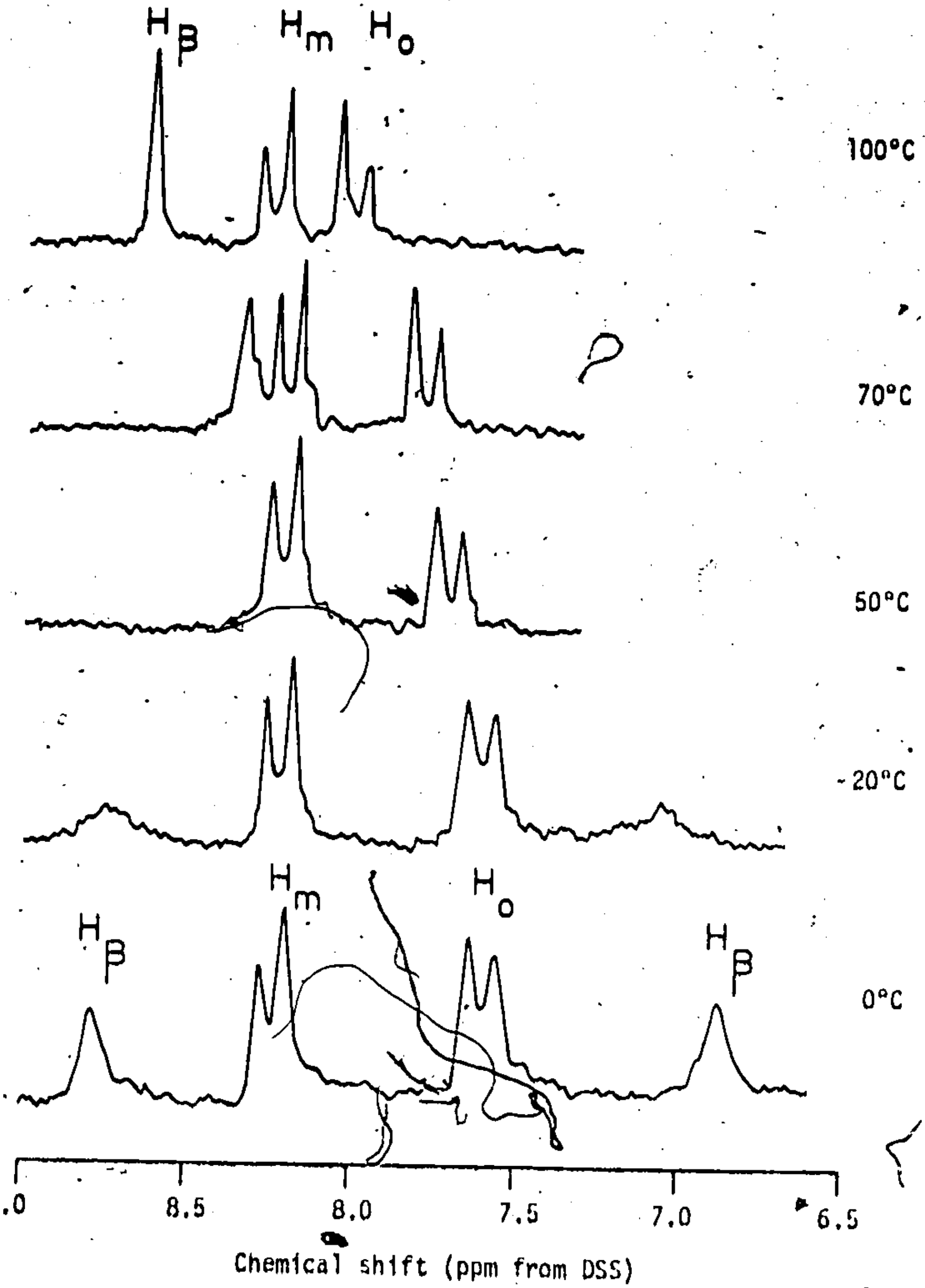

Figure IX. The effect of temperature on the [1]Hmr spectrum of $(NH_4)_4TPPS_4$ (0.0140 molal) in D_2O. Assignments are shown in Figure VI.

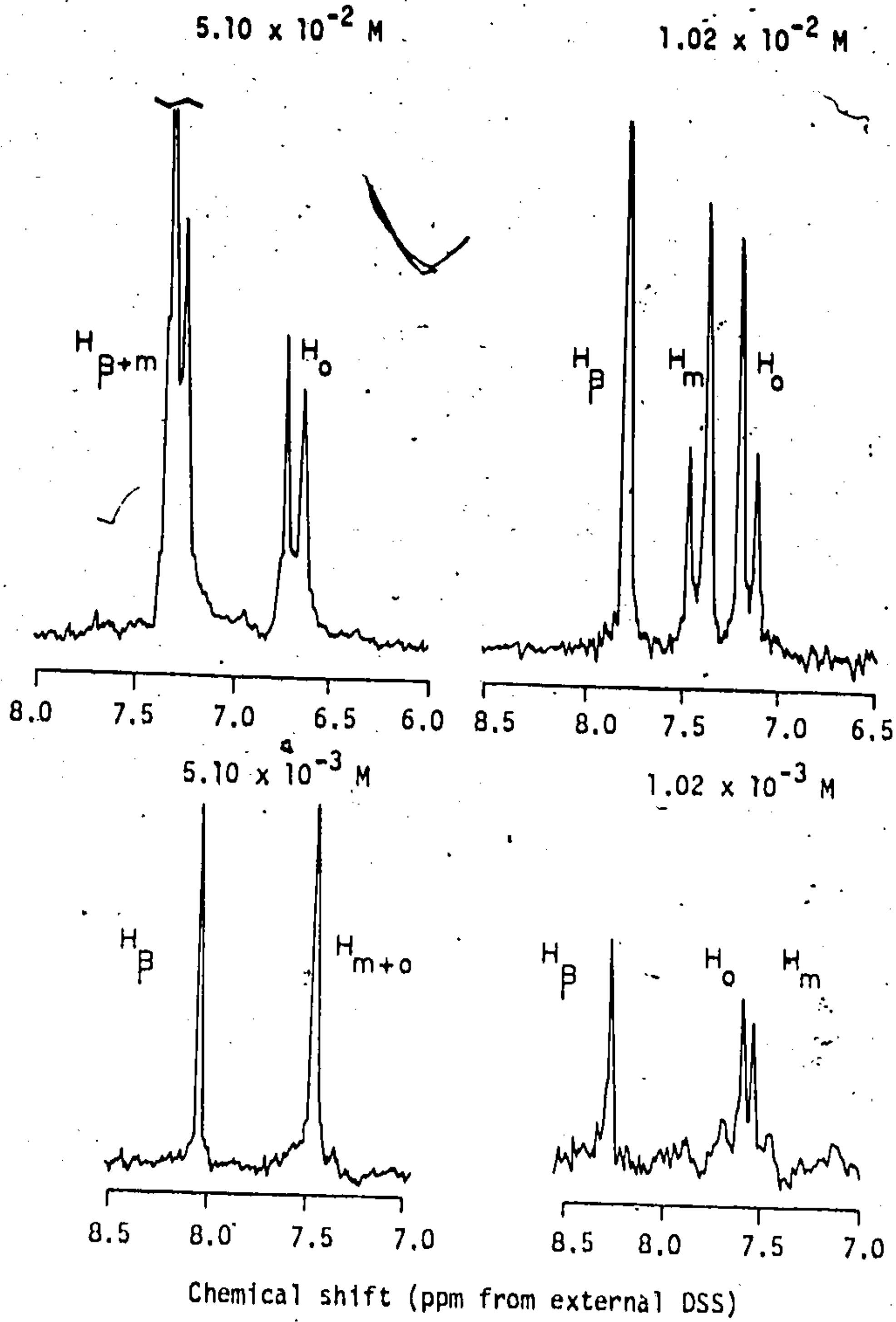

Figure X. The effect of increased temperature (100°C) on the ^{1}Hmr spectrum of $(NH_4)_4TPPS_4$ at various concentrations in D_2O. Assignments are shown in Figure VI.

The broad signal generally observed for moderately concentrated $TPPS_4$ solutions at ambient temperatures (the signal is too broad to observe at low concentrations) indicates moderately slow tautomerism.

The spectrum of the ammonium salt of $TPPS_4$ in DMSO-d_6 is shown in Figure XI. Use of the partially deuterated $TPPS_4$ derivative definitively showed that the positions of the H(ortho) and H(meta) signals are reversed relative to those in D_2O at ambient temperatures. Such a crossover was also observed for dilute solutions ($\leq 10^{-3}$ M) in D_2O at elevated temperatures (~ 100°C) where the extent of aggregation is relatively small. Thus it is concluded that the extent of aggregation in DMSO-d_6 must be minimal. Figure XII shows the effect of varying solvent composition while the porphyrin concentration and temperature remain constant. Increasing the proportion of DMSO-d_6 in D_2O results in a downfield shift of all signals (especially H(ortho) and H(β-pyrrole)), as expected for decreasing aggregation.

An interesting feature of DMSO-d_6 solutions of the ammonium salt is the sharp triplet* (Figure XI) for the ammonium protons (J_{NH} = 51 Hz, as expected for the ^{14}N nucleus). Solutions of other ammonium salts such as ammonium nitrate or ammonium p-toluenesulphonate exhibited only a broad singlet indicating the occurrence of proton exchange. The unusually slow exchange for $(NH_4)_4TPPS_4$ could be explained on the basis that traces of acidic impurities are removed by interaction with the porphyrin centre.

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* The triplet is observed only if the solutions are prepared carefully, e.g., no paramagnetic impurities or significant levels of acid or base are present.

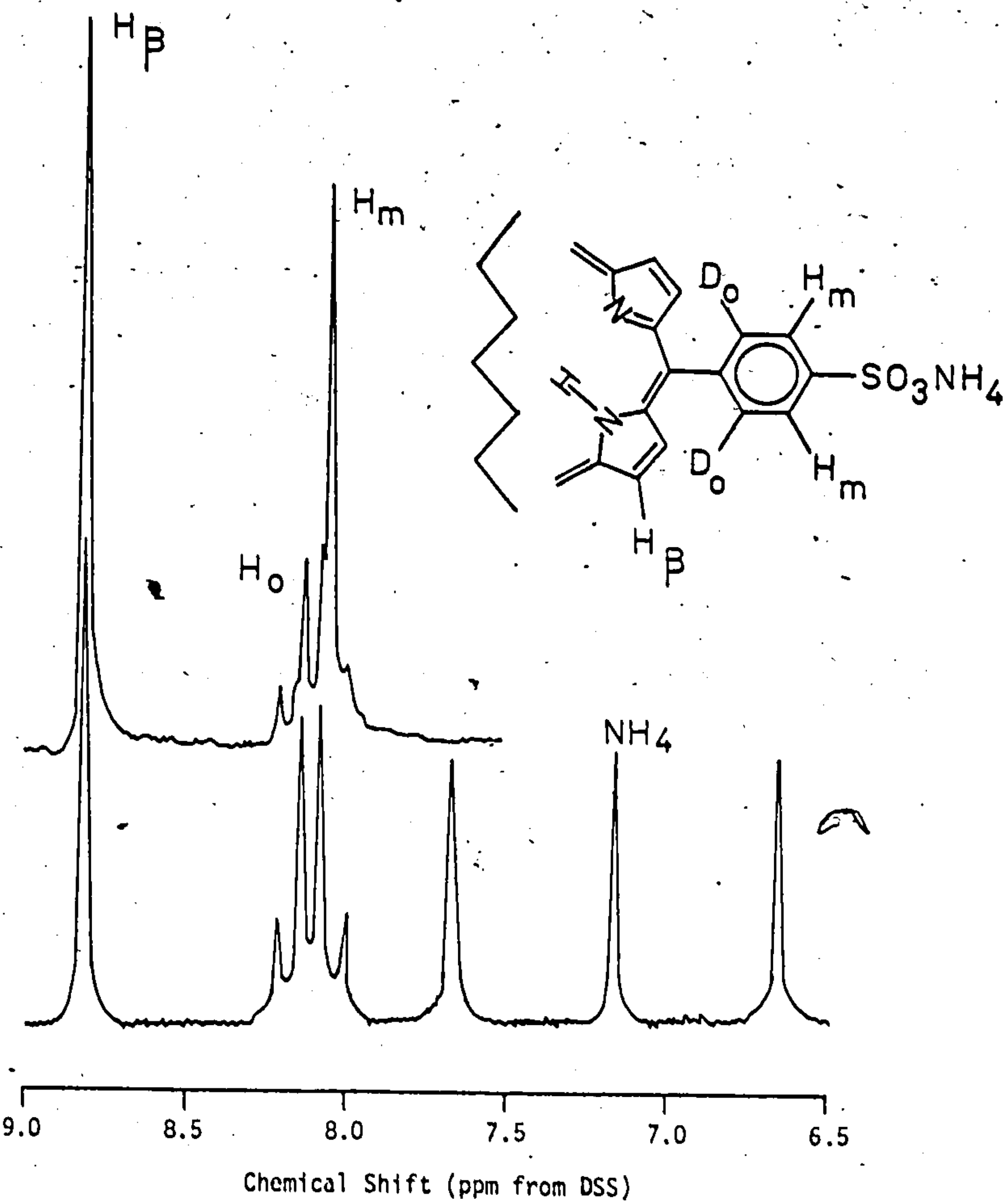


Figure XI. ^{1}Hmr spectra of $(NH_4)_4TPPS_4$ (0.0439 molal; bottom) and of its partially deuterated analogue (0.0413 molal; top) in DMSO-d_6 at 35°C.

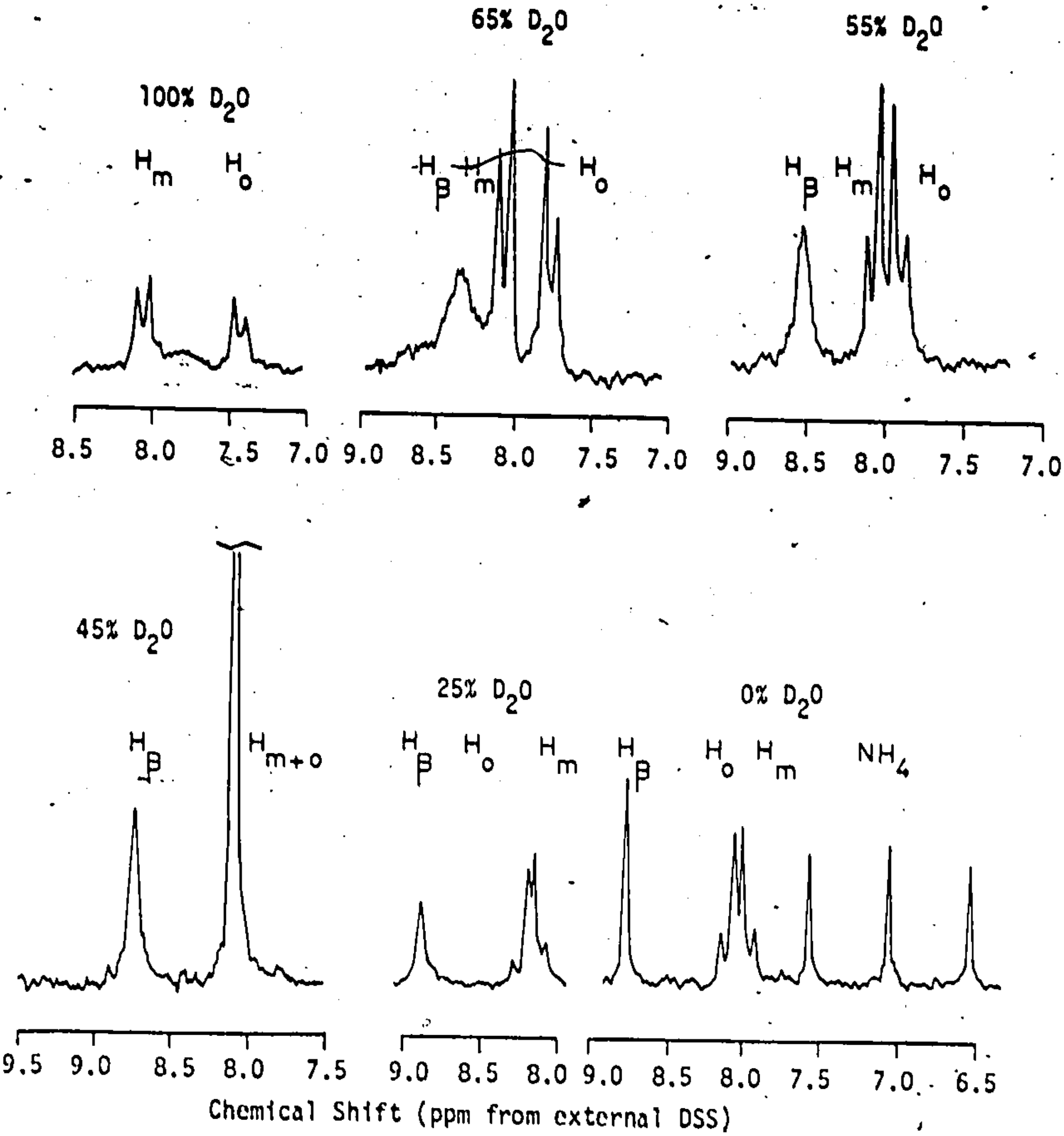


Figure XII. The effect of solvent composition on the ^{1}Hmr spectrum of $(NH_4)_4TPPS_4$ (0.0346 ± 0.0004 M) at 35°C. The percentage (v/v) of D_2O was varied in a mixture of D_2O and DMSO-d_6. Assignments are shown in Figure XI.

When ammonium salts (e.g., ammonium nitrate) are added to DMSO-d_6 solutions of $(NH_4)_4TPPS_4$, an enhancement in the intensity of the triplet signal was observed (Figure XIII). The triplet signal could be made to disappear by the addition of D_2O.

The β-pyrrole-proton signal is sharp for all the DMSO-d_6 solutions of $TPPS_4$, showing that the tautomerism is considerably more rapid in DMSO-d_6 than in D_2O at the same temperature. The difference in rates is primarily an isotope effect.

^{13}Cmr spectroscopy was used to further characterize $TPPS_4$ and a spectrum (D_2O solution) is shown in Figure XIV. The assignment of all the signals in the ^{1}Hmr spectrum allowed the use of selective decoupling for the unambiguous assignment of the proton-bearing carbons. Thus, the signals at 136.8 and 125.6 ppm were assigned to the phenyl carbons ortho (C_2) and meta (C_3) to the porphyrin ring, respectively. The assignment of the ortho-carbon resonance to lower field than the meta-carbon signal is consistent with that of Abraham <u>et al.</u> (141) for TPP, although in contrast to that found for a number of substituted benzenes (Table 2). The broad resonance at 131.3 ppm was assigned to the β-pyrrole carbon. Abraham <u>et al.</u> (71) have shown that replacement of the inner protons of the porphyrin by deuterium results in broadening of the β-pyrrole-carbon signal because of slower tautomerism. Because the α-pyrrole carbon signal is broadened by even rapid tautomerism, it is not surprising that no signal arising from the α-pyrrole carbons was observed in D_2O at ambient temperature.

The signals at 143.8 and 144.0 ppm were assigned to C_4 (para to

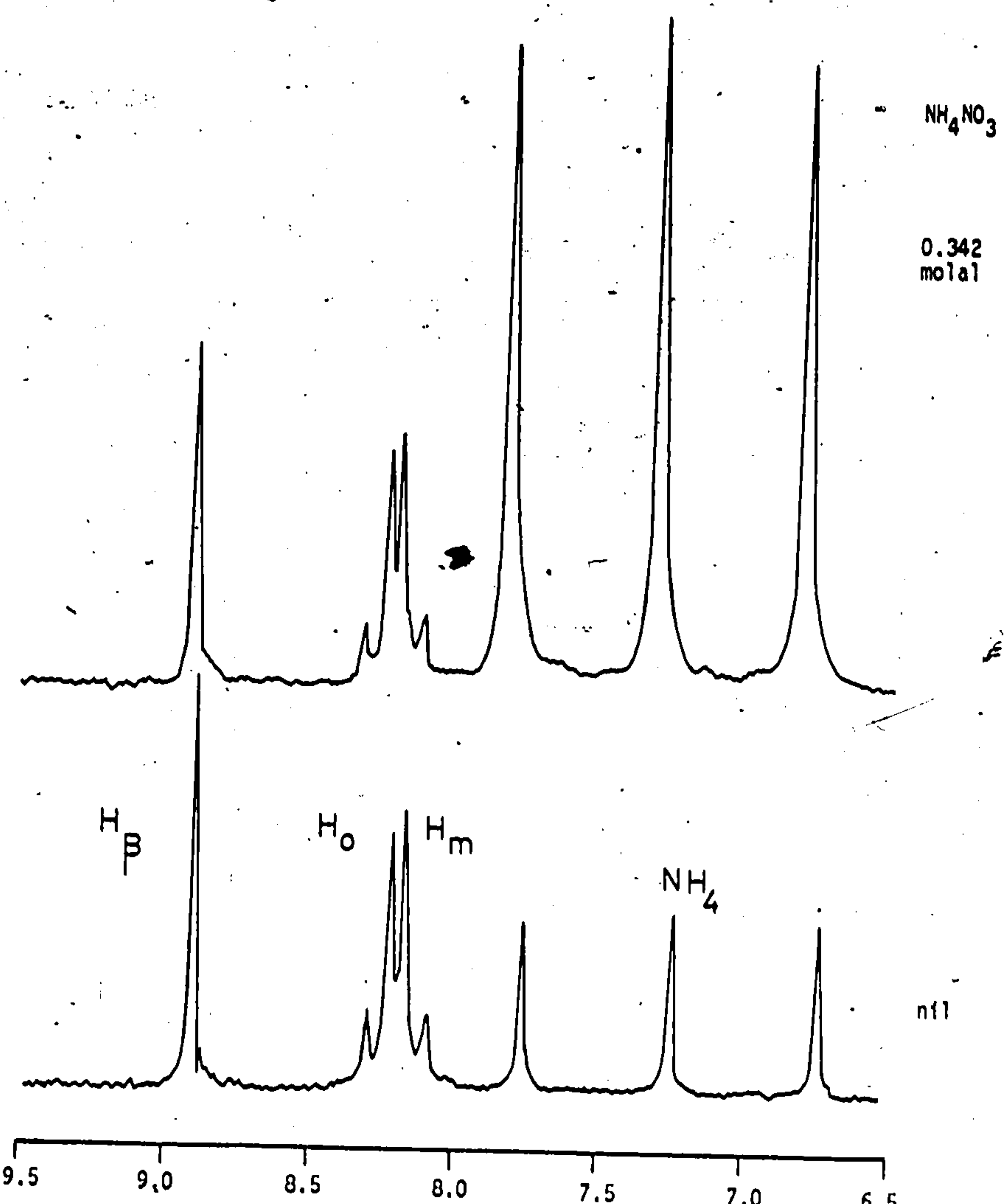


Figure XIII. The effect of ammonium salt on the ^{1}Hmr spectrum of $(NH_4)_4TPPS_4$ (0.0344 molal) in DMSO-d_6 at 35°C. Assignments shown in Figure XI.

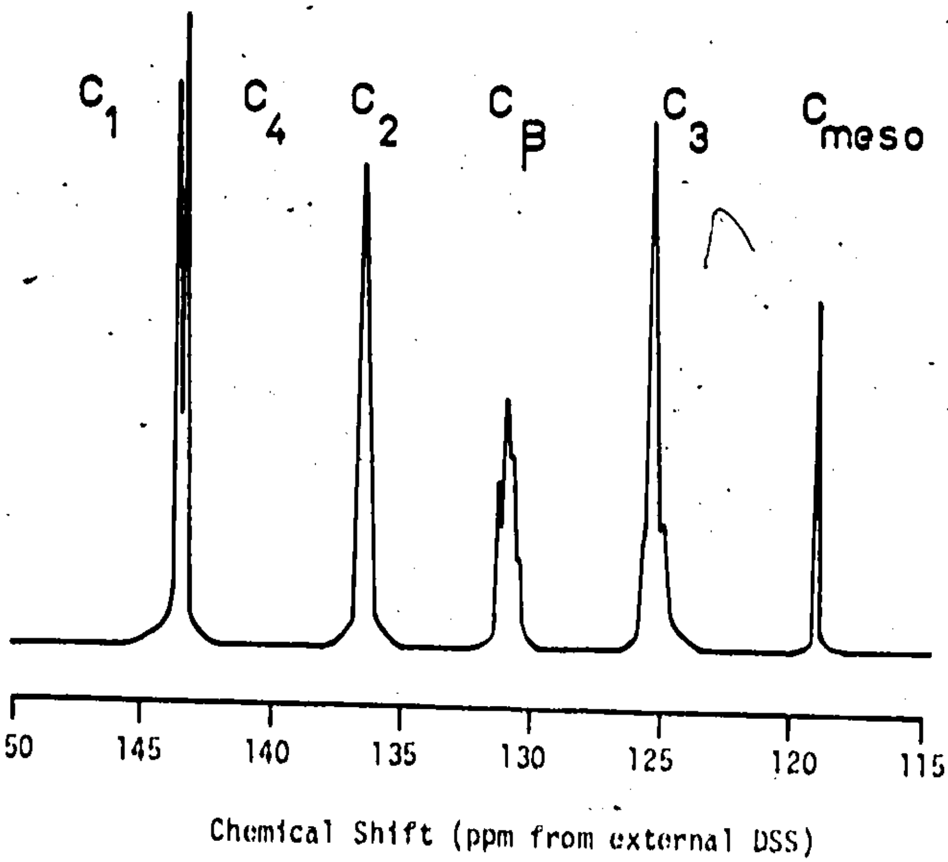
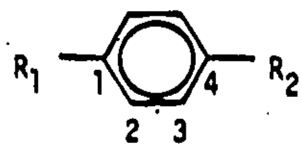
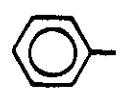
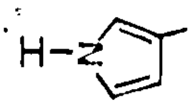


Figure XIV. ^{13}Cmr spectrum of Na_4TPPS_4 (0.0850 M) in D_2O at 28°C. See Table 2 for assignments.

TABLE 2

^{13}C Chemical Shifts of Some Substituted Benzenes



COMPOUND	R_1	R_2	Chemical Shift (ppm) C_1	C_2	C_3	C_4	Ref.
toluene	CH_3	-H	137.8	129.3	128.5	125.6	(142)
biphenyl		-H	141.7	127.6	129.2	127.7	(143)
styrene	CH_2-CH-	-H	138.2	126.7	128.9	128.2	(144)
3-phenylpyrrole		-H	135.5	124.9	128.4	124.5	(145)
sodium benzene sulphonate	H-	$-SO_3Na$	133.0	126.7	130.4	143.7	*
sodium toluene sulphonate	CH_3-	$-SO_3Na$	140.9	126.7	130.8	143.7	*

* Determined in this work.

the porphyrin ring) and C_1, respectively, by comparison with the values of substituted benzenes shown in Table 2. By comparison with the values found for other tetraarylporphines (141) and by the process of elimination, the meso-carbon resonance was considered to correspond to the signal at 119.2 ppm.

In conclusion, the NMR behaviour of $TPPS_4$ is very interesting. It is also extremely informative, especially with regard to the aggregative properties of $TPPS_4$ in solution, as seen above and further demonstrated in Section III.2(b).

(c) Metalloporphyrins

The biological importance and interesting chemical properties of metalloporphyrins has led to the synthesis of a wide range of metal complexes (40-42). No single general method exists for the preparation of metalloporphyrins because of the wide variety of properties of the porphyrin ligands and of the metallic salts and complexes used in synthesis. A recent review by Buchler (146) provides a convenient summary of the main synthetic methods. A factor common to all of these methods is the use of a solvent in which the porphyrin, metal salt (or complex) and metalloporphyrin are soluble. After the reaction, the excess metal salt must be separated from the metalloporphyrin. The heterogeneous metal insertion reaction described in this work has the immediate advantage over the more conventional methods in that the excess metal reactant is separated from the product by simple filtration of the excess solid phase rather than by the tedious methods (often in combination) of precipitation, extraction, dialysis and chromatography.

Fifty metals or metal oxides were tested for reactivity with $TPPS_4$. The results are summarized in Tables 3 and 4.

As Table 3 indicates, only divalent metal oxides and Ag(I) oxide react with $TPPS_4$. None of trivalent, tetravalent or mixed M(II)-M(III) oxides (e.g., Fe_3O_4, Ni_3O_4) were reactive. Although in solution, certain M(III) species are known to react with porphyrins (37), these reactions are extremely slow because of the high activation energies required to desolvate the metal ion. In the heterogeneous reaction, the high lattice energies of the M(III) and higher oxides (8000-17000 kJ/mole compared to about 4000 kJ/mole for M(II) oxides (147)) is the likely reason for their lack of reactivity with $TPPS_4$. It appears, however, that such a thermodynamic criterion as the lattice energy of the metal oxide versus the stability of the metalloporphyrin is not the only factor that governs the reactivity of the metal oxide with the porphyrin. For example, neither PdO (which has a structure similar to that of CuO (148)) nor PdS reacted even though $PdTPPS_4$ is known to be among the most stable of the metalloporphyrins and is readily formed from $PdBr_2$. It appears that kinetic considerations, governed by the surface properties of the solid, may also be involved.

With MgO, CaO, HgO and especially PbO, adsorption of the porphyrin on the surface of the metal oxide was found to be a serious problem and substantial amounts of porphyrin were lost. With other metal oxides, adsorption was less severe.

The reactivities of the metallic elements (Table 4) are even more capricious and do not correlate with standard reduction potentials. For

TABLE 3

Reactivity of Metal Oxides with $TPPS_4$

Metalloporphyrin formed		No observed reaction*		
MgO	ZnO	BeO	Cr_2O_3	ZrO_2
CaO	CdO	BaO	Mn_2O_3	RuO_2
MnO	SnO	TiO	MnO_2	In_2O_3
CoO	HgO	PdO	Fe_2O_3	La_2O_3
NiO	PbO	Sc_2O_3	Fe_3O_4	Bi_2O_3
CuO	Ag_2O	V_2O_3	Ni_3O_4	

* For 72 hours at 100°C.

TABLE 4

Reactivity of Metals with $TPPS_4$

Metalloporphyrin formed		No observed reaction*			
Mn	Zn	Ti	As	Sn**	Bi
Fe	Cd	V	Nb	Sb	
Co	Hg	Cr	Ru	W	
Cu	Pb	Ni	Ag	Au	

* For 72 hours at 100°C

** Unstable species formed on reaction

example, Ni and Ag did not react (their oxides did) but several elements whose standard electrode potentials are between those of Ni and Ag did react. Ni and Ag were non-reactive even when present in large quantities as a fine powder (i.e., a large exposed surface area).

In spite of prior etching of the metal surface to remove any oxide layer, it is possible, for at least some of the metals tested, that the reactive species is not the free metal but some other species, such as a hydrous oxide, formed on the metal surface. This is certainly true for the reactions of Mn, Fe and Pb in which a coating was immediately formed on the metal surface upon the addition of water. With Mn, the formation of the reactive green oxide was readily observed and reaction with the porphyrin followed. In the case of Fe, the reactive species is probably the transitory hydrated ferrous oxide (the higher oxides were found to be unreactive with the porphyrin (Table 3)). Thus, the only way to prepare the iron porphyrin heterogeneously is through reaction with the metal. With Pb, the formation of an oxide coating presents a problem due to extensive adsorption of porphyrin as discussed above.

As noted in Table 4, Sn metal reacted to form an unstable product. After the unreacted Sn was removed, the solution rapidly changed colour on exposure to air, forming a new product whose spectrum corresponded to neither that of the free porphyrin nor of the normal Sn(IV) metalloporphyrin (which can be obtained by reaction with SnO). The initial and oxidized species were not characterized further.

The stoichiometry of the heterogeneous oxide reaction is:

$$M(II)O(s) + H_2TPPS_4(aq) \rightleftharpoons M(II)TPPS_4(aq) + H_2O \quad [1]$$

where the hydrogens in the porphyrin centre are designated by the formulation H_2TPPS_4. For the zero-valent metals, two possible reactions exist. One possibility is that, as in the case of Mn and Fe, surface oxidation by dissolved oxygen in the solvent occurs; the metal insertion would then occur as by reaction [1]. The second possibility is:

$$\overset{0}{M}(s) + H_2TPPS_4(aq) \rightleftarrows M(II)TPPS_4(aq) + H_2 \qquad [2]$$

Because low concentrations of the porphyrin were used, very small amounts of hydrogen gas would have been evolved and thus would have been extremely difficult to detect. Tsutsui et al. (36) have represented the reaction of soluble zero-valent metal complexes with porphyrins by a similar equation. In the present study, a brief attempt was made to distinguish between the two reactions. The formation of $CuTPPS_4$ from Cu metal was carried out in two stoppered cuvettes at room temperature. Oxygen gas was bubbled through one and N_2 through the other before the reaction was initiated. Any reaction which occurred was followed by noting the increase of metalloporphyrin absorbance at 540 nm. It was observed that reaction occurred in both cases but was faster in the presence of excess O_2. This observation suggests that the oxide mechanism [1] is likely significant in the reaction of metals with $TPPS_4$ but that reaction [2] cannot be ruled out. A thorough investigation would be of considerable interest.

The most useful aspect of the heterogeneous metallation of porphyrins lies in the simplicity of the preparation of metalloporphyrins, particularly those of water-soluble porphyrins. As stated earlier, in the conventional procedures with $TPPS_4$ and other water-soluble porphyrins, the separation of excess metal salt from the product is cumbersome, usually by repeated precipitation from aqueous methanol (86) or by dialysis (149).

High purity of the metalloporphyrin is difficult to achieve. In the heterogeneous reactions, the product is not contaminated by counter-ions, excess metal or acid, provided that the solid phase is insoluble. Where the metal oxide is slightly soluble (MgO, CaO, ZnO, Ag_2O, PbO and HgO), contamination can be minimized by simple reduction of the volume of the filtrate and refiltration through the 0.22 μ Millipore filter. The solubilities of CaO and PbO are lower at increased temperatures and hence, solutions containing these oxides may be filtered advantageously while hot.

Heterogeneous metallations can be accomplished with a variety of insoluble compounds. For example, metallation was found to occur in reactions with Cu_2Cl_2, CuS and $PdBr_2$. The byproducts, HCl, H_2S and HBr, respectively, are not as readily removed. Facile removal of the byproducts is a prime requisite of the heterogeneous reaction otherwise its advantage is lost. In this connection, it would be worthwhile to extend the scope of the heterogeneous reaction by the use of metal carbonates.

The general applicability of the heterogeneous reaction was demonstrated with the water-insoluble porphyrins hematoporphyrin (HP, also soluble in strongly acidic or basic aqueous media), protoporphyrin IX dimethyl ester (PPDME), octaethylporphine (OEP) and meso-tetraphenylporphine (TPP). The structures of these compounds are shown in Figure XV. Representative reactions with Cu, CuO and NiO only were carried out. The reactions in non-aqueous solvents were slower. NiO, for example, which reacted with $TPPS_4$ in less than two hours, took two days to completely

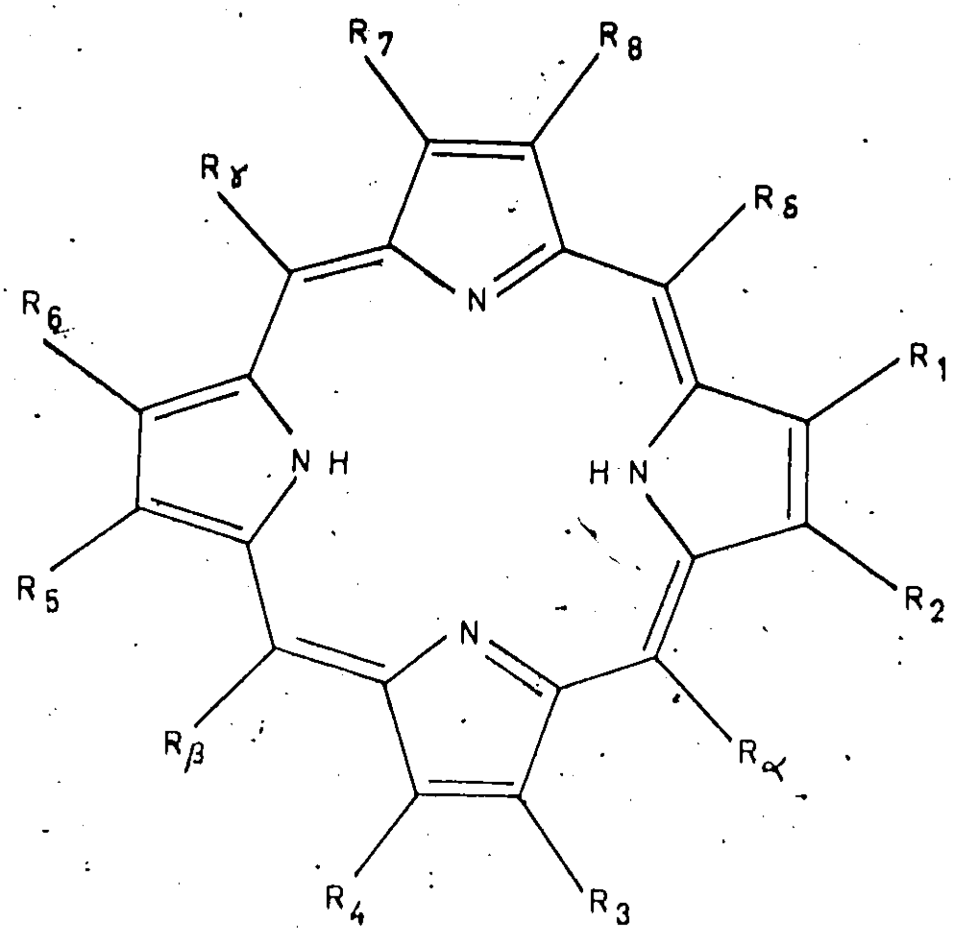


Figure XV. Structures of porphyrins tested for heterogeneous reaction.

(a) Hematoporphyrin (HP):
$R_2 = R_4 = -CHOHCH_3$; $R_6 = R_7 = -CH_2CH_2COOH$; $R_{\alpha-\delta} = -H$

(b) Protoporphyrin IX dimethyl ester (PPDME):
$R_1 = R_3 = R_5 = R_8 = -CH_3$; $R_2 = R_4 = -CH{=}CH_2$; $R_6 = R_7 = -CH_2CH_2COOCH_3$;
$R_{\alpha-\delta} = -H$

(c) Octaethylporphine (OEP):
$R_{1-8} = -CH_2CH_3$; $R_{\alpha-\delta} = -H$

(d) Meso-tetraphenylporphine (TPP):
$R_{1-8} = -H$; $R_{\alpha-\delta} = -C_6H_5$

(e) Meso-tetrasodium tetra(p-sulphophenyl)porphine (Na_4TPPS_4):
$R_{1-8} = -H$; $R_{\alpha-\delta} = -C_6H_4SO_3Na$

react with OEP in DMF/chloroform. The choice of non-aqueous solvent is important, especially for OEP and TPP. Thus, in both chloroform and DMF, TPP failed to react with CuO. However, in 50-75% v/v DMF in chloroform, reaction readily occurred. These solvent mixtures provided both a suitably high refluxing temperature and sufficient solubility of the porphyrins and metalloporphyrins. Also, these mixtures appeared to optimize the surface adsorption/desorption effects of the porphyrins. Such factors explain why some solvents are more suitable than others in the heterogeneous reaction.

The results of elemental analyses on selected metal complexes of $TPPS_4$ synthesized heterogeneously are shown in Table 5. Analyses for C, H, N and S were done before the extreme hygroscopicity of the metal complexes was fully appreciated. Conventional handling of these compounds resulted in the absorption of variable quantities of moisture, which accounts at least in part for the low values for C, N and S and the high values for H. Calculations based on the values for C and H indicate that no more than .5% of other impurities were present.

Thermogravimetric analysis disclosed the hygroscopicity of the $TPPS_4$ complexes as well as their thermal stability. The water content of the complexes was found to vary with ambient conditions. For example, the $ZnTPPS_4$ complex was found to contain as much as 27.5% H_2O, corresponding to 16.6 moles of water per mole of complex. Similarly, the $CuTPPS_4$ complex contained up to 18.5% H_2O (11.2 moles H_2O per mole of complex). This moisture was strongly retained. The $ZnTPPS_4$ and $CuTPPS_4$ complexes, for example, retained 2.8-3.3% H_2O, corresponding to 1.7-2.0

TABLE 5.

Elemental Analyses of $TPPS_4$ Complexes

COMPOUND	FORMULA	CALCULATED (%) C	H	N	S	M	FOUND (%) C	H	N	S	M
$CuTPPS_4$	$C_{44}H_{24}N_4S_4O_{12}Na_4Cu$	48.73	2.23	5.17	11.83	5.86	43.24	2.73	4.59	10.07	5.72
$ZnTPPS_4$	$C_{44}H_{24}N_4S_4O_{12}Na_4Zn$	48.65	2.23	5.16	11.81	6.02	43.79	2.53	4.71	10.29	6.50
$CoTPPS_4$	$C_{44}H_{24}N_4S_4O_{12}Na_4Co$	48.94	2.24	5.19	11.88	--	42.80	2.45	4.63	10.03	
$NiTPPS_4$	$C_{44}H_{24}N_4S_4O_{12}Na_4Ni$	48.95	2.24	5.19	11.88	--	42.84	2.71	4.70	8.09	
$CdTPPS_4$	$C_{44}H_{24}N_4S_4O_{12}Na_4Cd$	46.63	2.13	4.94	11.32	--	42.63	2.63	4.62	10.14	

moles of water per mole of complex at 90°C. Dried samples regained moisture very rapidly, indicating that significant uptake of water would occur even during the time required to transfer a sample from a desiccator to a balance pan. The metal complexes were more hygroscopic than the free ligand.

Representative thermograms are shown in Figures XVI and XVII. These show that gradual weight loss, corresponding to loss of H_2O, occurred up to about 200°C. Between 200-400°C, an almost constant weight region was observed. Above about 400°C, rapid decomposition occurred. For the $CoTPPS_4$, $NiTPPS_4$ and $CdTPPS_4$ complexes, additional regions of stability at higher temperatures were observed. With the exception of $CuTPPS_4$, the complexes were more stable than the free ligand.

For each complex, when the temperature reached 875-900°C, it was maintained in that range until little change in weight occurred with time. The total weight loss, in general, was found to be consistent with the formation of two moles of Na_2SO_4 (the complexes were prepared as the sodium salts) and one mole of metal oxide (Table 6).

TABLE 6

Comparison of calculated weight loss (based on formation of two moles of Na_2SO_4* and one mole of M(II)O) and experimental results.

COMPOUND	CALCULATED LOSS (%)	OBSERVED LOSS (%)
Na_4TPPS_4	72.2	72.2
$CuTPPS_4$	66.5	64.5
$ZnTPPS_4$	66.4	66.5
$CoTPPS_4$	66.7	61.7
$NiTPPS_4$	66.8	65.3
$CdTPPS_4$	63.6	63.7

* Two moles of Na_2SO_4 would be formed rather than four moles because of the limiting amount of sodium. The other two sulphonate groups would (presumably) decompose to SO_2.

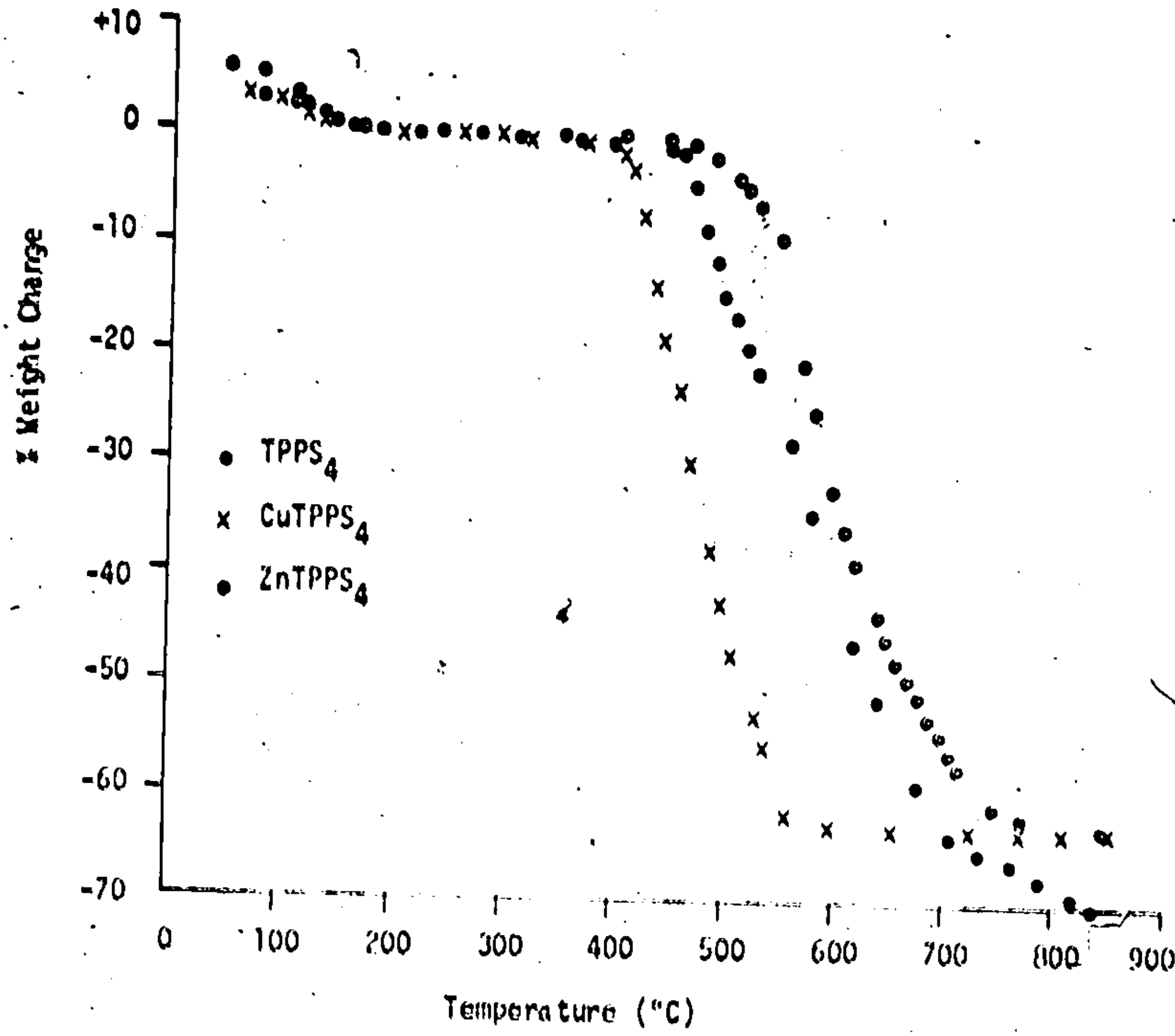


Figure XVI. Percent weight change (relative to anhydrous compound) of $TPPS_4$ and of its Cu(II) and Zn(II) complexes as a function of temperature.

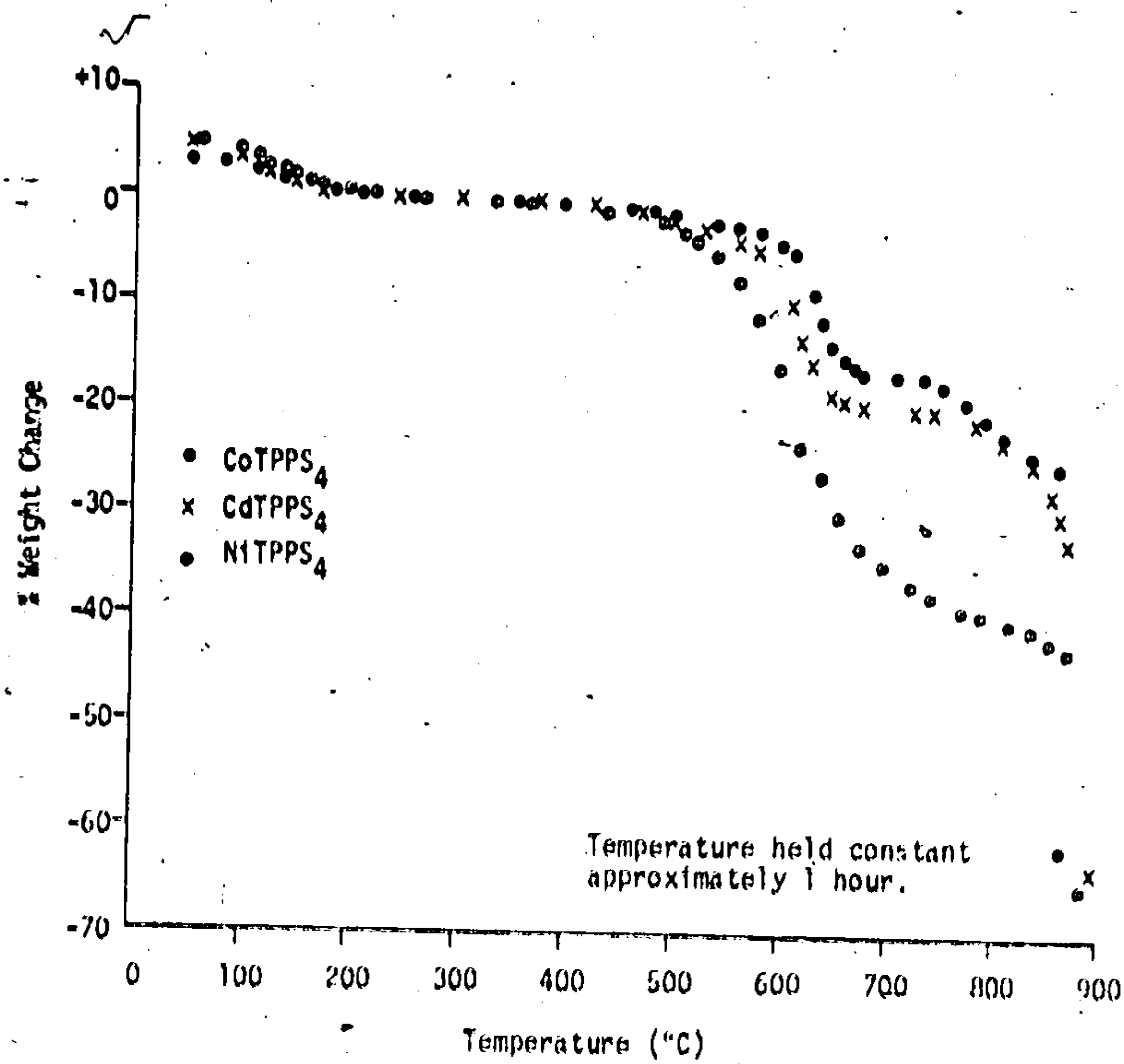


Figure XVII. Percent weight change (relative to anhydrous compound) of the Co(II), Ni(II) and Cd(II) complexes of $TPPS_4$ as a function of temperature.

The electronic absorption spectra of some metalloporphyrins formed by heterogeneous reaction are shown in Figures XVIII to XXV. These spectra indicate that the oxidation state of the metal in the complex is that which is usually stabilized by the porphyrin (35). Thus, Ag(II), Fe(III) and Mn(III) are all cases where the higher oxidation state is stabilized. For $SnTPPS_4$, the stability of the complex to 1M hydrochloric acid suggests that the Sn(IV) state is stabilized (35). The presence of Sn(IV) was confirmed by the Mössbauer spectrum (150) (isomer shift 0.12 $\pm$ 0.05 mm/sec at $80^{\circ}K$ versus calcium stannate at room temperature; line width 0.99 $\pm$ 0.05 mm/sec; no quadrupole splitting detected). In the remaining complexes, including that of Co, the metal was in the divalent state. There is some suggestion from the electronic spectra that different complexes result when $TPPS_4$ is reacted with metallic Hg and HgO. Further study is merited.

The Mg(II), Ca(II), Cd(II), Hg(II) (prepared from HgO) and Pb(II) complexes were isolated by concentration of the solutions. Only the Hg(II) complex has been previously reported (151). The complexes dissociate extensively in solution and hence the spectra shown are combinations of the porphyrin and metalloporphyrin spectra.

The novel method of heterogeneous reaction has been shown to be a convenient way to prepare a number of metalloporphyrins, especially those of $TPPS_4$, and should facilitate studies by other workers.

III.2 Studies on Aggregation

(a) Spectrophotometry

As discussed in the INTRODUCTION, previous studies on the aggregation of $TPPS_4$ in solution failed to reach uniform conclusions. In three

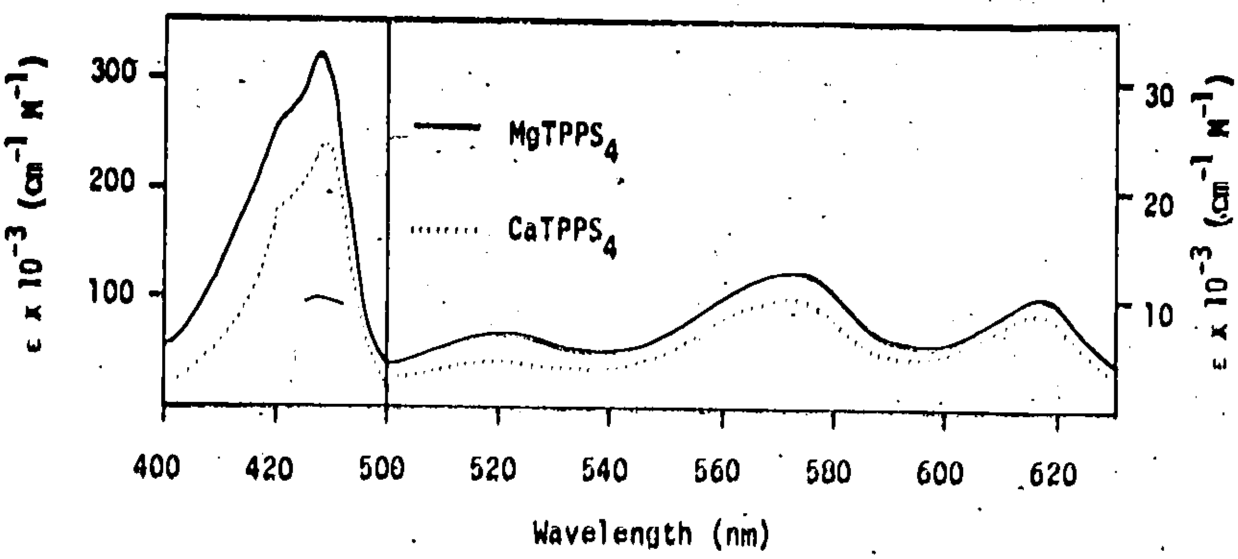


Figure XVIII. Absorption spectra of $MgTPPS_4$ and $CaTPPS_4$ (both 1×10^{-4} M; 0.10 mm cells for $\lambda < 500$ nm; 1.0 mm cells for $\lambda > 500$ nm).

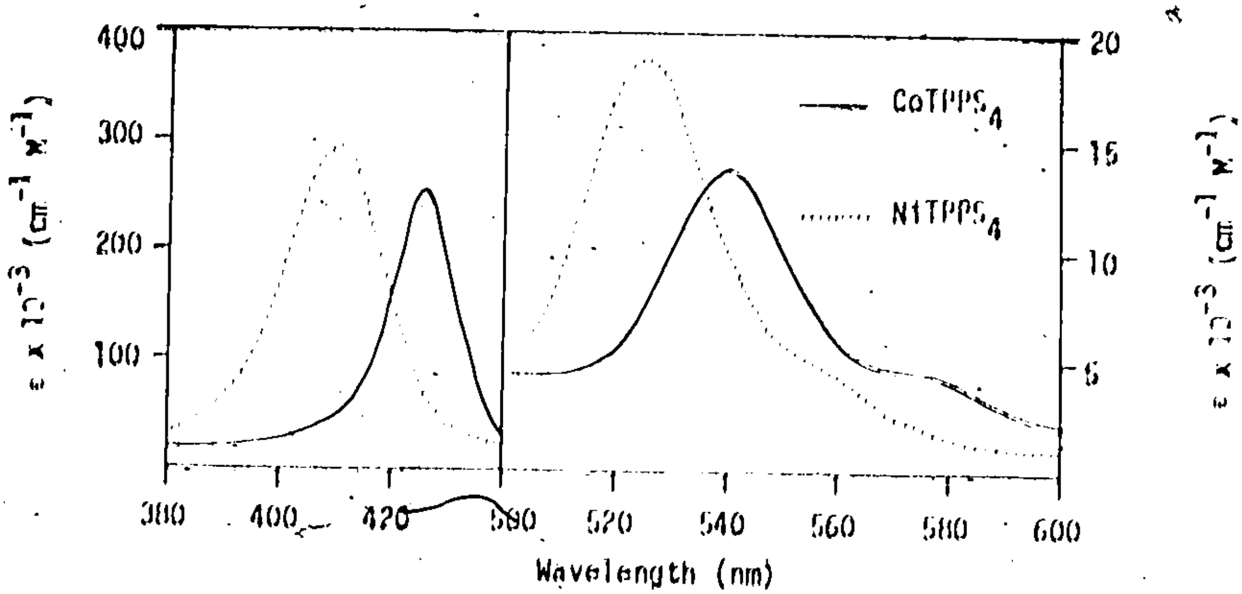
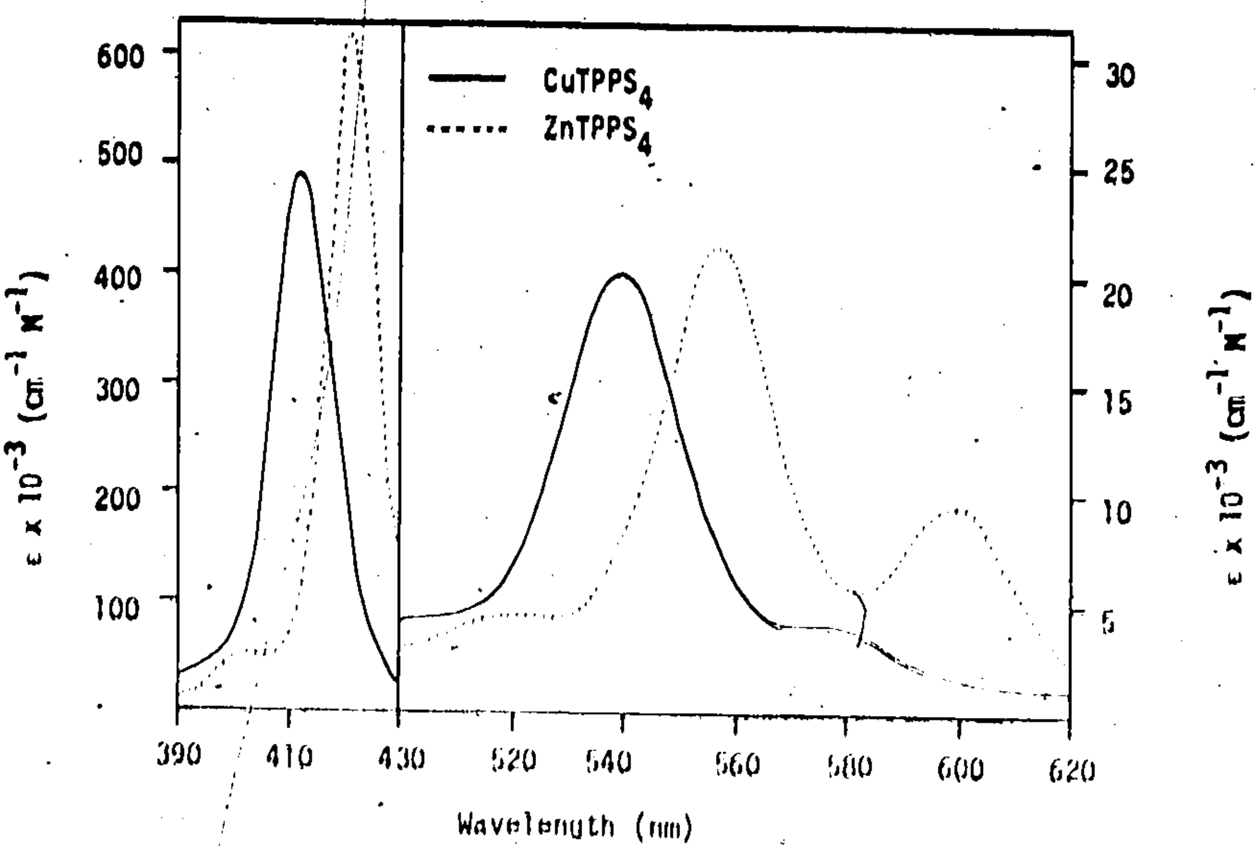


Figure XIX. Absorption spectra of $CoTPPS_4$ and $NiTPPS_4$ (both 1×10^{-6} M, 1 cm cells for $\lambda < 500$ nm; 2×10^{-5} M, 1 cm cells for $\lambda > 500$ nm).

Figure XX. Absorption spectra of $CuTPPS_4$ and $ZnTPPS_4$ (conditions same as in Figure XIX).



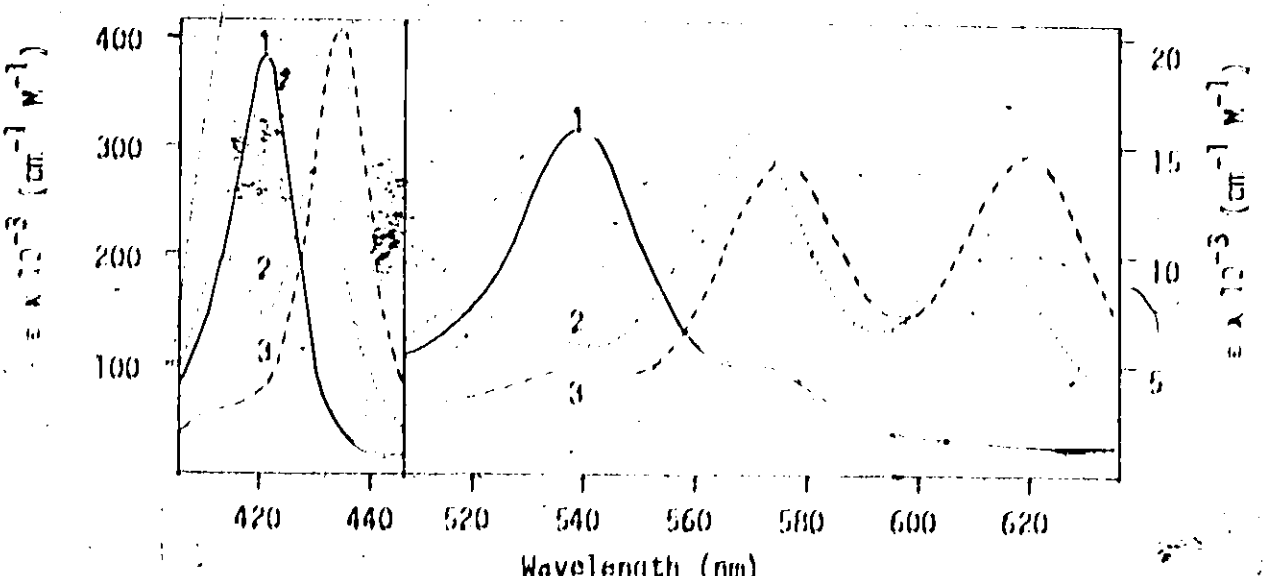


Figure XXI. Absorption spectra of $AgTPPS_4$ (1: conditions as in Figure XIX) and of $CdTPPS_4$ (2, neutral solution; 3, strongly alkaline solution; both 2×10^{-4} M, 0.10 mm cells for $\lambda < 500$ nm, 1.0 mm cells for $\lambda > 500$ nm).

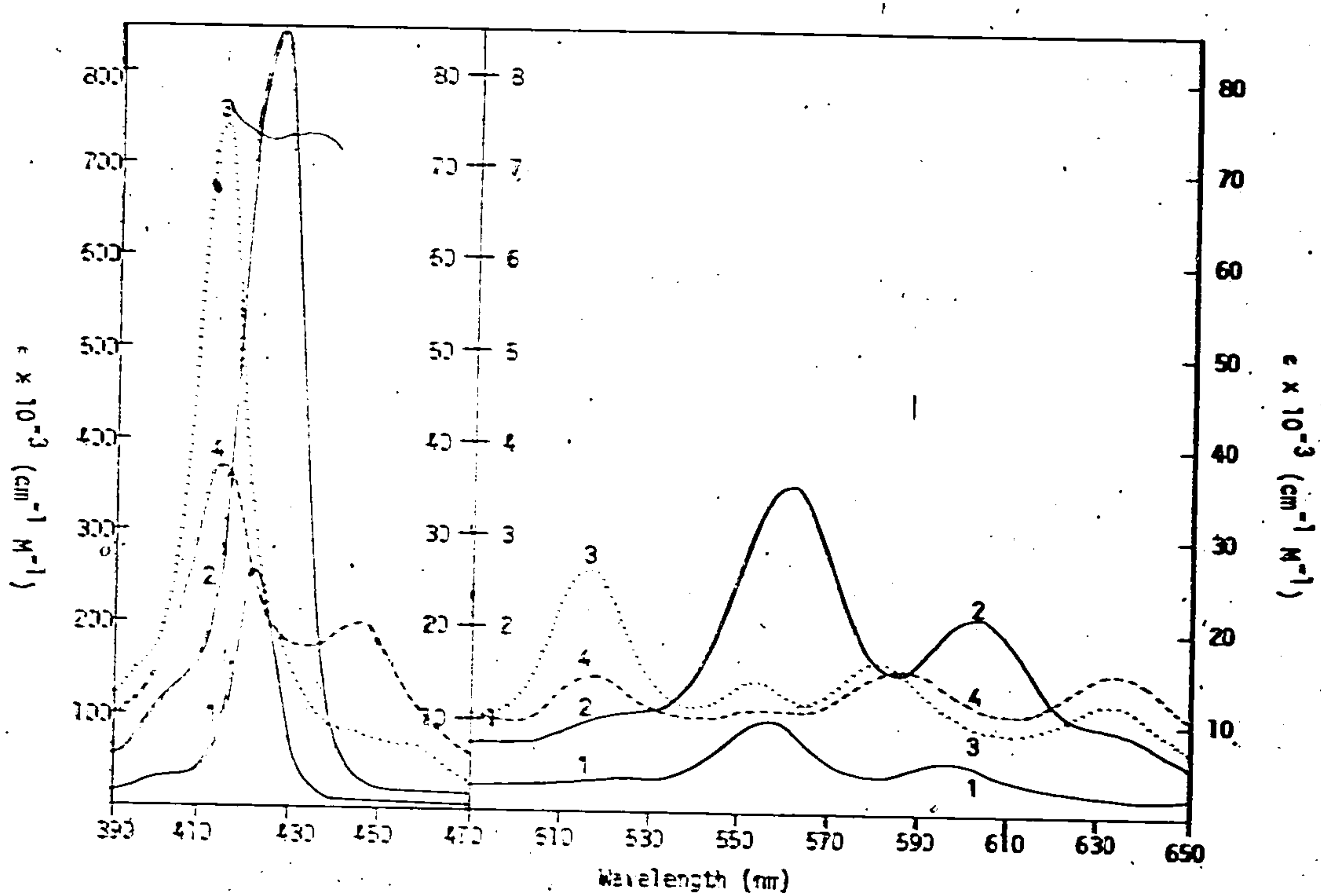


Figure XXII. Absorption spectra of $HgTPPS_4$. (a) Complex prepared from Hg metal (1×10^{-5} M; 1 mm cells for $\lambda < 500$ nm, 1 cm cells for $\lambda > 500$ nm; 1 neutral solution; 2 strongly alkaline solution). (b) Complex prepared from HgO (1×10^{-4} M; 1 mm cells for $\lambda < 500$ nm, 1 cm cells for $\lambda > 500$ nm; 3 neutral solution; 4 strongly alkaline solution). Outer scales refer to spectra 1 and 2, inner scales refer to 3 and 4.

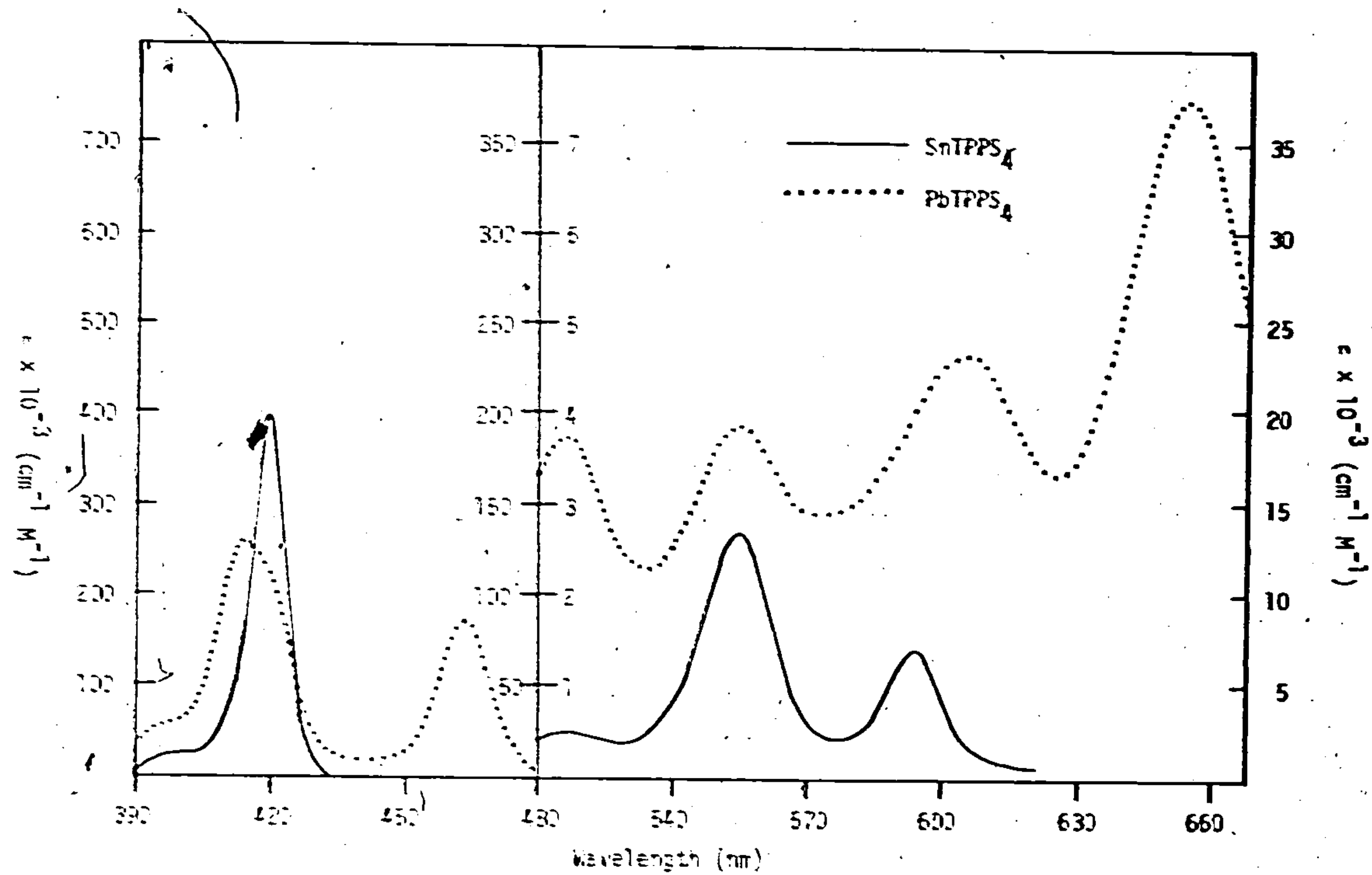


Figure XXIII. Absorption spectra of $SnTPPS_4$ (conditions as in Figure XIX; outer scales) and of $PbTPPS_4$ (2×10^{-2} M, 0.10 mm cells for λ < 500 nm, 1 cm cells for λ > 500 nm; inner scales).

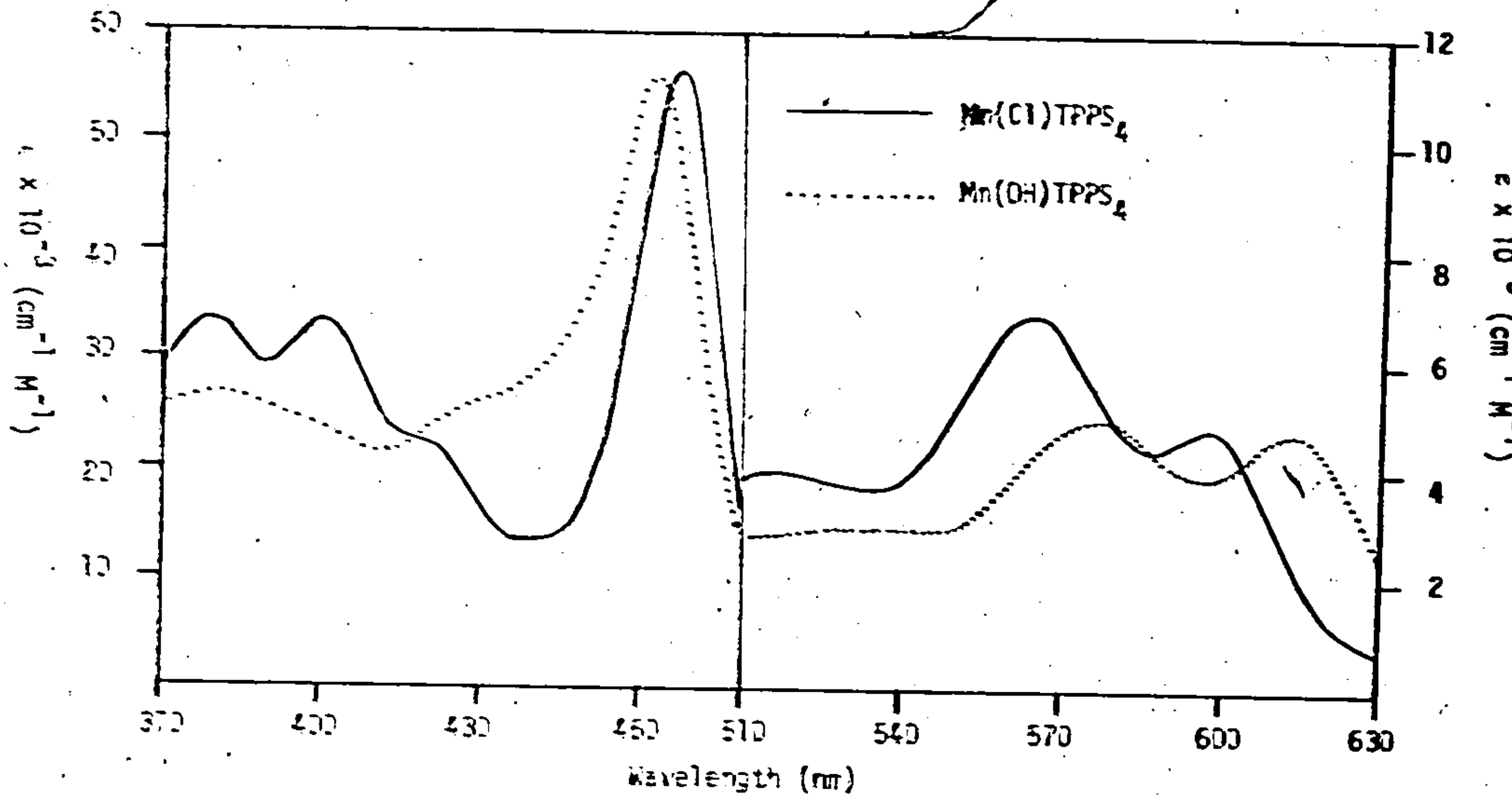


Figure XXIV. Absorption spectra of Mn(III)TPPS$_4$ (1×10^{-5} M for $\lambda < 500$ nm; 1×10^{-4} M for $\lambda > 500$ nm; 1 cm cells in both cases) in 0.25 M HCl and in 0.25 M NaOH.

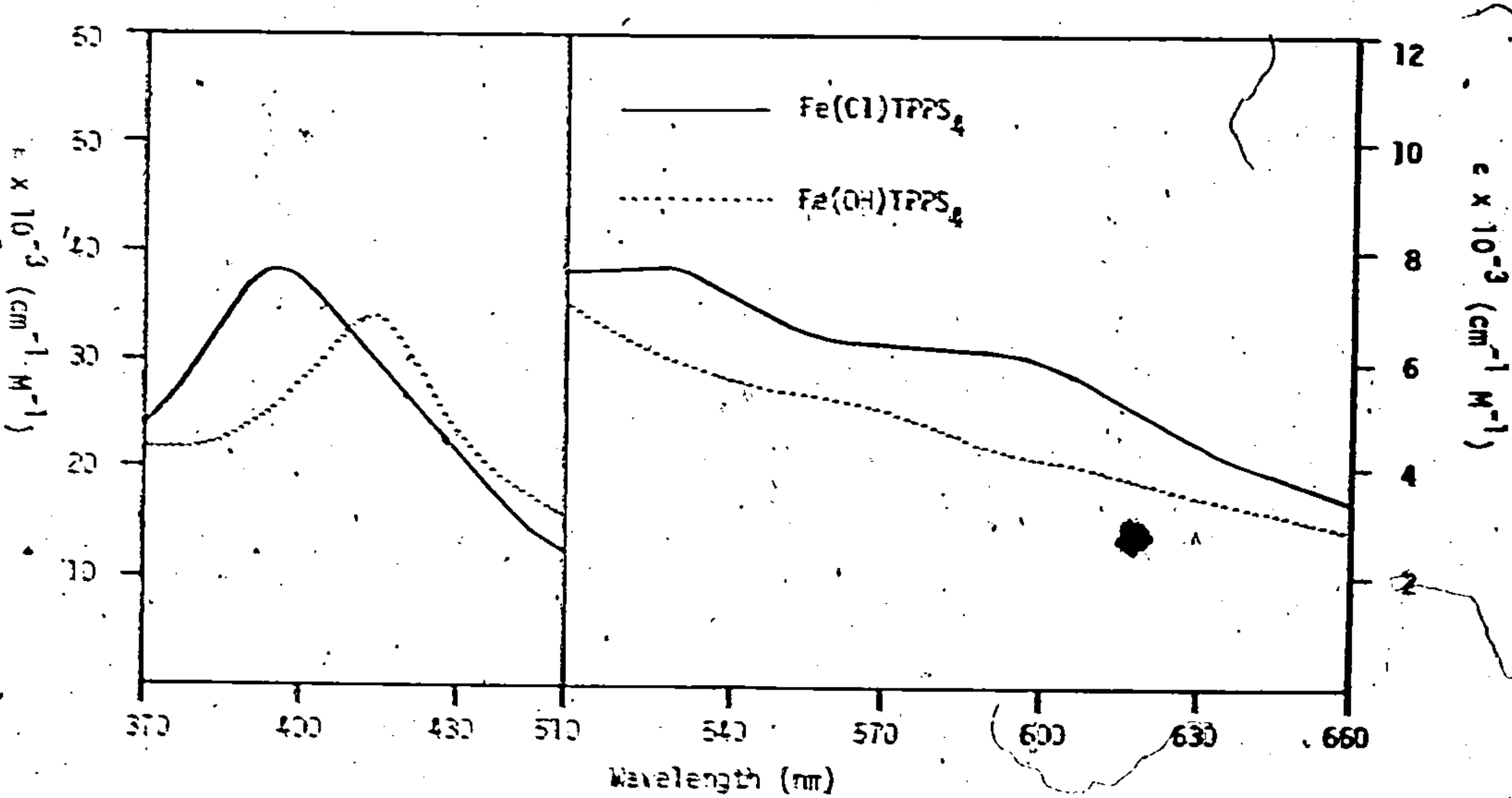


Figure XXV. Absorption spectra of Fe(III)TPPS$_4$ (1×10^{-5} M for $\lambda < 500$ nm; 1×10^{-4} M for $\lambda > 500$ nm; 1 cm cells in both cases) in 0.25 M HCl and in 0.25 M NaOH.

separate studies, the compound or its copper complex (whose behaviour is expected to be qualitatively similar) was said to be (i) monomeric over a wide concentration range (86); (ii) readily dimerized, with $K_D = 9.6 \times 10^4\ M^{-1}$ (91); and, (iii) a mixture of the monomer and a polymer with little or no evidence for a dimer (93). Thus, it was necessary to reexamine the behaviour of the ligand in solution before proceeding with further studies.

Qualitative examination of the electronic absorption spectra of $TPPS_4$ solutions showed that in non-acidic media, the Soret band decreased in intensity* and broadened as the concentration of $TPPS_4$ was increased. A concurrent small hypsochromic shift (about 1 nm) on passing from 1.8×10^{-6} to 1.8×10^{-4} M (0.1 M ionic strength) was also observed. In contrast, the bands in the visible region were shifted bathochromically. The shift was most noticeable for the band at 635 nm, for which overlap with other bands was smallest. The magnitude of the shift was 14 nm for a concentration change from 1.8×10^{-5} to 1.8×10^{-4} M. These observations suggest behaviour such as aggregation and are in agreement with recent findings on the spectral characteristics of some cofacial porphyrins (152). These are porphyrin dimers held in rigid configuration by bridging alkyl chains.

Quantitative data on aggregation were obtained from the deviations from Beer-Lambert law. In these studies it was assumed that only the monomer and dimer existed in significant quantities under the conditions employed. A non-linear least-squares curve-fitting program (NLWOOD, supplied

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* Here, intensity is defined as the apparent molar absorptivity, which is A(measured)/C(analytical)·path length.

by the McMaster University Data Processing and Computing Centre) was used to fit the data to the following equation:

$$A = (\varepsilon_2 C_T/2) + (2\varepsilon_1 - \varepsilon_2)(\sqrt{1 + 8K_D C_T} - 1)/8K_D \qquad [3]$$

where A = absorbance

C_T = analytical concentration of porphyrin

ε_1 = molar absorptivity of the monomer

ε_2 = molar absorptivity of the dimer

K_D = dimerization constant

This equation is derived in Appendix II(a). A description of this program may be found in the monograph by Daniel and Wood (153). Absorbance and concentration data were fitted to the above equation and estimates of ε_1, ε_2 and K_D were obtained. Alternatively, ε_1 was determined separately by linear least-squares analysis of low-concentration data ($\leq 2 \times 10^{-6}$ M) and this value was substituted directly into the above equation. This latter technique yielded an improved value of ε_1 and better precision for the values of ε_2 and K_D. The improved precision could be explained largely by the fact that the data was fitted to fewer parameters and to a lesser extent by the fact that the variance of ε_1 was not incorporated into equation [3].

In the following description, the above methods of data anlysis are referred to as "direct" methods. By splitting the data into two portions, as above, and determining ε_1 using one portion, another technique, known as the "ratio" method, could also be used. In this technique, the ratio of the calculated absorbance (assuming only the monomer to be present) to the observed absorbance was analyzed as a function of the analytical concentration. The data was fitted to the equation:

$$R = A(CALC.)/A(OBS.)$$

$$= \varepsilon_1 C_T/A(OBS.)$$

$$= 8\varepsilon_1 C_T K_D/[(4K_D\varepsilon_2 C_T) + (2\varepsilon_1 - \varepsilon_2)(\sqrt{1 + 8K_D C_T} - 1)] \qquad [4]$$

where ε_1, ε_2, C_T and K_D are defined as for equation [3] for the direct methods.

Data points which showed significant discrepancy from the general trend ("outliers") were much more obvious in the ratio method. This technique was useful when the data showed a moderate degree of scatter. In such cases, the ratio method gave improved precision for K_D values although the precision for ε_2 was sometimes decreased. However, in the analysis of data with little scatter, the ratio method showed no advantage over the direct method.

Figure XXVI illustrates typical data as analyzed by the direct and ratio methods. Table 7 gives a summary of the results obtained for unprotonated $TPPS_4$ in three media (0.100 M ionic strength).

Examination of the data showed that a major contribution to the variance in ε_1 was due to the variable amount of adsorbed moisture on the $TPPS_4$. Although the level of precision was adequate for this work, better precision could be obtained by weighing all samples on a thermobalance or in a dry-box. For further studies, such weighing techniques are recommended, especially for metalloporphyrins because they are considerably more

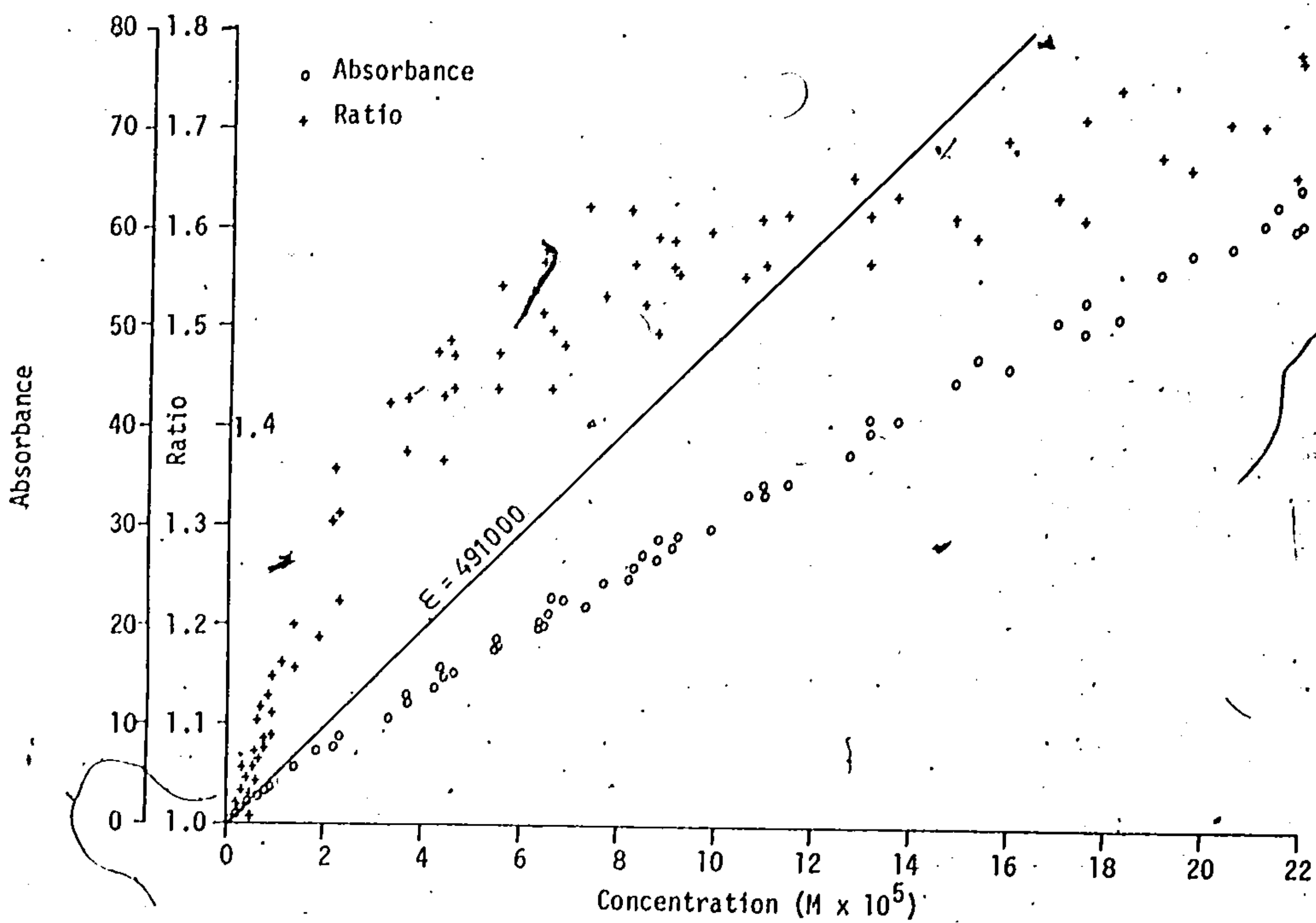

Figure XXVI. $TPPS_4$ in 0.100 M potassium nitrate solution at 25°C. The straight line corresponds to the absorbance expected in the absence of aggregation. Ratio = $A_{calc.}/A_{obs.}$

TABLE 7

Spectrophotometric Study of the Dimerization of Free Ligand

λ = 413 nm	N = total number of data points
T = 25.0°C	R = number of replicate experiments
μ = 0.100 M	$\pm$ values = 95% confidence limits

LINEAR REGRESSION

Concentration range = 1.1×10^{-7} M - 2.1×10^{-6} M

MEDIUM	pH	N	R	ε_1 (cm^{-1} M^{-1})
Potassium nitrate	5.4 - 6.4	36	3	$(491.2 \pm 1.5) \times 10^3$
Phosphate buffer	7.0	42	4	$(510.0 \pm 3.2) \times 10^3$
Sodium acetate	9.0	36	4	$(473.5 \pm 3.5) \times 10^3$

NON-LINEAR REGRESSION

Potassium nitrate medium

	DIRECT	DIRECT	RATIO
N	127	91	91
Range	1.1×10^{-7} - 4.2×10^{-4} M	2.1×10^{-6} - 4.2×10^{-4} M	2.1×10^{-6} - 4.2×10^{-4} M
ε_1 (cm^{-1} M^{-1})	$(473 \pm 70) \times 10^3$	*	*
ε_2 (cm^{-1} M^{-1})	$(419 \pm 20) \times 10^3$	$(422 \pm 14) \times 10^3$	$(408 \pm 20) \times 10^3$
K_D (M^{-1})	$(20 \pm 17) \times 10^3$	$(25.4 \pm 4.3) \times 10^3$	$(22.3 \pm 3.5) \times 10^3$

Continued....

TABLE 7 (Continued)

Phosphate buffer medium

	DIRECT	DIRECT	RATIO
N	101	59	59
Range	$1.1 \times 10^{-7} - 3.4 \times 10^{-4}$ M	$2.1 \times 10^{-6} - 3.4 \times 10^{-4}$ M	$2.1 \times 10^{-6} - 3.4 \times 10^{-4}$ M
ϵ_1 (cm^{-1} M^{-1})	$(517 \pm 17) \times 10^3$	*	*
ϵ_2 (cm^{-1} M^{-1})	$(397.9 \pm 8.9) \times 10^3$	$(394.4 \pm 7.4) \times 10^3$	$(384 \pm 16) \times 10^3$
K_D (M^{-1})	$(17.3 \pm 3.5) \times 10^3$	$(15.8 \pm 1.0) \times 10^3$	$(15.4 \pm 1.3) \times 10^3$

Sodium acetate medium

	DIRECT	DIRECT	RATIO
N	74	38	38
Range	$1.1 \times 10^{-7} - 5.2 \times 10^{-4}$ M	$2.1 \times 10^{-6} - 5.2 \times 10^{-4}$ M	$2.1 \times 10^{-6} - 5.2 \times 10^{-4}$ M
ϵ_1 (cm^{-1} M^{-1})	$(404 \pm 51) \times 10^3$	*	*
ϵ_2 (cm^{-1} M^{-1})	$(360 \pm 51) \times 10^3$	$(399 \pm 27) \times 10^3$	$(422 \pm 19) \times 10^3$
K_D (M^{-1})	$(4 \pm 5) \times 10^3$	$(12.9 \pm 3.6) \times 10^3$	$(16.4 \pm 3.0) \times 10^3$

* Value determined by linear regression used in calculations.

hygroscopic than the free ligand. Variable quantities of moisture not only increase the variance of the data but give low values for ε_1, ε_2 and K_D. To test the magnitude of the effect of moisture on the various parameters, one of the data sets was altered by assuming the presence of 10% moisture and the values of the parameters were then recalculated. The results are shown in Table 8 together with the original data obtained on the assumption of no moisture.

TABLE 8

Effect of Moisture (Phosphate-Buffer Medium)*

	Dry	10% Moisture
ε_1 (cm^{-1} M^{-1})	$(517 \pm 17) \times 10^3$	$(471 \pm 15) \times 10^3$
ε_2 (cm^{-1} M^{-1})	$(397.9 \pm 8.9) \times 10^3$	$(361.4 \pm 8.5) \times 10^3$
K_D (M^{-1})	$(17.3 \pm 3.5) \times 10^3$	$(15.8 \pm 3.3) \times 10^3$

* N = 101, direct method

As the moisture content is increased, there is a corresponding decrease in the values of the parameters which are all affected to approximately the same extent on a percent basis.

Table 7 shows that the value of ε_1 in different media varies widely. This fact invalidates the practice (e.g., 90, 92, 149) of determining ε_1 under non-aggregative conditions (e.g., no electrolyte present, increased temperature), and then using this value in calculations involving data obtained for conditions under which aggregation becomes significant. The ε_1 value and consequently the value of K_D are dependent on medium effects.

The more precise values of K_D yielded by the two-parameter direct method and the ratio method are considered to be the more acceptable ones. Agreement between the two methods is good within each electrolyte solution. Comparison among electrolytes shows that the value of K_D is the same in phosphate as in acetate media (within experimental error) but the value in potassium nitrate solution is substantially greater. However, since the pH of the potassium nitrate solutions varied between 5.4 and 6.4, there is some doubt that the value of K_D in solutions of this electrolyte is indeed significantly different from the K_D values in the other solutions. The lower end of this pH range is close to the reported values of pK_4 and pK_3 of $TPPS_4$* so that significant quantities of porphyrin would have been protonated. Because the protonation of $TPPS_4$ results in a spectral shift of the Soret band from 413 nm to 434 nm, any protonation of $TPPS_4$ would cause a decrease in the absorbance at 413 nm. Since aggregation also results in decreased absorbance, a reduction in the absorbance at 413 nm due to protonation would be interpreted as increased aggregation, thus yielding spuriously high values for K_D. Since the value of K_D in potassium nitrate media is, indeed, higher than in the other media, it is reasonable to assume that K_D is not intrinsically different in potassium nitrate and that the aggregation of unprotonated $TPPS_4$ is probably dependent only on the ionic

* pK_4 and pK_3 are essentially indistinguishable and have been reported as 4.8 (86) and 4.55 (99).

strength and not on specific-ion effects in simple ionic media.

No explanation can be advanced to account for the failure of Fleischer et al. (86) to observe any aggregation with $TPPS_4$. In contrast, Hambright et al. (91) found $TPPS_4$ to aggregate readily and determined the value of K_D to be about $9.6 \times 10^4\ M^{-1}$. This value is considerably greater than that found in the present study ($1.51 \times 10^4\ M^{-1}$, average of results in phosphate and acetate media). The larger K_D value may be the result of their use of Hepes buffer (4-(2-hydroxyethyl)-1-piperazineethanesulphonic acid). It has been shown in NMR studies that specific interactions can occur between $TPPS_4$ and organic compounds, especially large molecules such as DSS (sodium 2,2-dimethyl-2-silapentane-5-sulphonate). Thus interaction with Hepes buffer is a distinct possibility and logic similar to that explaining the effect of protonation leads to the conclusion that high values of K_D would result.

The aggregation of the Cu(II) and Zn(II) complexes of $TPPS_4$ was also examined. Because of metallation, aggregation of the metalloporphyrins is pH-insensitive over a wider range of acidity. Studies were carried out only at pH 7 and the results are summarized in Table 9. Although the Cu(II) complex aggregates more readily than the free ligand, no evidence was found to support the claim (93) that the predominant species were monomeric and polymeric, with very little dimer formation. In the present study, a monomer-dimer model allows the best fit of the data.

In other studies (e.g., 90, 91), no evidence was found for dimerization of the Zn(II) complexes of $TPPS_4$ (and of meso-tetra(p-carboxyphenyl)-porphine). It was concluded that the Zn(II) complexes were either monomeric

TABLE 9

Spectrophotometric study of metalloporphyrin dimerization

λ = 412 nm for $CuTPPS_4$	Medium = phosphate buffer, pH 7.0
= 556 nm for $ZnTPPS_4$	N = Total number of data points
T = 25.0°C	R = number of replicate experiments
μ = 0.100 M	$\pm$ values = 95% confidence limits

LINEAR REGRESSION

COMPOUND	N	R	Conc. Range	ε_1 (cm^{-1} M^{-1})
$CuTPPS_4$	32	4	$7 \times 10^{-8} - 6 \times 10^{-7}$ M	$(414.0 \pm 5.0) \times 10^3$
$ZnTPPS_4$	16	2	$1 \times 10^{-5} - 2 \times 10^{-4}$ M	$(20.57 \pm 0.11) \times 10^3$

NON-LINEAR REGRESSION

$CuTPPS_4$ (R = 3).

	DIRECT	DIRECT	RATIO
N	88	69	69
Range	$1.0 \times 10^{-7} - 5.5 \times 10^{-4}$ M	$1.0 \times 10^{-6} - 5.5 \times 10^{-4}$ M	$1.0 \times 10^{-6} - 5.5 \times 10^{-4}$ M
ε_1 (cm^{-1} M^{-1})	$(391 \pm 60) \times 10^3$	*	*
ε_2 (cm^{-1} M^{-1})	$(216.7 \pm 8.5) \times 10^3$	$(219.1 \pm 5.5) \times 10^3$	$(224.9 \pm 6.0) \times 10^3$
K_D (M^{-1})	$(33 \pm 20) \times 10^3$	$(41.5 \pm 3.8) \times 10^3$	$(45.0 \pm 3.1) \times 10^3$

$ZnTPPS_4$ (R = 5)

	DIRECT	DIRECT	RATIO
N	65	41	41
Range	$1.0 \times 10^{-5} - 6.2 \times 10^{-3}$ M	$2.0 \times 10^{-4} - 6.2 \times 10^{-3}$ M	$2.0 \times 10^{-4} - 6.2 \times 10^{-3}$ M
ε_1 (cm^{-1} M^{-1})	$(23.5 \pm 6.0) \times 10^3$	*	*
ε_2 (cm^{-1} M^{-1})	$(29.9 \pm 2.0) \times 10^3$	$(27.5 \pm 1.$[illegible]$) \times 10^3$	$(25.3 \pm 2.2) \times 10^3$
K_D (M^{-1})	$(1.2 \pm 2.8) \times 10^3$	221 ± 81.	153 ± 45

* Value determined by linear regression used in calculations

or that K_D was less than 100 M^{-1} (the assumptions used to obtain this value were not stated (90)). In the present study, the $ZnTPPS_4$ was indeed found not to aggregate up to a concentration of about 2×10^{-4} M (the limit for use of the Soret band); however, at concentrations above about 3×10^{-4} M (concentrations which necessitate the use of the less intense bands at 556 and 596 nm), dimerization was evident. The use of these lower intensity bands resulted in a decrease in precision due to the small difference between ε_1 and ε_2. In fact, this difference was too small in the case of the 5[illegible]6-nm band to yield a reliable value for K_D.

The behaviour of protonated $TPPS_4$ (see INTRODUCTION) is quite different from that of the non-protonated free ligand or of the metal complexes. The following portion of this work describes the experimental findings and proposes a model which is consistent with both these results and those of other workers.

Spectra of protonated $TPPS_4$ in its monomeric and aggregated forms are shown in Figure XXVII. Figure XXVIII shows the change in absorbance at 435 and 491 nm as a function of the analytical concentration of $TPPS_4$ (in formate buffer). Similar results were obtained in other media. The sudden change in absorbance which occurs over a narrow concentration range in the region of 3×10^{-6} M cannot be reconciled with a simple model of aggregation. Such a change is, however, characteristic of micellization.*

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* Molecules which possess both a hydrophilic and a hydrophobic portion can form organized aggregates (i.e., the molecules in the aggregate are aligned so that their hydrophobic portions are adjacent, as are their hydrophilic portions). Such aggregates, usually consisting of 10-100 monomer units, are known as micelles. The sizes of the aggregates in a system fall within a narrow range for a fixed set of conditions.

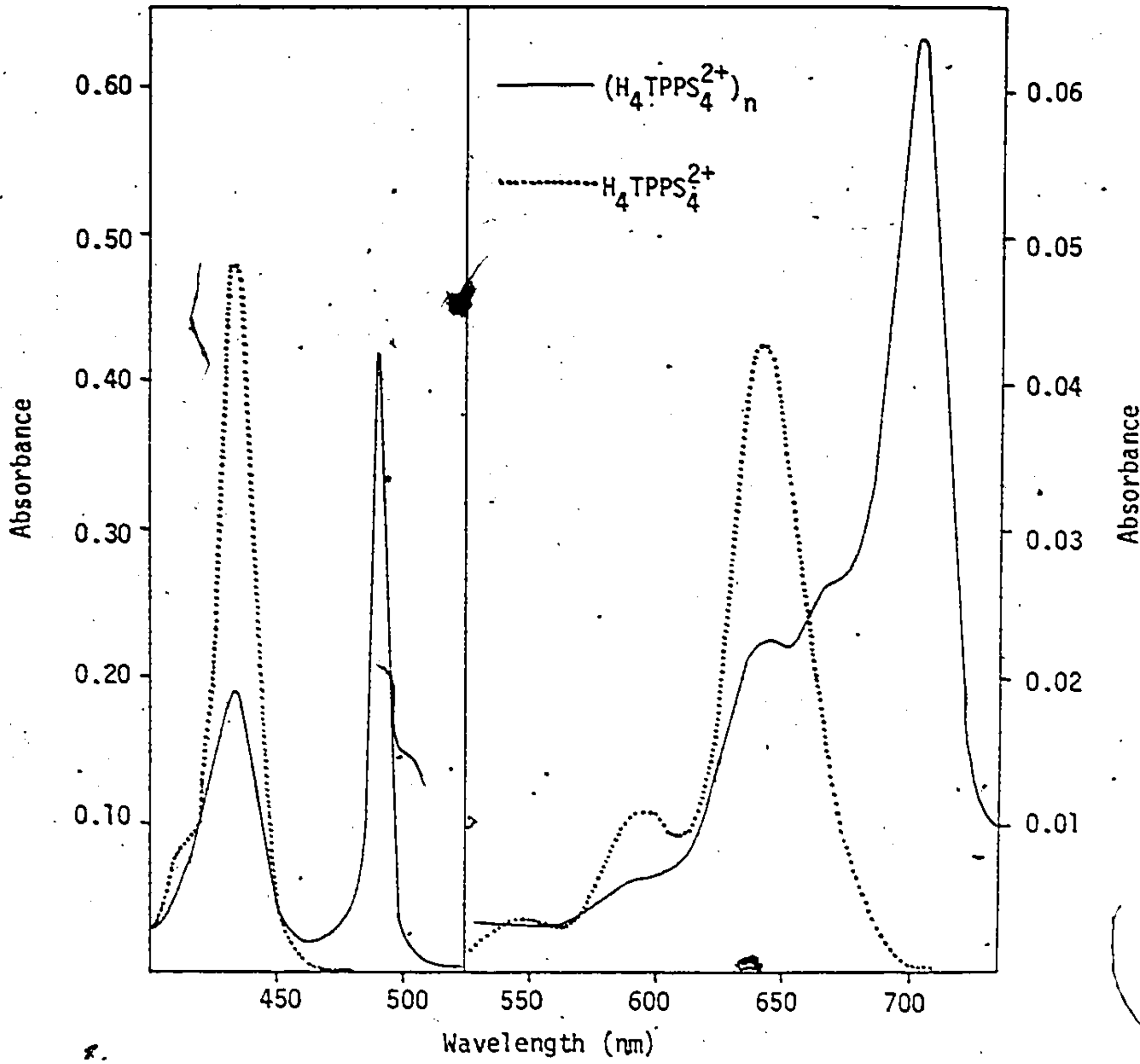

Figure XXVII. Absorption spectra of $TPPS_4$ diacid as the monomer (1×10^{-6} M; 1 cm cells) and as an aggregate (1×10^{-5} M; 1 mm cells) in pH 3.55 formate buffer. Spectra at $\lambda > 525$ nm obtained using expanded scale (0.1 A full scale).

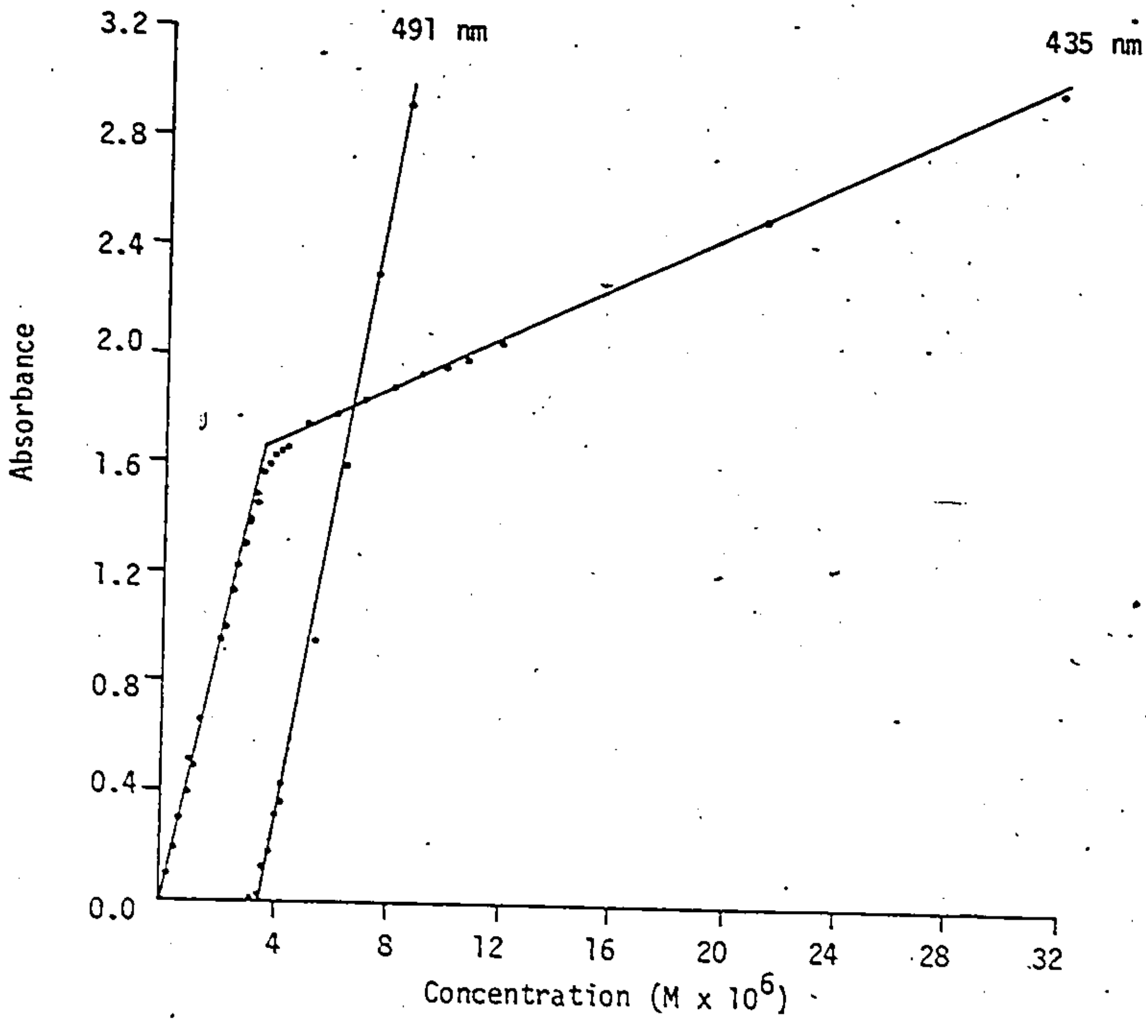

Figure XXVIII. $TPPS_4$ in pH 3.55 formate buffer at 25°C; μ = 0.100 (0.095 M KNO_3).

This can be seen by the application of the law of mass action to the equilibrium between monomers and aggregates (155).

$$nP \rightleftarrows (P)n$$
$$C(1-x) \rightleftarrows Cx/n \qquad [5]$$

where P represents a porphyrin molecule

n = number of monomer units per aggregate

C = analytical concentration of porphyrin

x = fraction of monomer units aggregated

Therefore, the equilibrium constant will be given by

$$K = (Cx/n)/[C(1-x)]^n \qquad [6]$$

For small values of n, x will change gradually as a function of C even when the value of K is large. Any physical property which is related to the concentration of either monomer or aggregate will also deviate from the expected response (based on the presence of monomer only). Thus, the gradual deviation of the absorbance from the Beer-Lambert law as a function of the analytical concentration, as observed for the non-protonated free ligand for example, is characteristic of the formation of small aggregates. At larger values of n, x will remain small up to a certain value of C and will then increase rapidly. The sharpness of the change increases as n increases (Figure XXIX).

The appearance of two new intense bands at 491 nm and 708 nm also indicated that the species formed are not simple dimers or tetramers since similar bands were not evident for any concentration of the unprotonated

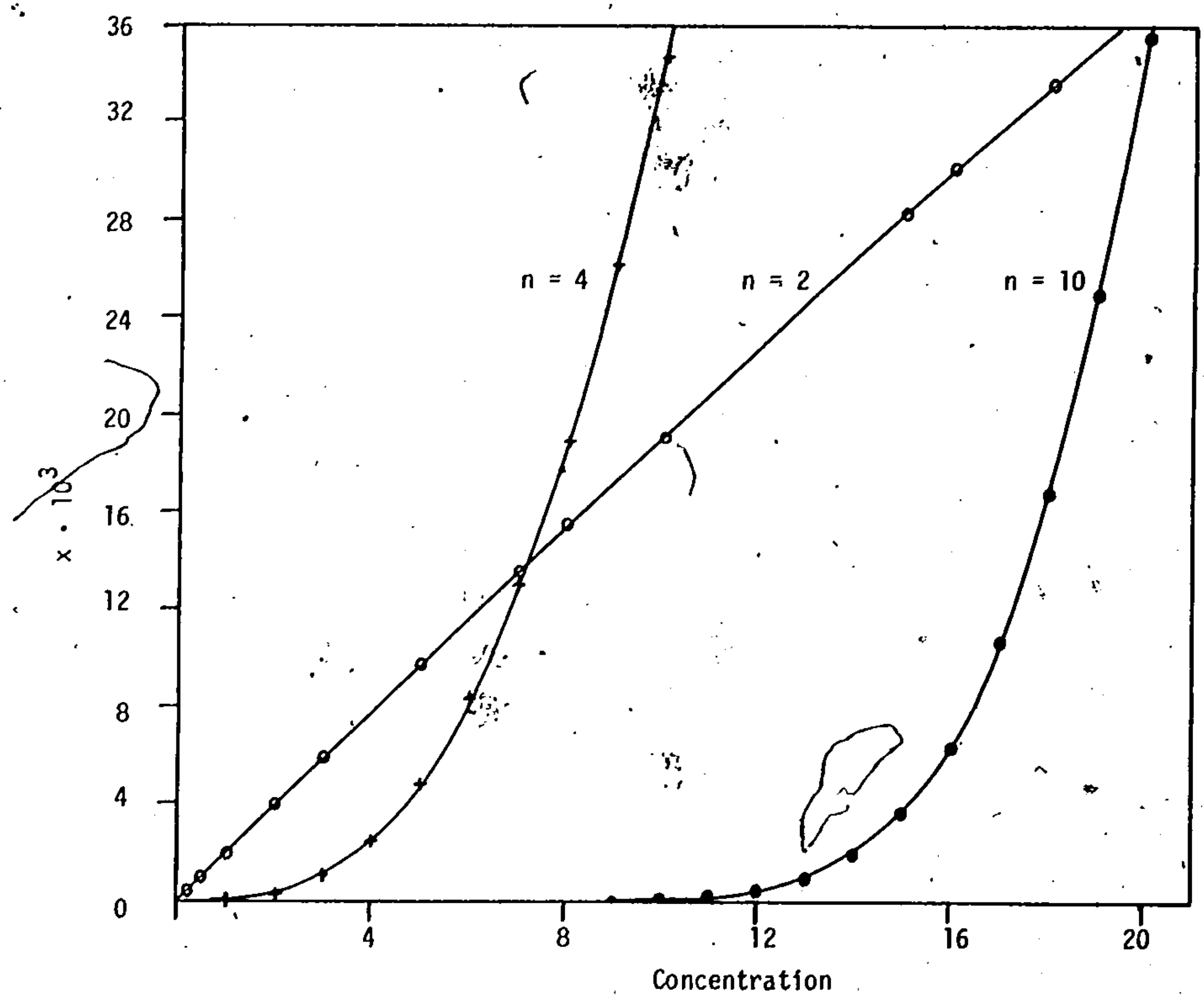

Figure XXIX. Fraction of monomer units aggregated as a function of concentration for different values of n (number of monomer units in aggregate). Concentration scale is different for each case (n = 2, M x 10^7; n = 4, M x 10^3; n = 10, M x 10^2). K = 10^4 for all cases.

ligand where aggregation is known to occur.

It is interesting to note that the behaviour described above has not been observed with other water-soluble porphyrins (such as the well-studied meso-tetra (4-N-methylpyridyl)porphine). This fact suggests that the development of opposite charges within the monomer is necessary for the formation of this type of micellar species. The micelles formed with $TPPS_4$ are unusual in being so highly charged* and in having a relatively small hydrocarbon portion. The sharpness and intensity of the 491-nm band indicate an interaction involving the large aromatic system of the molecule. Further information could be obtained by examining the variation in the critical micelle concentration (CMC) in different ionic media. The CMC is the concentration region where the abrupt change in a physical property occurs which is associated with the formation of micelles (156). The CMC is expressed as a single concentration as though the change in the physical property being measured were perfectly sharp. This value is obtained by extrapolation of the straight-line portions of the graph as shown in Figure XXX. In the present case, the CMC may be determined from the absorbance/concentration data for either the 435- or 491-nm band. The CMC

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* Among the most familiar micellar systems are those formed by ionic detergents such as the hexadecyltrimethylammonium (cationic detergent) and dodecyl sulphate (anionic detergent) salts. In these systems, the monomeric molecule has a hydrophilic portion containing only a single charged group and a fairly large hydrophobic portion.

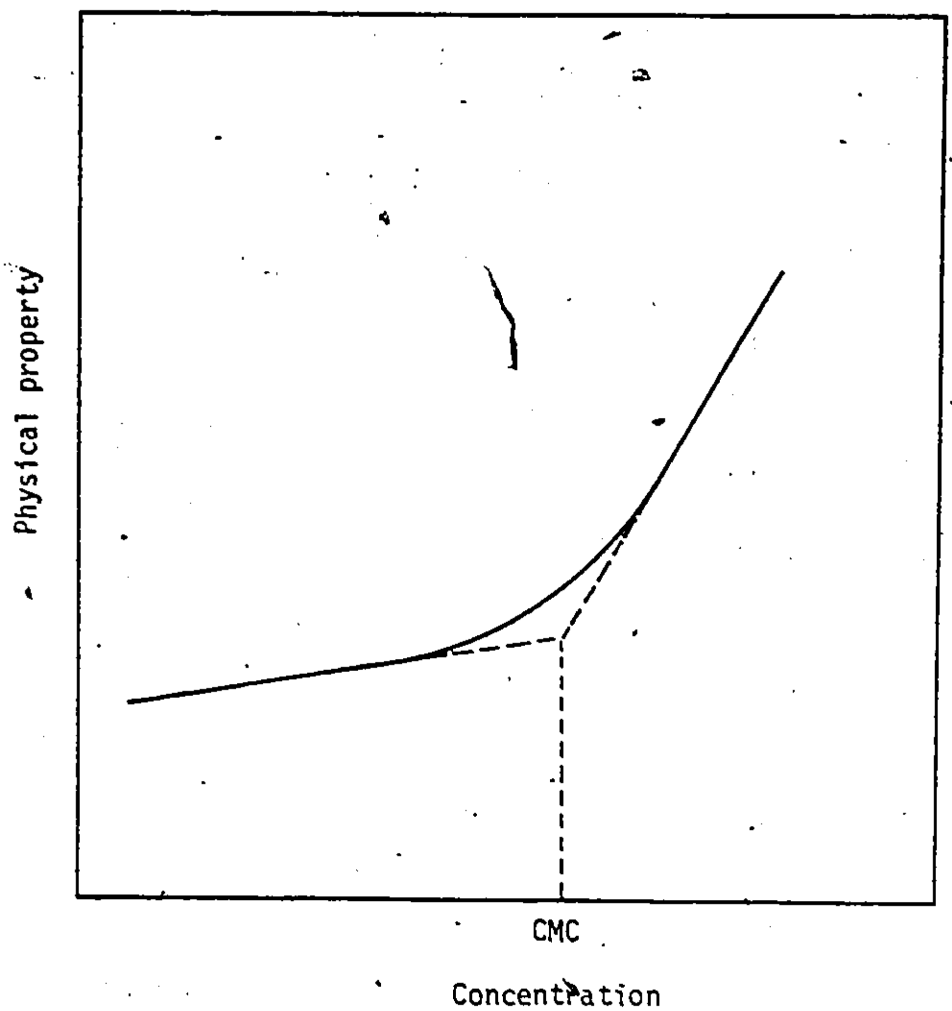

Figure XXX. Determination of the critical micelle concentration (CMC) from the change in a physical property as a function of the concentration of the micelle-forming species.

for $TPPS_4$ was determined in various media (0.1 M ionic strength) and the results are shown in Table 10. A range of concentrations (~ 10%) is

TABLE 10

Critical Micelle Concentration of Protonated $TPPS_4$ in Various Media

pH = 3.46 ± 0.02 μ = 0.100 M

T = 25.0°C λ = 435 and 491 nm

ANION PRESENT	CMC (M x 10^6)
Chloride	2.8 - 2.9
Nitrate	4.4 - 4.6
Formate	4.1 - 4.5
Acetate	14.8 - 15.2
Trichloroacetate	6.7 - 7.1
Lauryl sulphate	7.2 - 7.8
p-Toluene sulphonate	9.0 - 9.8
2-Naphthalene sulphonate	58.5 - 64.0

given for the CMC in each medium to reflect the fact that the 435- and 491-nm bands yielded different values. This range can be made narrower by the use of more extensive data, but the values obtained were considered sufficiently precise to note any trends. It was found that, in general, the CMC increased with the bulkiness of the anion. In conjunction with the strong interaction of the aromatic π-systems, as indicated by the intense 491-nm band, these findings suggest that a possible structure for the micellar species would involve stacking of the porphyrins parallel to each other with anions trapped between porphyrin molecules, perhaps even serving as bridging species. Qualitatively, this "stacking" model resembles that generally accepted for the non-protonated species.

The strong interactions with the anions would allow close approach of adjacent molecules by reducing the repulsion between the protonated centres.* Another possible structure is that the porphyrin molecules are stacked with their planes parallel, as above, but with adjacent molecules displaced horizontally relative to each other so that the sulphonate group of one porphyrin molecule interacts with the protonated centre of the next. In this model, other anions present in the medium would compete with the sulphonate groups for interaction with the positively-charged porphyrin centre. The binding power of acetate (greater basicity) and the high value of the CMC in this medium are consistent with this model. Such a model would also explain the apparent need for oppositely charged groups at the periphery and centre in order to observe micellization.

In the present work, it was found that increasing the ionic strength or the concentration of porphyrin resulted in increased aggregation with a concommittant increase in the intensity of the 491- and 708-nm bands. At intermediate levels of ionic strength or concentration, Tyndall phenomena became apparent. At still higher levels, a visible precipitate gradually formed with a simultaneous decrease in and eventual disappearance of the

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* The high CMC value for acetate, which indicates weaker micellar interaction, is difficult to rationalize on the basis of this simple model.

491- and 708-nm bands. The rate of micellization was found to vary with the identity of the anion but this was not investigated in detail. In general, the above observations are in agreement with those reported by other workers (25,86,89,92) for sulphonated TPP derivatives in acidic media.

Because of the intensity of the Soret bands and the limit to which the cell pathlength can be reduced (0.1 mm) and still be known with reasonable accuracy, the upper concentration range for the study of aggregation by spectrophotometric means is about 5×10^{-4} M. Measurement of less intense bands allows the use of higher concentrations but the difference in the molar absorptivities of the monomer and aggregate is frequently much smaller, thereby decreasing the precision of measurements. Since at higher concentrations (in neutral solutions), the possibility of species larger than dimers exists, other experimental methods were sought. ^{1}Hmr spectroscopy proved to be most appropriate for studies at the higher concentrations, as described in the following section.

(b) Nuclear Magnetic Resonance

The qualitative changes which occur in the ^{1}Hmr spectrum of $TPPS_4$ upon aggregation were discussed in Section III.1(b) as part of the characterization of the compound. The present section describes the quantitative aspects of these changes. The study was undertaken to learn more about the changes which occur in solution at concentrations greater than those accessible by spectrophotometry.

Quantitative ^{1}Hmr studies of porphyrin aggregation are rendered more difficult by the need to distinguish those spectral changes which occur as a result of aggregation from those due to interactions between the porphyrin and the solvent and/or the internal reference compound (76,79). The study by Hand and Cohen (157) illustrates the importance of interactions in aqueous solutions. This study showed that coaggregation between aromatic compounds and other organic species may occur, resulting in concentration-dependent upfield shifts of the ^{1}Hmr signals. This effect is especially significant for DSS (sodium 2,2-dimethyl-2-silapentane-5-sulphonate), a frequently used internal reference in aqueous solution. In the present work, the chemical shifts (relative to DSS as an <u>external</u> reference) of methanol, acetone, tetramethylammonium chloride and DSS were measured as a function of $TPPS_4$ concentration (6×10^{-3} to 1×10^{-1} M). Bulk diamagnetic changes resulting from the addition of each compound to D_2O would cause an equal change in the chemical shifts of all the signals relative to an external reference. As the HOD peak did not shift within experimental error, the contribution to the chemical shift changes of the other signals due to diamagnetic susceptibility changes were probably not large. Another contribution to chemical shift changes which must be considered is that due to the diamagnetic anisotropy of the aromatic porphyrin ring system. If no specific interactions occurred between the porphyrin and any other molecules present, this diamagnetic anisotropy would cause a general upfield shift of all the signals due to random averaging of the porphyrin ring-current over the sample volume. Abraham <u>et al.</u> (76) showed that when the effects of bulk diamagnetic susceptibility changes were small, the change in chemical shift of the solvent signal with porphyrin concentration could be used to estimate the effect of random averaging of the porphyrin ring-current. Thus, any change in the chemical shifts of the porphyrin signals in excess of these two effects (bulk diamagnetic susceptibility changes and random averaging of the ring current) could be attributed to aggregation of the porphyrin molecules (provided that the internal reference showed no specific interactions with the other sample components). Good agreement was found between the upfield shift of the solvent peak ($CHCl_3$) and the value calculated (0.14 ppm for 0.12 M solutions of coproporphyrin tetramethyl ester) for random averaging of the porphyrin ring current over the sample volume. $TPPS_4$ would be expected to produce somewhat greater shifts for corresponding concentrations because of the presence of the phenyl groups. In aqueous solution, however, this effect would be more than offset by the fact that the mole fraction of porphyrin is much less (by a factor of 4.43) than in chloroform solutions (the magnitudes of the shifts vary with the mole fraction of porphyrin present). Indeed, in the present work, the change in chemical shift of the solvent (HOD) signal did not appear to exceed the experimental error. This error was large (~ 0.02 ppm) because of the sensitivity of the signal to changes in hydrogen bonding which occur as a result of small temperature fluctuations and the addition of any solutes. The signals of all compounds tested as internal references exhibited upfield shifts with increasing porphyrin concentration in excess of any shift of the HOD signal. This suggests that specific interactions occur between these compounds and $TPPS_4$. These shifts were especially large for DSS (0.5 ppm in the presence of 0.1 M $TPPS_4$), a frequently used internal reference for aqueous solutions.

Because of the above problems, the absolute shifts of the phenyl ring protons could not be used to monitor aggregation. Rather, the <u>difference</u> in chemical shift between the phenyl protons ortho and meta to the porphyrin ring was used. This procedure effectively eliminated the above effects and provided a direct measure of the extent of aggregation. The data was fitted to the equation:

$$\Delta\delta_{OBS} = (\Delta\delta_M\,[\text{MONOMER}] + 2\Delta\delta_D\,[\text{DIMER}] + 4\Delta\delta_{TT}\,[\text{TETRAMER}])/C_o \qquad [7]$$

where $\Delta\delta_{OBS}$ = observed difference in chemical shifts, (δH(meta) - δH(ortho))

$\Delta\delta_M$ = (δH(meta) - δH(ortho)) for the monomer

$\Delta\delta_D$ = (δH(meta) - δH(ortho)) for the dimer

$\Delta\delta_{TT}$ = (δH(meta) - δH(ortho)) for the tetramer

C_o = analytical concentration of $TPPS_4$ in moles/litre

This equation and its variations are derived in Appendix II(b). The equation was varied by the deletion or addition of terms depending on whether the monomer and dimer only or the trimer and tetramer as well were being considered. The equation resembles those generally used in aggregation studies (e.g., 76) except that the chemical shift of a given proton has been replaced by the <u>difference</u> in chemical shifts (δH(meta) - δH(ortho)). The concentrations of the various species were obtained by solution of the appropriate equilibrium and mass balance equations. The Newton-Raphson method (e.g., 158) was used to solve higher-order equations. An example of the calculations involved is given in Appendix II(c). The value of K_D (phosphate buffer) obtained from the spectrophotometric studies was used in these calculations, together with the non-linear least-squares computer program, NLWOOD.

The ^{1}Hmr data for potassium nitrate, phosphate and sodium acetate media were fitted to equations similar to equation [7]. Three models were considered based on the assumptions that the main species in solution are (i) monomers and dimers; (ii) monomers, dimers and trimers; and, (iii) monomers, dimers and tetramers. Although a mathematically-good fit does not necessarily reflect physical reality, data-fitting can indicate which model among several will correspond most closely to experimental observations to provide a reasonable explanation of the data.

In the simplest model, it is assumed that only the monomeric and dimeric species are present in significant amounts and that the same equilibrium prevails as observed in the low-concentration spectrophotometric studies. This model was applied both with K_D fixed (value from spectrophotometry) and with it as a variable. In each situation, a very poor fit of the data resulted.

The models involving higher aggregates were tested similarly. The use of a trimer as a major species at higher concentrations provided an improved fit but was found unsuitable for two reasons. ~~First~~, the values of the various parameters had a very poor precision (i.e., the 95% confidence limits were frequently several times larger than the value itself). Second, the presence of a trimer as a major species required that $\Delta\delta_{TR}$ be $\geq$ 10 ppm. Not only is this value intrinsically too large, but it is unreasonable in comparison with the $\Delta\delta_D$ value of 0.6 ppm obtained simultaneously.

The model involving simultaneous equilibria between the monomer, dimer and tetramer yielded the best fit of the data (Figure XXXI). Also, values of the various parameters were physically reasonable. A more complex model involving all species including the trimer could not be adequately tested because of the limited number of data points. The much larger number of data points needed for such a complex model was considered to require a prohibitive quantity of the porphyrin. Also, because of the large value of K_D (15,800 M^{-1}), the equilibrium concentration of monomer would be relatively small. Consequently, the concentration of trimer would be even smaller and would result in only a minor contribution in data fitting.

Thus, the most reasonable model to account for the experimental data involves two main equilibria:

$$2\ \text{MONOMER} \xrightleftharpoons{K_D} \text{DIMER}$$

$$2\ \text{DIMER} \xrightleftharpoons{K_{TT}} \text{TETRAMER}$$

The results obtained using this model are shown in Table 11. K_{TT} is not a true equilibrium constant because the ionic strength could not be maintained constant at the high concentration levels of $TPPS_4$. The variation in ionic strength arises from the contribution of $TPPS_4$ itself. The use of a background electrolyte to minimize this variation was not effective because the solubility of $TPPS_4$ was greatly reduced. Thus, K_{TT} must be

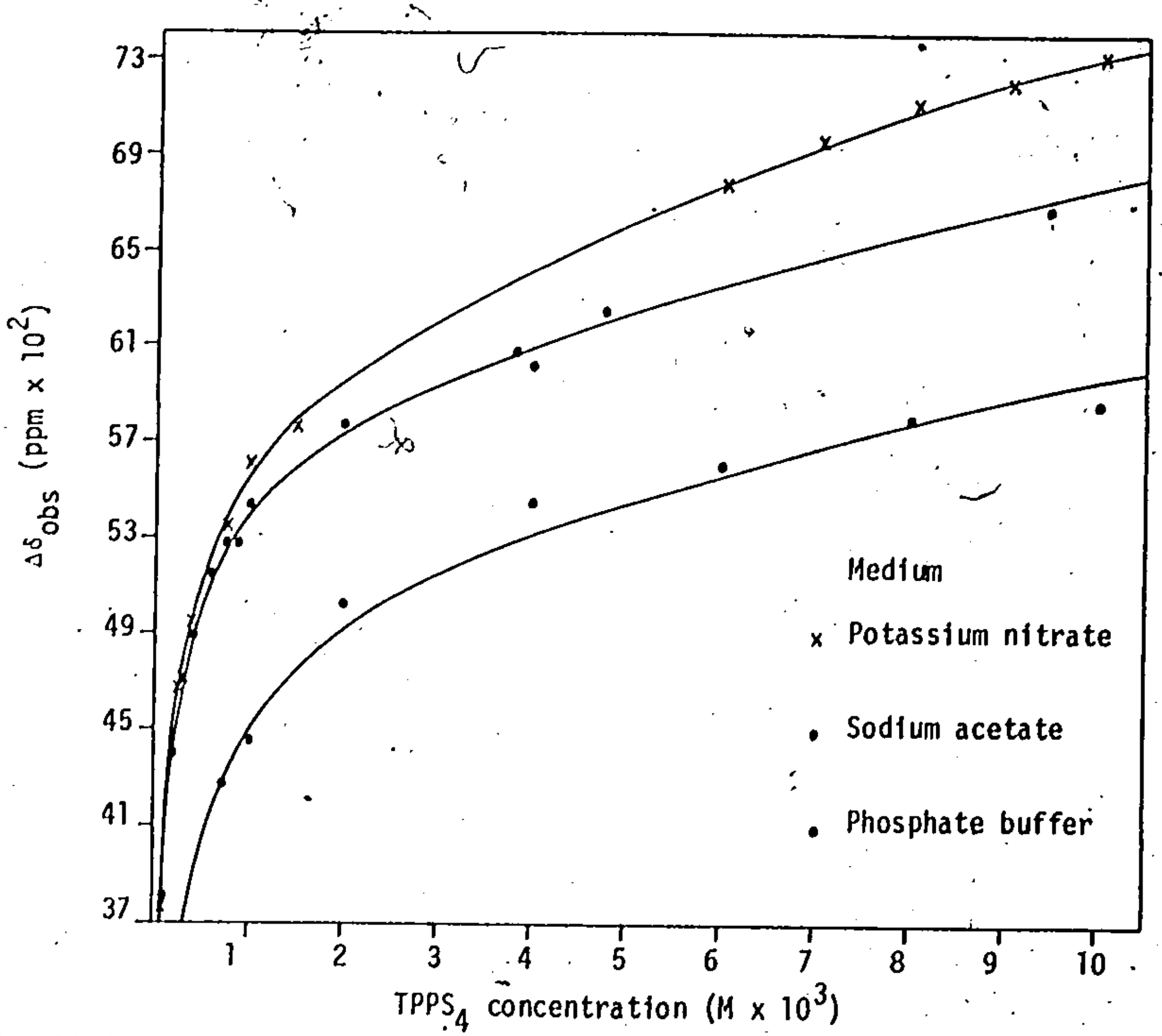

Figure XXXI. Difference in chemical shifts, (δH(meta) - δH(ortho)) as a function of the analytical concentration of $TPPS_4$ in three media. The solid lines are calculated based on the model involving monomer, dimer and tetramer and the values of the parameters listed in Table 11.

TABLE 11

NMR Study of Aggregation

T = 25.0°C

R = number of replicate experiments

N = total number of data points

CR = concentration range

$\pm$ values = 95% confidence limits

MEDIUM	Potassium nitrate	Phosphate buffer	Sodium acetate
R	2	1	2
N	17	10	24
CR (M)	$5.02 \times 10^{-5} - 5.02 \times 10^{-2}$	$1.00 \times 10^{-5} - 1.00 \times 10^{-1}$	$9.86 \times 10^{-5} - 9.46 \times 10^{-2}$
$\Delta\delta_M$ (ppm)	0.01 ± 0.02	-0.14 ± 0.03*	0.05 ± 0.02
$\Delta\delta_D$ (ppm)	0.647 ± 0.008	0.56 ± 0.02	0.626 ± 0.006
$\Delta\delta_{TT}$ (ppm)	1.77 ± 0.11	1.91 ± 0.62	3.21 ± 0.27
K_{TT}	15 ± 3	7 ± 6	3.7 ± 0.6

* The negative value of δ_{MON} indicates that crossover has occurred, i.e., the ortho phenyl signal is to low field of the meta phenyl signal whereas it is usually to high field.

regarded as a useful empirical parameter valid only for the conditions defined and having only qualitative significance.

Table 11 and Figure XXXI indicate that definite medium effects are detectable via ^{1}Hmr. Because of the instability of the higher aggregates, it is not surprising that changes in the supporting medium can have significant effects. This observation shows that extreme care must be exercised in the interpretation of any ^{1}Hmr studies involving $TPPS_4$.

For accurate interpretation of porphyrin complexation chemistry and knowledgeable application to analytical chemistry, an understanding of the aggregative behaviour of the free ligand and of its complexes is imperative. For example, aggregation of the free ligand affects its concentration in solution and, depending on the relative value of K_D and the metal-complex stability constants, quantitative complexation may or may not be achieved. Similarly, aggregation of the metalloporphyrins would cause deviation from the Beer-Lambert law in spectrophotometric determinations and could affect values of distribution ratios in separation processes. Thus, knowledge of the extent of aggregation and of values such as K_D are crucial and accounts for the emphasis placed on such studies in this thesis. The spectrophotometric studies have provided values of K_D for $TPPS_4$ and two metal complexes. These studies have also shown that at low concentrations below about 3×10^{-6} M, the ligand exists essentially as the free monomer (in non-acidic solutions); above this concentration level, dimerization occurs and its possible significance must be assessed for a given reaction. Similarly, for solutions of $CuTPPS_4$ and $ZnTPPS_4$, aggregation of the complexes cannot be ignored above concentrations of about 5×10^{-7} and 2×10^{-4} M; respectively. The ^{1}Hmr work extended the studies to more concentrated solution (e.g., to 0.1 M) and revealed that aggregation was severe in D_2O solutions at these high concentrations. In the non-aqueous solvent, DMSO-d_6, aggregation was considerably less pronounced even at the higher concentrations.

The spectrophotometric studies also showed that aggregation of protonated $TPPS_4$ is considerably more complex than that of the non-protonated ligand or the metal complexes since the process of aggregation is highly medium-dependent. This behaviour was considered especially interesting because of its possible influence on the kinetics of metallation of $TPPS_4$ in acidic medium. The results of studies in this area are discussed in the following section.

III.3 Kinetics of Metallation

The kinetics of metallation of $TPPS_4$ in neutral solution have been previously studied (99,102,159). In the present work, metallation in acidic medium (as exemplified by Cu(II) incorporation) was investigated. It has been shown that aggregates of non-protonated water-soluble porphyrins (including $TPPS_3$) dissociate rapidly (91,92,160). Therefore, these aggregates should not play a major role in the mechanism of metallation, apart from dissociating to provide more free monomer for metallation. Indeed, a study by Das (161) showed that dimerization of a water-soluble porphyrin has no effect on the rate of Cu(II) or Zn(II) incorporation. In acidic medium, aggregation is more extensive and its effects on the kinetics of metallation have not been previously examined. With $TPPS_4$, the kinetics of metal-ion incorporation in acidic medium is of special significance because the selectivity of $TPPS_4$ as an analytical reagent is greatest at lower pH values. In this study, the reaction rates in acidic medium were found to be conveniently slower than in neutral medium and could be measured by routine spectrophotometric means.

The kinetics of metallation were studied initially in the absence of aggregation by maintaining the porphyrin concentration below 3×10^{-6} M. Figure XXVIII shows the variation in absorbance at 435- and 491-nm with the analytical concentration of $TPPS_4$ and suggests that aggregation becomes significant above 3×10^{-6} M.

Derivations of the equations used in these studies may be found in standard textbooks (e.g:, 162,163). For a reaction between two substances, X and B, which is first-order with respect to one of the components, (X), (i.e., rate = $k[X][B]^n$), it is readily shown that under pseudo-first-order conditions (i.e., $[B]/[X] \geq 20$):

$$-d[X]/dt = k'[X] \quad \text{where} \quad k' = k[B]^n \qquad [8]$$

Integration yields:

$$\log [X] = -k't/2.303 + \log [X]_0 \qquad [9]$$

If the reaction is followed spectrophotometrically by measuring the absorbance of a species which follows the Beer-Lambert law, an alternate expression can be derived:

$$\log (A_\infty - A_t) = -k't/2.303 + \log (A_\infty - A_0) \qquad [10]$$

A plot of $\log (A_\infty - A_t)$ versus time will yield a straight line with slope

$-k'/2.303$ and intercept $\log [A_\infty - A_0]$. If the reaction is first-order in X, variation of the initial concentration of X will give a series of parallel lines. In the reaction between Cu(II) and $TPPS_4$, the initial concentration of Cu(II) was always at least twenty times that of $TPPS_4$. Also, the absorbance of $TPPS_4$ at 435-nm obeys the Beer-Lambert law at concentrations below 3×10^{-6} M. Thus, Figure XXXII indicates that the reaction is first-order with respect to $TPPS_4$.

A series of values for k' may be obtained by variation of the concentration of B (Cu(II)), still under pseudo-first-order conditions. From [8],

$$\log k' = \log k + n \log [B] \qquad [11]$$

and a plot of log k' versus log [B] will give a straight line whose slope, n, will be equal to the order of the reaction with respect to B. Figure XXXIII ($n = 1.00 \pm 0.04$) shows that the reaction between Cu(II) and $TPPS_4$ is first-order with respect to Cu(II). The finding that the reaction in acid solution at low concentrations of $TPPS_4$ is first-order with respect to both Cu(II) and $TPPS_4$ is in keeping with results obtained at a higher pH and concentration (99) and with studies on Zn(II) incorporation (102, 159).

An attempt was made to determine whether $Cu(H_2O)_6^{2+}$ or $Cu(H_2O)_5HCOO^+$ was the reactive species. These species (henceforth referred to simply as Cu(II) and $CuHCOO^+$) are the main ones present under the experimental conditions. The concentrations of $TPPS_4$ and total Cu(II) ($Cu(II)_T$) were held constant while the total formate concentration was varied. In general, the rate of reaction increased only modestly (about a 50% increase in k')

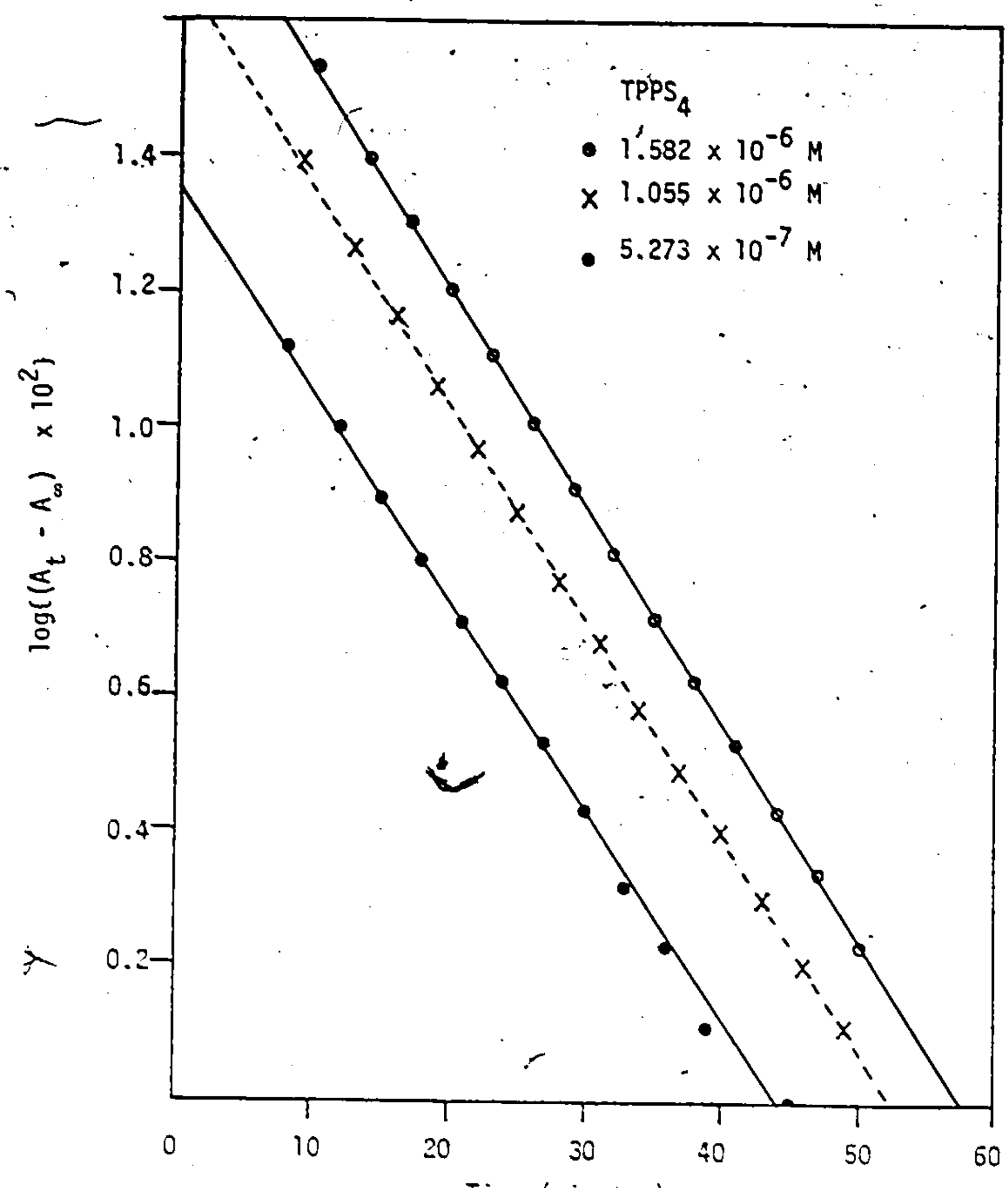

Figure XXXII. Incorporation of $Cu(II)_T$ into $TPPS_4$ at various concentrations of monomeric $TPPS_4$. $[Cu(II)_T] = 1.054 \times 10^{-3}$ M; $[formate]_T = 9.92 \times 10^{-3}$ M; pH = 3.55; λ = 434 nm.

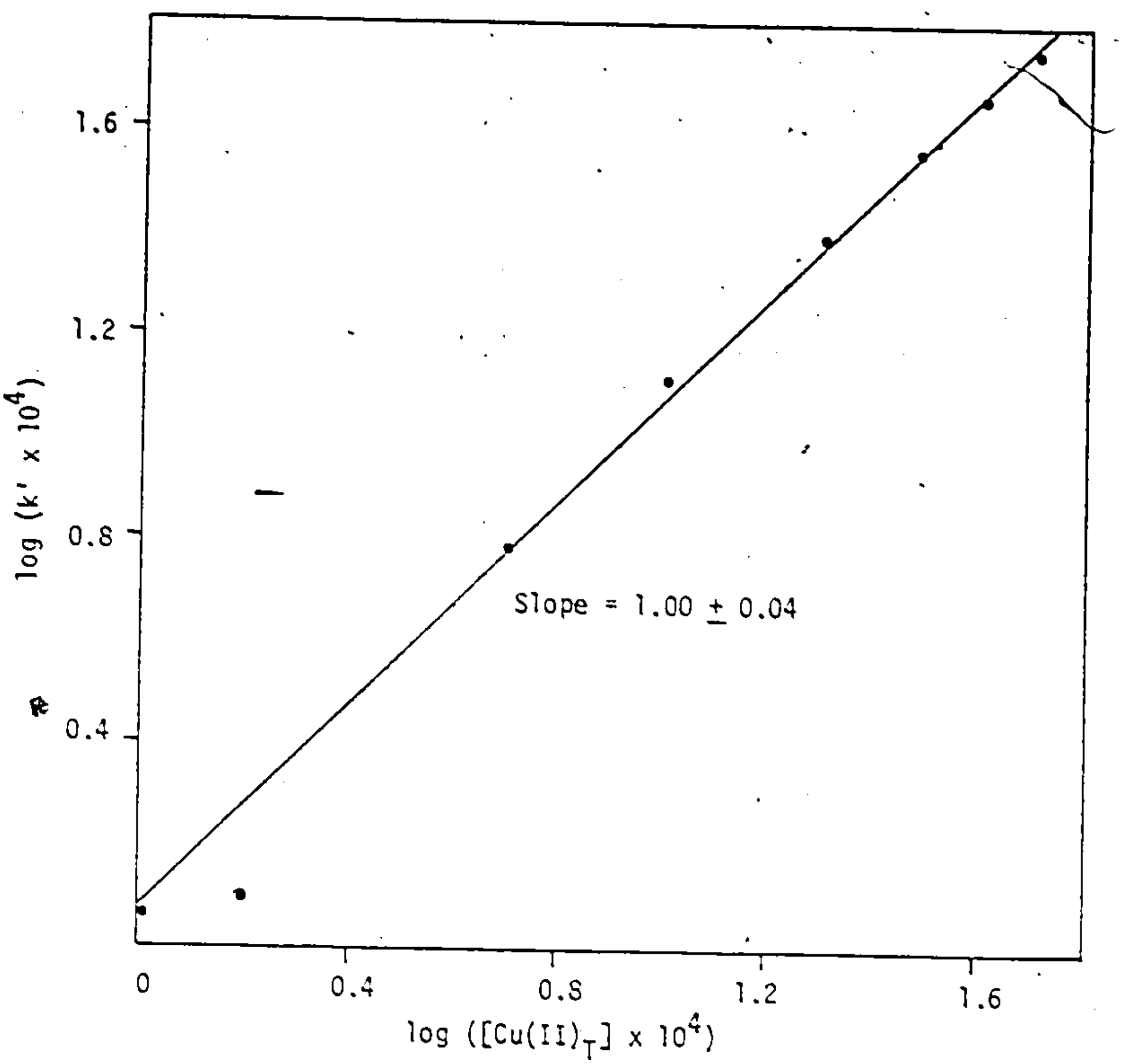

Figure XXXIII. Determination of the order of reaction with respect to $[Cu(II)_T]$. $[TPPS_4]$ = 2.00 x 10^{-6} M; $[formate]_T$ = 9.92 x 10^{-3} M; pH = 3.55; λ = 434 nm.

from zero to 0.20 M total formate (Figure XXXIV), which suggests that both species are important in the metallation of $TPPS_4$. This result is consistent with previous studies showing that some carboxylic acids accelerate porphyrin metallation (116-118,164). Hambright et al. (99) found that the rate of $Cu(II)_T$ incorporation into $TPPS_4$ in acetate buffer was dependent upon the acetate concentration at low pH (where a significant portion of the porphyrin is protonated). At higher pH (where the major porphyrin species is H_2TPPS_4*), no acetate dependence was observed, indicating that Cu(II) and $CuCH_3COO^+$ reacted with H_2TPPS_4 at the same rate. They suggested that the acetate dependence at lower pH was due to a direct reaction with $H_4TPPS_4^{2+}$ (porphyrin dications are usually considered to be unreactive). In the present study, the work of Hambright et al. was extended by investigating the formate dependence in detail at a fixed pH (3.55). Lower concentrations of $TPPS_4$ were used to eliminate the effects of aggregation.

At low $TPPS_4$ concentrations, the following reactions can be proposed consistent with the results of the present study and that of Hambright et al.:

$$HCOOH \xrightleftharpoons{K_a} HCOO^- + H^+ \qquad [12]$$

$$Cu(II) + HCOO^- \xrightleftharpoons{K_f} CuHCOO^+ \qquad [13]$$

$$H_4TPPS_4^{4+} \xrightleftharpoons{K_3K_4} H_2TPPS_4 + 2H^+ \qquad [14]$$

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* H_2TPPS_4 and $H_4TPPS_4^{2+}$ are used here to distinguish between the non-protonated porphyrin and the porphyrin dication, respectively. The charges associated with the sulphonate groups at the periphery of the molecule have been ignored.

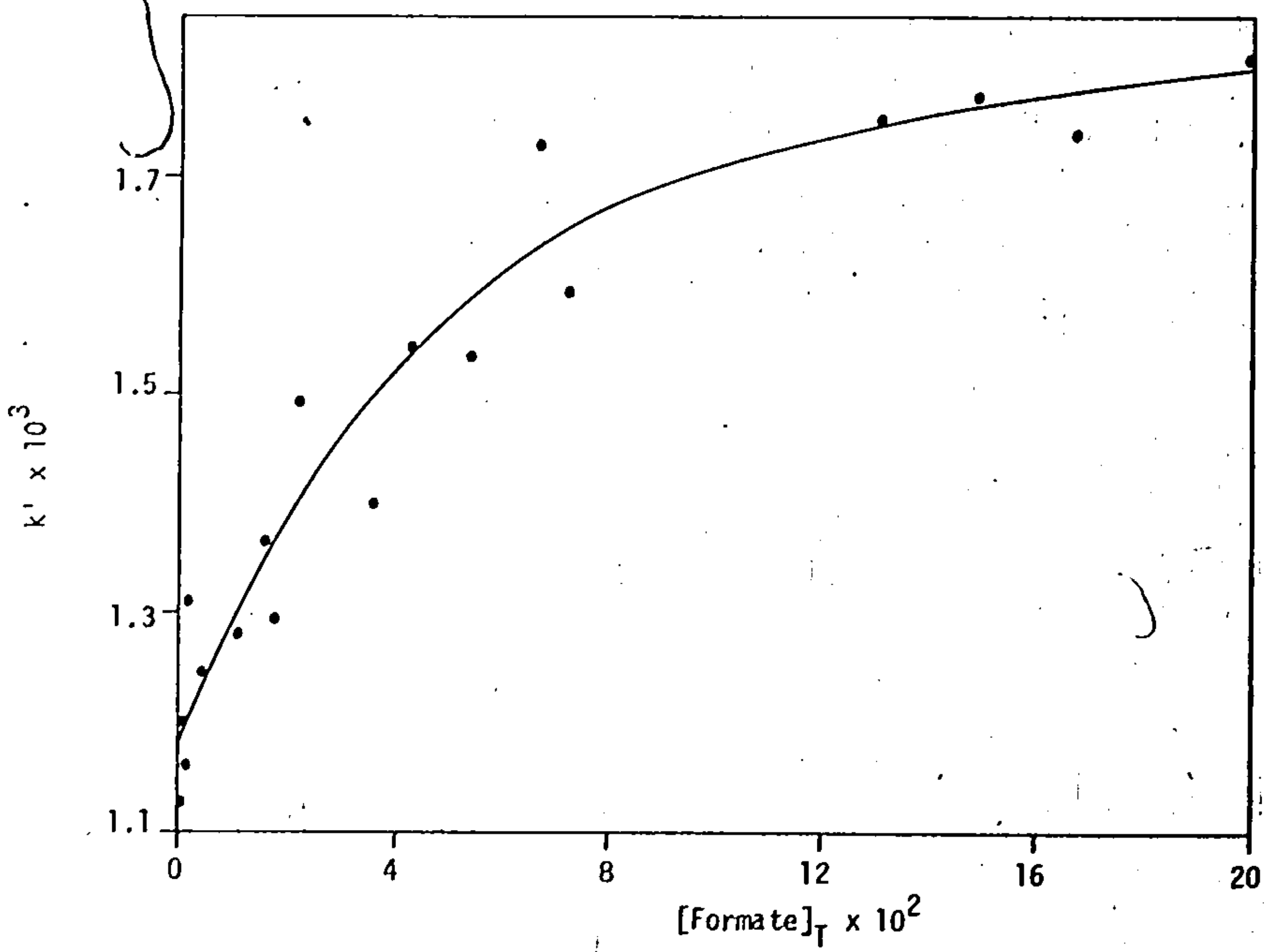

Figure XXXIV. Variation of the observed rate constant as a function of the total formate concentration. $[Cu(II)_T] = 1.118 \times 10^{-3}$ M; $[TPPS_4] = 2.00 \times 10^{-6}$ M; pH = 3.55

$$Cu(II) + H_2TPPS_4 \xrightleftharpoons{k_1} CuTPPS_4 + 2H^+ \qquad [15]$$

$$CuHCOO^+ + H_2TPPS_4 \xrightleftharpoons{k_2} CuTPPS_4 + 2H^+ + HCOO^- \qquad [16]$$

$$Cu(II) + HCOO^- + H_4TPPS_4 \xrightleftharpoons{k_3} CuTPPS_4 + 4H^+ + HCOO^- \qquad [17]$$

$$CuHCOO^+ + H_4TPPS_4^{2+} \xrightleftharpoons{k_4} CuTPPS_4 + 4H^+ + HCOO^- \qquad [18]$$

Reactions [12] - [14] come to equilibrium much faster than reactions [15] - [18]. The reverse reactions for [15] - [18] are so much slower than the forward reactions that they can be considered essentially irreversible. Therefore:

$$d[CuTPPS_4]/dt = k_1[Cu(II)][H_2TPPS_4] + k_2[CuHCOO^+][H_2TPPS_4] + k_3[Cu(II)][H_4TPPS_4^{2+}][HCOO^-] + k_4[CuHCOO^+][H_4TPPS_4^{2+}] \qquad [19]$$

From the rapid prior equilibria, [12] - [14], the following relationships may be obtained:

$$[H_2TPPS_4] = K_3K_4[H_4TPPS_4^{2+}]/[H^+]^2 \qquad [20]$$

$$[Cu(II)] = [Cu(II)_T]/(1 + K_f[HCOO^-]) \qquad [21]$$

$$[CuHCOO^+] = K_f[Cu(II)_T][HCOO^-]/(1 + K_f[HCOO^-]) \qquad [22]$$

Substitution of these terms into equation [19] yields the equivalent expression:

$$d[CuTPPS_4]/dt = -d[H_4TPPS_4^{2+}]/dt = \{[H_4TPPS_4^{2+}]/(1 + K_f[HCOO^-])\}\cdot\{k_1K_3K_4[Cu(II)_T]/[H^+]^2 + k_2K_3K_4K_f[Cu(II)_T][HCOO^-]/[H^+]^2 + k_3[Cu(II)_T][HCOO^-] + k_4K_f[Cu(II)_T][HCOO^-]\} \qquad [23]$$

Equation [23] is consistent with the observation that the reaction is first-order with respect to both $[H_4TPPS_4^{2+}]$ and $[Cu(II)_T]$.

Equation [23] also shows that $k'(k_{observed})$ should vary non-linearly as a function of $[HCOO^-]$, but that $k'(1 + K_f[HCOO^-])$ versus $[HCOO^-]$ should be linear. The results obtained, based on $K_f = 100$ (1,2), are shown in Figure XXXV. The slope is equal to $(k_2K_3K_4K_f[Cu(II)_T]/[H^+]^2 + k_3[Cu(II)_T] + k_4K_f[Cu(II)_T])$, and the intercept corresponds to $k_1K_3K_4[Cu(II)_T]/[H^+]^2$. Substitution of values for the various parameters allows an estimation of the rate constants. Thus, for $K_3K_4 = 10^{-9.10}$ (99), $[Cu(II)_T] = 1.118 \times 10^{-3}$ M and $[H^+] = 2.82 \times 10^{-4}$ M, k_1 was found to be 93 ± 12 M^{-1} sec^{-1} (from the intercept). In acetate buffer (99), the value of k_1 was comparable (161.1 ± 2.9 M^{-1} sec^{-1}). Since [17] and [18] are not distinguishable experimentally, $k_3[Cu(II)_T] + k_4K_f[Cu(II)_T]$ may be combined to form $k_5[Cu(II)_T]$. Assuming that $k_1 = k_2$, as was found in acetate buffer (99), k_5 can be obtained from the slope and was found to be 74 ± 13 M^{-1} sec^{-1} (the main error arises from k_1). A much larger value (1886 ± 104 M^{-2} sec^{-1}) was found in acetate buffer. The large difference is difficult to explain and cannot be accounted for on the basis that the assumption (i.e., $k_1 = k_2$) is incorrect.

With regard to ionic strength (μ), the rate of Cu(II) incorporation was found to decrease by a factor of approximately two when μ was varied from 0.02 to 0.42 M. The precision of the results was too poor, however, to define an exact relationship.

At porphyrin concentrations greater than about 3×10^{-6} M (the CMC), very different results were obtained. Figure XXXVI shows results

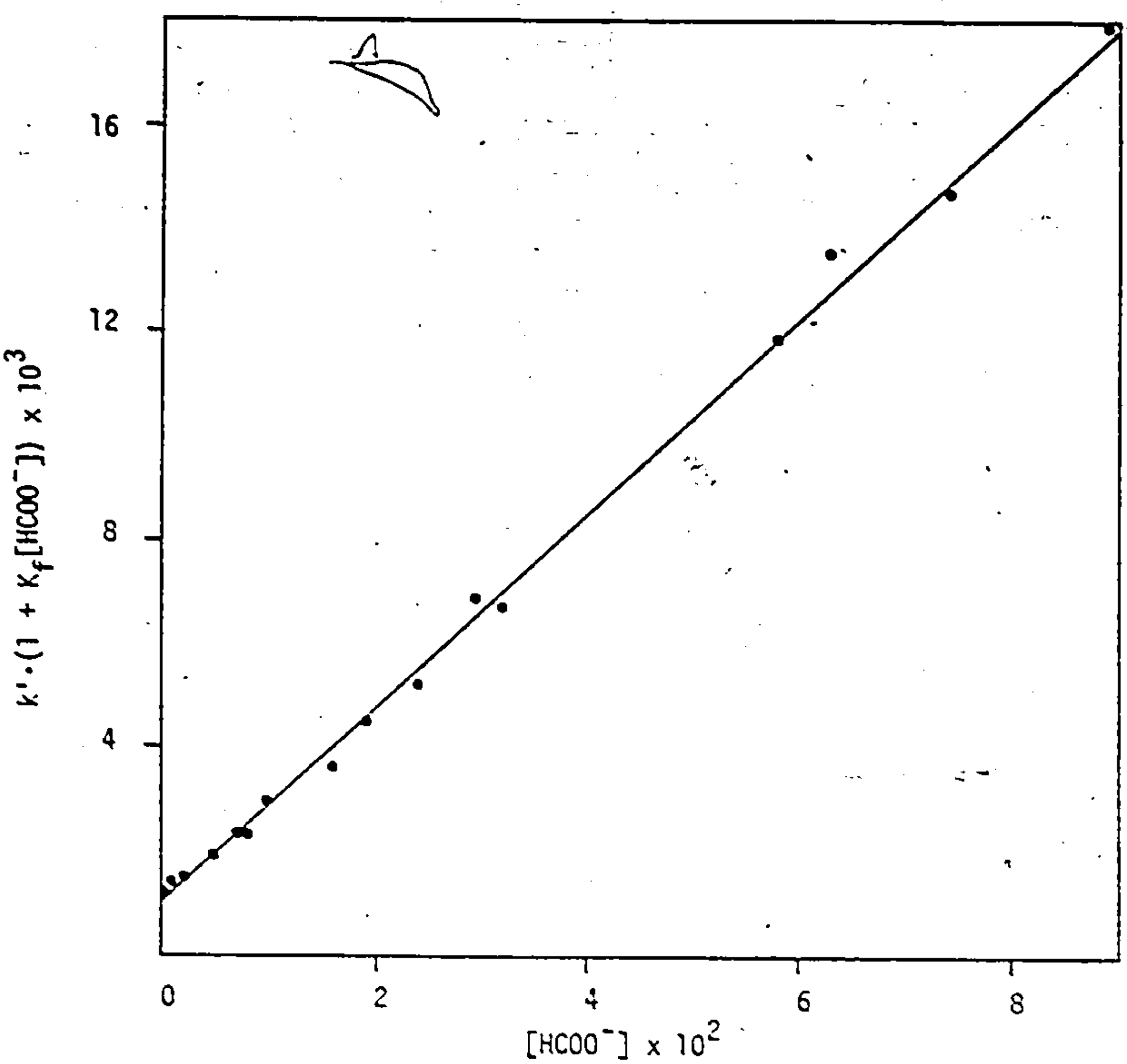

Figure XXXV. Variation of $k'(1 + K_f[HCOO^-])$ as a function of $[HCOO^-]$. $[Cu(II)_T] = 1.118 \times 10^{-3}$ M; $[TPPS_4] = 2.00 \times 10^{-6}$ M; pH = 3.55. Calculations based on data shown in Figure XXXIV with $K_f = 100$ and $K_a = 2.288 \times 10^{-4}$.

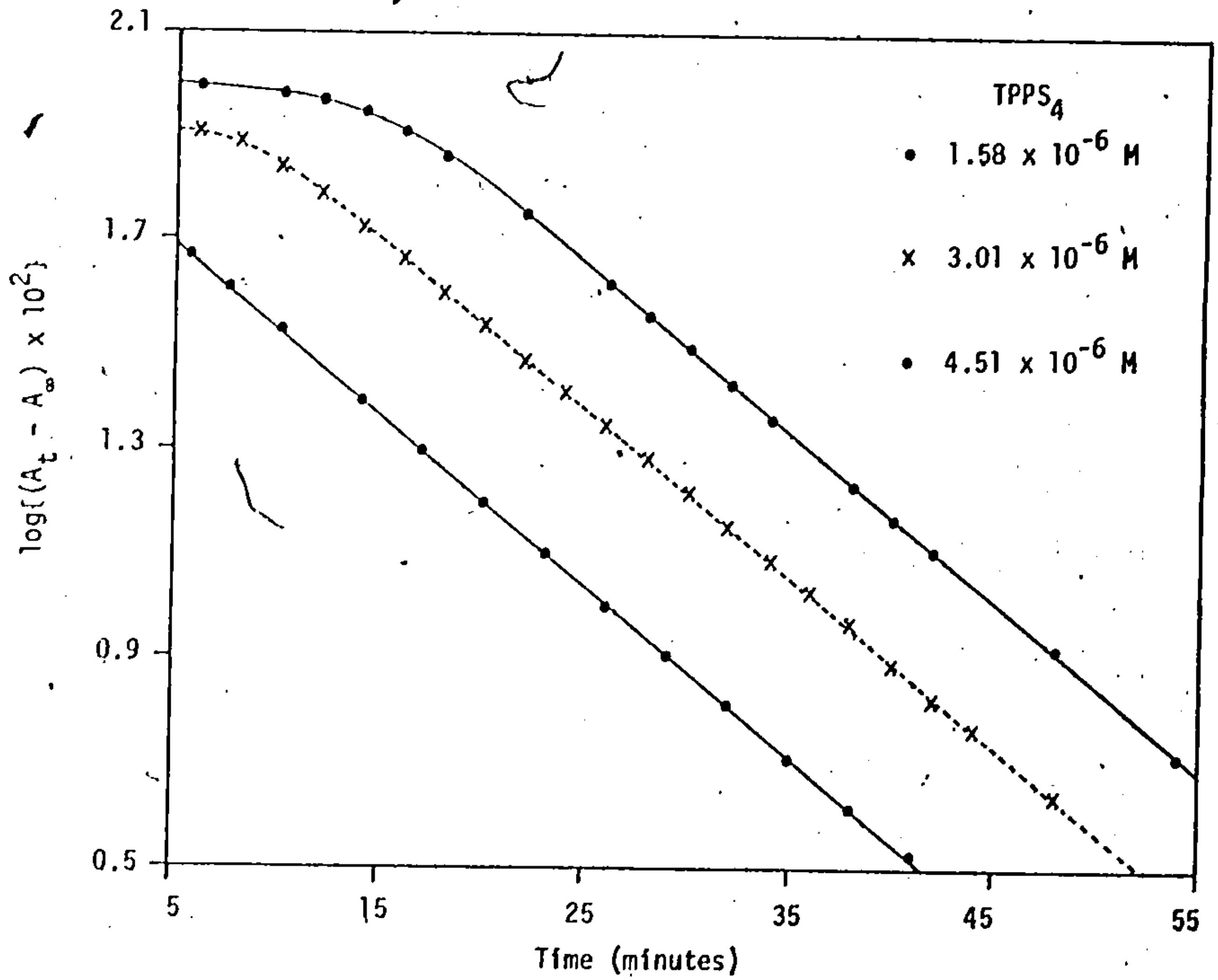

Figure XXXVI. Incorporation of $Cu(II)_T$ into $TPPS_4$ which is monomeric (1.58×10^{-6} M) or initially aggregated ($\geq 3 \times 10^{-6}$ M). $[Cu(II)_T] = 1.055 \times 10^{-3}$ M; $[formate]_T = 9.92 \times 10^{-3}$ M; pH = 3.55; λ = 434 nm.

for concentrations above and below the CMC (all other conditions are the same). At porphyrin concentrations greater than the CMC, the reaction is distinctly slower initially. The data corresponded to no simple order for the reaction over its entire duration. Thus, the presence of ~~at~~ least one other process in addition to the metallation reaction was indicated. Because the only change in conditions was the increased concentration of $TPPS_4$, the additional process was considered to be associated with micellization.

$$(H_4TPPS_4^{2+})_n \rightleftarrows nH_4TPPS_4^{2+} \qquad [24]$$

This additional process would significantly affect the reaction rate until sufficient porphyrin reacted to reduce the analytical concentration below the CMC. Conditions favouring an increase in micellization such as greater analytical concentration of porphyrin or increased ionic strength resulted in an expected prolongation of the initial slow portion of the reaction. In addition, other reactions such as the direct metallation of micelles ([25]) and the formation of micellar complex ([26]) could occur.

$$(H_4TPPS_4^{2+})_n + Cu(II)_T \rightarrow CuTPPS_4 + 2H^+ + (H_4TPPS_4^{2+})_{n-1} \qquad [25]$$

$$(aCu(II)_T \cdot (H_4TPPS_4^{2+})_n) \rightleftarrows aCu(II)_T + (H_4TPPS_4^{2+})_n \qquad [26]$$

Considerable work would be required to elucidate the mechanism under conditions of significant micellization.

In summary, at concentrations of $TPPS_4$ greater than the CMC, the presence of micellar species results in a significant decrease in the rate of metalloporphyrin formation. As the $TPPS_4$ concentration decreases below the CMC during the reaction, the rate increases, eventually reaching a value obtained under monomeric conditions. Thus, in any analytical applications, it is desirable to maintain the concentration of $TPPS_4$ below the CMC in order to obtain a simpler and faster metallation reaction.

III.4 Applications

(a) Reactivity towards metal ions

One of the factors governing the choice of analytical applications of $TPPS_4$ is its selectivity toward metal ions. The tests in neutral solution indicate the general selectivity of $TPPS_4$ (Table 12). Among the ions

TABLE 12

Reactivity of $TPPS_4$ with Metal Ions in Neutral Solution

Metalloporphyrin formed		No observed reaction			
Mn^{2+}	Pd^{2+}	Mg^{2+}	Cr^{3+}	Gd^{3+}	In^{3+}
Fe^{2+}	Ag^{+}	Ca^{2+}	Y^{3+}	Ho^{3+}	
Co^{2+}	Cd^{2+}	Sr^{2+}	La^{3+}	Er^{3+}	
Ni^{2+}	Hg^{2+}	Ba^{2+}	Ce^{3+}	Th^{4+}	
Cu^{2+}	Sn^{2+}	Sc^{3+}	Nd^{3+}	UO_2^{2+}	
Zn^{2+}	Pb^{2+} *	VO^{2+}	Eu^{3+}	Al^{3+}	

* Reaction indefinite.

tested only ten divalent metal ions and Ag(I) reacted. The reaction with Pb(II) was indefinite. These ions can be separated from other divalent ions (e.g., alkaline earths) and all +3 and +4 ions. As noted in Section III.1(c), however, the complexes of $TPPS_4$ with Cd(II), Hg(II) and Pb(II) are unstable and hence their quantitative isolation may prove difficult.

In acid solution, $CdTPPS_4$, $HgTPPS_4$ and $PbTPPS_4$ are dissociated, thereby increasing the selectivity of $TPPS_4$. In addition to thermodynamic (solution stability) factors, the difference in the rates of reaction of $TPPS_4$ with various metal ions is significant in acid solution, thus providing a degree of kinetic selectivity.* Cu(II), Zn(II) and Ag(I) were found to react considerably faster than all other ions tested. Their relative rates were found to be Cu(II) (1.00), Zn(II) (0.013) and Ag (0.0083). The combination of thermodynamic and kinetic factors impart a high degree of selectivity to $TPPS_4$, making it potentially a very valuable analytical reagent, particularly with regard to the separation and preconcentration of metal ions frequently implicated in environmental, marine and physiological studies (e.g., 165-168). Thus, a means of extracting $TPPS_4$ and its complexes was investigated.

(b) Extraction of $TPPS_4$ and its complexes

Tetraphenylarsonium chloride (Ph_4AsCl) has been used in numerous studies (e.g., 169) for the ion-pair extraction of anionic species. Because $TPPS_4$ has four peripheral sulphonate groups, its extraction with Ph_4AsCl seemed promising. Chloroform has been extensively used as a sol-

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* The selectivity displayed by $TPPS_4$ in neutral solution is at least partly kinetically controlled. For example, Fe(III) reacts extremely slowly in neutral solution (37). Once formed, the Fe(III) complex is very stable. One factor contributing to the slow reaction is hydrolysis of Fe(III).

vent for inorganic extractions (169); also, ion-pairs are known to be stabilized by alcohols (169). Therefore, chloroform and 1-pentanol were chosen for initial testing as organic phases. Both $TPPS_4$ and $CuTPPS_4$ (1×10^{-5} M) were found to be readily extracted into chloroform and 1-pentanol at high concentrations (5×10^{-2} M) of Ph_4AsCl. Qualitatively, they were more soluble in 1-pentanol. Upon addition of HCl or $HClO_4$ to the aqueous phase, phase separation was much slower and incomplete. A white precipitate, especially voluminous with $HClO_4$ (possibly Ph_4AsClO_4), was formed and concentrated mainly at the interface of the two phases. No precipitate was observed with HNO_3. In acid solution, the $TPPS_4$ was protonated (indicated by a colour change from reddish-purple to green) and was concentrated in the aqueous phase.

The very presence of $TPPS_4$ itself was found to adversely affect the phase separation. However, the addition of KNO_3 increased the rate of phase separation except at high concentrations (> 1 M), where poor separation again occurred. (KNO_3 improved phase separation more than an equivalent amount of HNO_3.) In all tests, much poorer phase separation occurred with chloroform and its use was therefore terminated.

In 1-pentanol, no significant extraction of porphyrin (1.8×10^{-6} M) was found in the pH range 1.5 to 7.0 using 2×10^{-4} M Ph_4AsCl. At 1×10^{-2} M Ph_4AsCl, extraction of $TPPS_4$ occurred and the results are shown in Figure XXXVII. In 1 M HNO_3, the extraction behaviour was the same as at pH 1.5.

The extraction of $CuTPPS_4$ was found to be independent of pH from 0 to 7 at 1×10^{-2} M Ph_4AsCl. As with $TPPS_4$, no significant extraction of $CuTPPS_4$ was found using 2×10^{-4} M Ph_4AsCl.

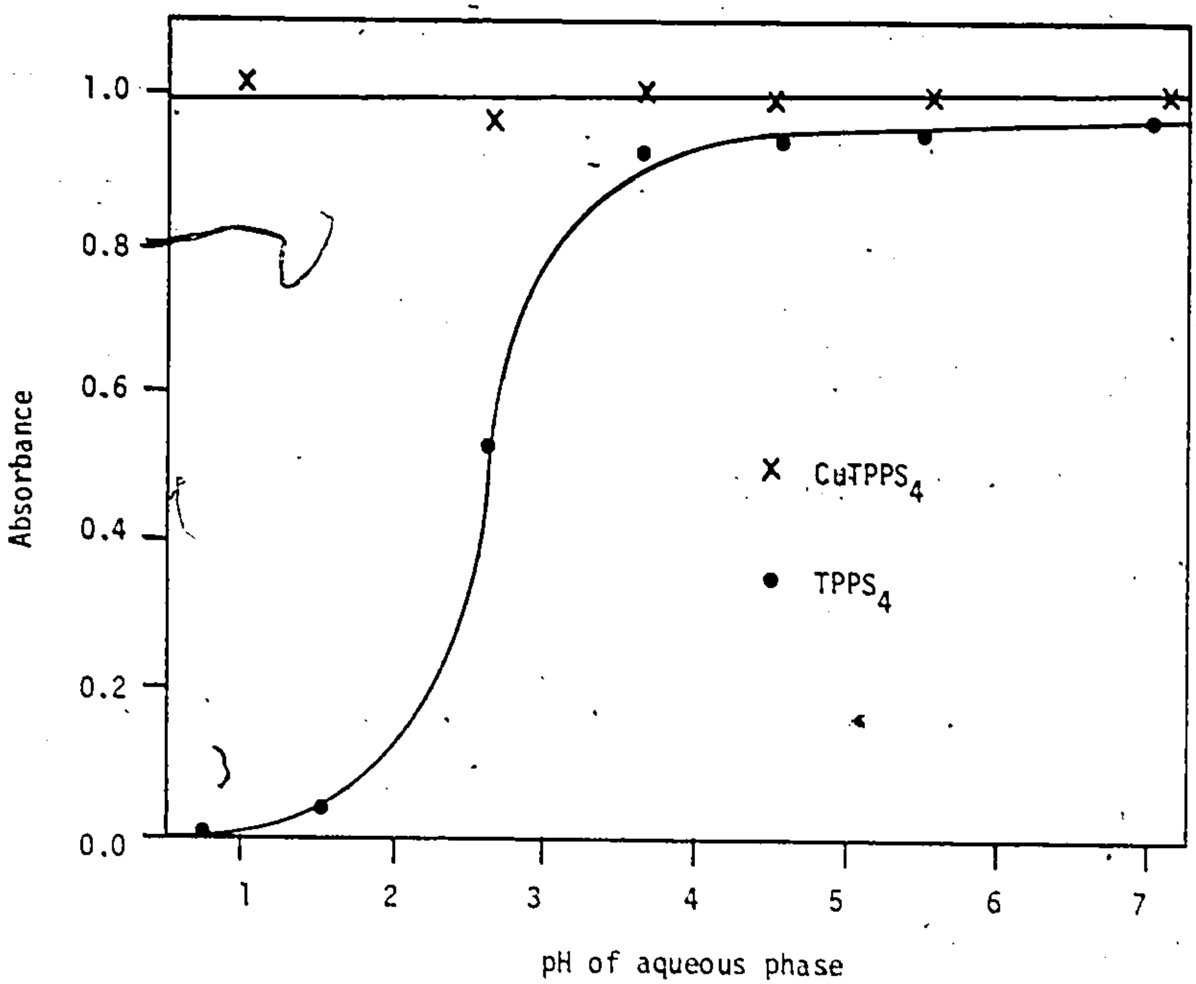

Figure XXXVII. pH-Dependence of the extraction of $TPPS_4$ and $CuTPPS_4$ from aqueous solution into an equal volume of 1-pentanol. Absorbance of 1-pentanol phase measured at 418.5 nm ($TPPS_4$) and 415.5 nm ($CuTPPS_4$). Concentration of both species 1.8×10^{-6} M in aqueous phase.

(c) Extraction-spectrophotometric determination of Cu(II) at trace levels

The intense absorption exhibited by porphyrins and metalloporphyrins in the Soret region makes their use as spectrophotometric reagents in trace metal analysis an attractive alternative to the experimentally more difficult techniques of atomic absorption and emission. The analytical sensitivity of porphyrins has, indeed, been exploited recently by a number of workers (e.g., 25-27, 29, see INTRODUCTION). A major problem in the spectrophotometric determination of metal ions is the severe overlap of the Soret bands of the metalloporphyrin and the excess free porphyrin. This problem can be largely alleviated in the case of porphyrins containing aromatic meso substituents (e.g., $TPPS_3$, $TPPS_4$, TMPyP) by protonation of the excess porphyrin after metallation. Protonation causes a substantial shift in the Soret absorption (see Figures V, XVII-XXV for spectra of $TPPS_4$ and various metalloporphyrins).

In the present study, protonation of excess $TPPS_4$ after reaction with Cu(II) was used as a means of separating the reagent from the complex. Not only does this procedure circumvent the spectral interference problem but it improves the detection limits for Cu(II) by virtue of preconcentration. The following work describes the application of this technique to the determination of Cu(II) at the ppb level, a thousand-fold improvement in detection over previous work (25,27).

The procedure, as it was developed and used in the determination, was described previously in the EXPERIMENTAL SECTION, but the main features are summarized below. The long reaction times which occur at very low concentrations of Cu(II) necessitated heating the solution. The complex thus formed could be separated from the excess porphyrin by extraction at any pH below 1.5, but its lability in hot acidic solution made it necessary to cool the reaction solution prior to acidification and extraction. At room temperature, $CuTPPS_4$ was found to be stable to 1 M HNO_3 for several hours. Addition of KNO_3 (after acidification) improved phase separation in the extraction without contaminating the solution. The problem of contamination is a serious one at low concentrations as recognized by others (e.g., 170). However, in the present study, it was less critical after the solution was cooled and acidified because the reaction of $TPPS_4$ with (contaminating) metal ions is very slow under these conditions.

A calibration curve for copper is shown in Figure XXXVIII. The high blank value is due to contamination from the reagents. The major portion of the blank was found to arise from Cu(II) in the formate buffer. The detection limit for Cu(II), defined as three standard deviations above the background (1 S.D. = 0.026; n = 12), is 1.1 ppb. This limit compares favourably with flame atomic absorption (4 ppb (171)) and inductively-coupled plasma atomic emission (1 ppb (172)).

Interferences may be classified into three groups: (i) substances which compete with $TPPS_4$ and its complexes for available extractant (Ph_4AsNO_3) (the most serious interferences will be large, singly-charged anions such as perrhennate or perchlorate (169)); (ii) complexing agents which compete with $TPPS_4$ for Cu(II); and, (iii) other metal ions which can react with $TPPS_4$. The presence of interferences of types (i) and (ii) must be considered and their effect evaluated for a given individual

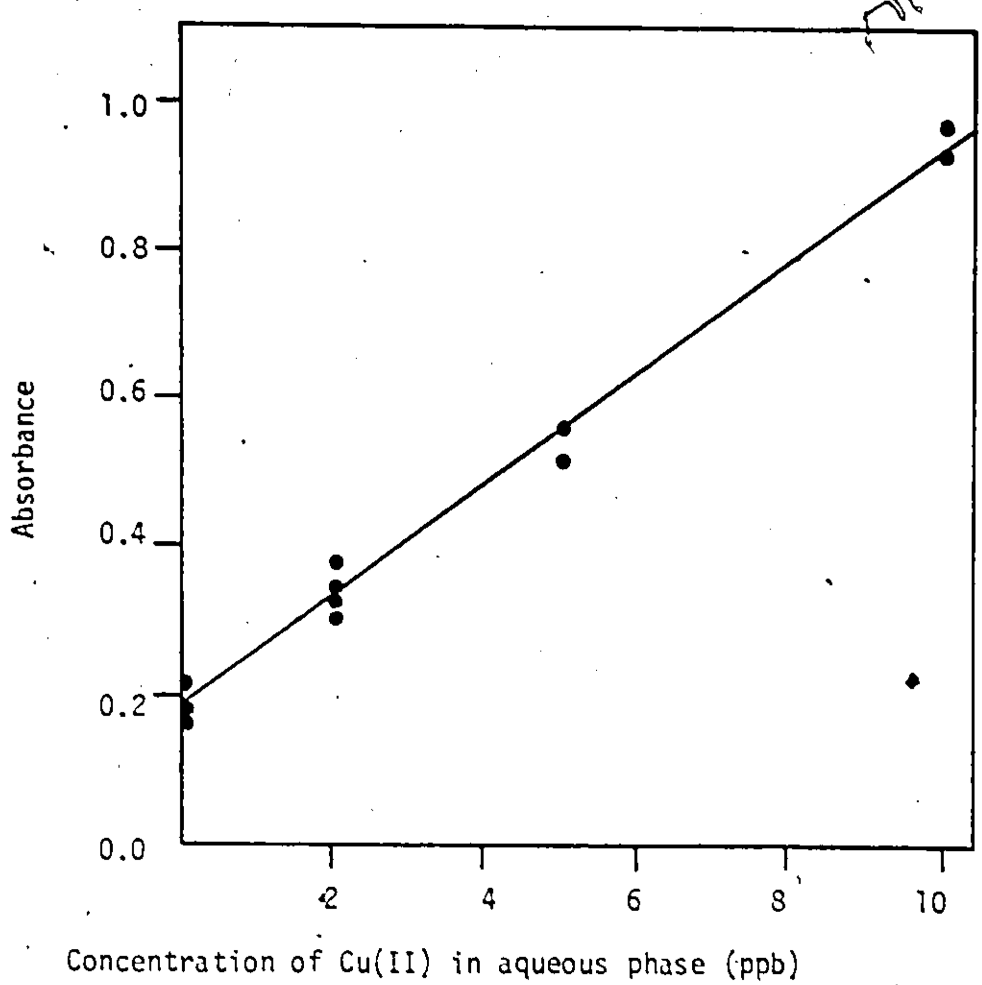

Figure XXXVIII. Calibration curve for the extraction-spectrophotometric determination of Cu(II). Concentration factor 10; 1 cm cells; absorbance of 1-pentanol phase at 415.5 nm.

matrix (e.g., biological fluids). Such interferences are less likely to occur in natural water samples for which the present technique seems to be best suited. The effect of selected metal ions on the determination of Cu(II) is examined below.

The choice of metal ions tested as interferences was based on the results of tests of $TPPS_4$ reactivity and provided a cross-section of rates of reaction and stability of products. Zn(II) and Ag(I) react most rapidly following Cu(II), Co(II) reacts at a moderate rate, while Ni(II) and Cd(II) react relatively slowly, with Cd(II) forming an unstable complex. An ion was considered to interfere if the absorbance of the extract differed from that obtained with Cu(II) alone by more than two standard deviations. The results of these tests are summarized in Table 13.

TABLE 13

Metal-ion interferences in the determination of Cu(II)*

Metal ion	No Interference	Interference
Zn(II)	3.1×10	1.2×10^2
Ag(I)**	3.2×10^2	1.2×10^3
Co(II)	3.2×10^2	1.6×10^3
Ni(II)	1.6×10^3	4.8×10^3
Cd(II)	1.7×10^5	---

* Cu(II) present at 2 ppb concentration. Values given represent the mole ratio of metal ion being tested to Cu(II).

** The behaviour of Ag(I) is complex. See discussion below.

The extent of the interference is determined by the relative reaction rates and stabilities of Cu(II) and the competing metal ion with $TPPS_4$, and by the spectral overlap of the metalloporphyrin formed. Various combinations of these factors can result in both positive and negative interferences. In the case of Ag(I), additional complications arise due to the formation of a precipitate from the reaction of Ag(I) with the formate buffer. At low concentrations of Ag(I), coprecipitation of Cu(II) results in a decrease in absorbance with increasing concentrations of Ag(I). This counterbalances the increase in absorbance expected from spectral overlap with the Ag(I) complex. Thus, although Ag(I) does not interfere at concentrations 320 times that of Cu(II) according to the definition given above, this is the result of a cancellation of effects.

Definite trends in absorbance were noted as a function of Ag(I) concentration. At higher concentrations, sufficient $AgTPPS_4$ was formed so that the absorbance increased with Ag(I) concentration despite precipitate formation. Therefore, the absorbance readings again fell within the two standard-deviation limit of no interference (e.g., at 9.5×10^3-fold molar excess of Ag(I)). At a 6.4×10^4-fold molar excess, a positive deviation was obtained. The effect of Ag(I) as an interference is complex.

On the whole, the above method shows good selectivity towards Cu(II), with Zn(II) and Ag(I) as the major interfering metal ions.

The efficiency of the extraction process was examined with the aid of radioactive copper (^{64}Cu). Only an estimate of the distribution ratio (D) could be obtained because the solubility of 1-pentanol in water precluded the use of exact volumes of organic solvent (see EXPERIMENTAL SECTION). D was found to be about 60, resulting in 81.5% extraction of copper. Therefore, a three-fold extraction with the same total volume of 1-pentanol, as described in the EXPERIMENTAL SECTION, should yield 93% extraction. Experimentally, 86% of the copper was found to be extracted. The slight improvement over a single-stage extraction does not warrant the additional work required for a three-stage extraction. Although the extraction is not quantitative, this is not a problem with a comparative method such as spectrophotometry as long as identical procedures are used for the preparation of the calibration curve and the analysis. A large value for D is desirable, however, since it would provide higher preconcentration values and allow the use of different preconcentration values for the preparation of the calibration curve and for the analysis. Thus, an important extension of this work would be the optimization of the extraction conditions. Higher concentrations of Ph_4AsNO_3 would likely improve the extraction (note that no significant extraction occurred at a concentration of 2×10^{-4} M) but the cost would be prohibitive. Also, the solubility of 1-pentanol in water and the relatively slow phase separation are unfavourable characteristics which make it desirable to find another organic solvent.

Preliminary experiments indicated that commercial trialkylamines and tetraalkylammonium salts (e.g., Alamine 336 and Aliquat 336, General Mills) might prove to be satisfactory reagents for extraction of $TPPS_4$ complexes. These compounds can be readily used in conjunction with more suitable organic solvents (e.g., non-water-soluble) such as methyl isobutyl ketone (MIBK) (the $TPPS_4$ ion pairs formed with Ph_4As^+ failed to extract into MIBK). In an experiment with trilaurylamine, 91% of the $^{64}Cu(II)$ was extracted into MIBK in a single step (10 ml of MIBK containing 4 g of the amine; aqueous phase 0.6 M in HNO_3). The greater quantities of amine which were required (50-fold greater than the quantity of Ph_4AsNO_3 for equivalent extraction) are not costly as the amine and related compounds are very inexpensive. The optimization of the extraction would greatly extend the utility of this method.

The high sensitivity of $TPPS_4$ and its metal complexes towards spectrophotometric measurement permits determination of Cu(II) in acid solution at trace levels. With a combination of separation methods such as ion-exchange chromatography, the $TPPS_4$ extraction-spectrophotometric method can readily be extended to other ions; its inherent simplicity would be advantageous. An interesting alternative to preliminary separation of metal ions would be the alteration of the selectivity by changing the relative rate of reaction of $TPPS_4$ with various metal ions with the aid of auxilliary complexing agents (e.g., 37, 115-118).

As noted in Table 12, $TPPS_4$ is selective towards a small number of metal ions, many of which are of contemporary importance. Thus, the ultimate application of $TPPS_4$ to important analytical problems should be feasible. Consider the following two examples. (a) The detection limits of today's sophisticated analytical instruments are constantly being challenged in environmental and material-purity problems. The extractability of $TPPS_4$ complexes would enhance the present-day detection limits in a preconcentration step. For example, the atomic absorption and inductively-coupled plasma emission spectroscopy. Detection limits for Cu(II) (4 and 1 ppb, respectively) could be improved readily by a factor of 10. With further development, perhaps a factor of 25-40 could be achieved. Similar procedures for the limited number of metal ions that react with $TPPS_4$ could also be developed. (b) Natural samples such as encountered in marine and physiological environments often present severe matrix problems (e.g., 165,167) in the trace determination of various metal ions. The development of extraction procedures that cleanly separate a <u>group</u> of metal ions from the matrix and serve as a means of preconcentration is of great current interest (e.g., 173 and references therein). The inherent selectivity of the instruments (e.g., atomic absorption and ICP emission spectrometers) would then distinguish among the individual ions of the group and the quantitative estimation made without matrix effects. Such an extraction procedure seems to be well suited to $TPPS_4$. Reaction with a group of metal ions rather than a single ion can be readily achieved by the use of less selective reaction conditions such as higher pH and longer heating times. In addition to eliminating matrix effects, the use of $TPPS_4$ could also eliminate the spectral interference encountered in atomic emission spectroscopy due to the ubiquitous presence of iron. Since the reaction of $TPPS_4$ with Fe(III) is extremely slow (e.g., 37) prior oxidation of the iron would effectively eliminate interference from this source.

Clearly, the development of applications of $TPPS_4$ to such problems would have been within the scope of this thesis but limitations of time prevented exploration beyond the application to Cu(II). There is no question, however, that the fundamental work reported in this thesis lays open the application of $TPPS_4$ to very interesting analytical problems.

IV. Suggestions for Further Work

1. Although the synthesis of $TPPS_4$ is extremely simple, its purification is tedious and leads to extensive loss of porphyrin. A simpler and more economical purification scheme would be extremely advantageous in studies with this compound and, if the method were general, with porphyrins as a whole.

2. The elucidation of the mechanism of the heterogeneous reaction of $TPPS_4$ with metals and insoluble metal compounds would be interesting and could lead to more general applicability of the reaction. For example, whether the reaction with metals is direct or through oxide formation should be investigated. Study of the factors (e.g., the nature of the solvent) affecting the adsorption and desorption of $TPPS_4$ and its metal complexes would be relevant, especially in the case of water-insoluble porphyrins.

3. Absorption spectra indicate that the complexes formed by the reaction of $TPPS_4$ with Hg and HgO are not the same. Further study is required to show how these complexes differ or whether one of them is a complex of a trace impurity.

4. The formation of $TPPS_4$ micelles in acidic solution is intriguing. Additional information on the structure, stability and rates of formation and dissociation of these micelles in different media would be of interest (e.g., the relation between the CMC and the structure and pKa of a wider range of ionic media). Such information would not only enhance our fundamental knowledge but could well be of practical signifi-

cance. In the present work, it was shown that the presence of micelles significantly alters the rate of Cu(II) incorporation into $TPPS_4$. The effect on the rates of incorporation of other metal ions should be investigated. It has also been shown that micelles can serve as catalysts for a number of reactions (156). The micelles formed by $TPPS_4$ might have unusual properties in this respect, particularly in view of the fact that their very formation was unexpected.

5. Potential applications of $TPPS_4$ to significant analytical problems, as noted in RESULTS AND DISCUSSION, should be explored.

CHAPTER V

V.1 APPENDIX I - CALIBRATION OF THE MICROBURETTE

The essential role of the Gilmont microburette in this work makes it necessary to indicate details of its calibration. The microburette was calibrated as described by Vogel (119).

Nominal Volume	Volume Delivered
0.0250	0.024958
0.0500	0.050063
0.0500	0.049936
0.1000	0.099865
0.1000	0.099705
0.2000	0.199760
0.3000	0.299435
0.4000	0.399248
0.4000	0.399600
0.5000	0.499045
0.6000	0.598933
0.6000	0.598592
0.7000	0.699166
0.7000	0.704033
0.8000	0.793700
0.9000	0.898134
1.0000	0.998051
1.1000	1.09767
1.2000	1.19817
1.3000	1.29779

1.4000	1.39798
1.5000	1.49695
1.6000	1.59786
1.7000	1.69785
1.8000	1.79779
1.9000	1.89770
2.0000	1.99695
2.1000	2.09750
2.2000	2.19800
2.3000	2.29735
2.4000	2.39797
2.5200	2.51705
2.5000	2.49697

A graph of volume delivered versus nominal volume gave a line of slope 0.99870 $\pm$ 0.00034 (95% confidence limits).

No systematic error as a function of volume delivered was found, as ascertained by a graph of the correction factor (C.F. = volume delivered/nominal volume) versus nominal volume. A line with a slope of $(2.7 \pm 8.9) \times 10^{-4}$ and an intercept of 0.9982 ± 0.0013 was obtained.

The correction factor was found to be insignificant for this work as the accuracy and precision were limited by the remaining equipment.

V.2 APPENDIX II

(a) Derivation of Equation Used in Spectrophotometric Studies

An equation which predicts the absorbance of a solution of $TPPS_4$ as a function of the analytical concentration is derived below. This equation is applicable when the major species present are the monomer and dimer but may be readily extended to include higher aggregates.

$$2\ \text{MONOMER} \overset{K_D}{\rightleftharpoons} \text{DIMER}$$

$$K_D = D/M^2$$

where K_D = dimerization constant

D = concentration of dimer

M = concentration of monomer

If the analytical concentration is C_T, then

$$M = C_T - 2D$$
$$K_D = D/(C_T - 2D)^2$$
$$K_D(C_T - 2D)^2 - D = 0$$
$$K_D C_T^2 - 4K_D C_T D + 4K_D D^2 - D = 0$$

Solution of the above quadratic equation yields

$$D = (4K_D C_T + 1 \pm \sqrt{16K_D^2 C_T^2 + 8K_D C_T + 1 - 16K_D^2 C_T^2})/8K_D$$

Since D cannot be negative nor greater than C_T,

$$D = (4K_D C_T + 1 - \sqrt{8K_D C_T + 1})/8K_D$$

$$M = C_T - 2D$$

$$= (8K_DC_T - 8K_DC_T - 2 + 2\sqrt{8K_DC_T + 1})/8K_D$$

$$M = (-2 + 2\sqrt{8K_DC_T + 1})/8K_D$$

Assuming that absorbances are additive,

$$A = \varepsilon_1 M + \varepsilon_2 D$$

where A = absorbance

ε_1 = molar absorptivity of the monomer

ε_2 = molar absorptivity of the dimer

M,D as defined above.

$$A = \varepsilon_1 M + \varepsilon_2 D$$

$$= (-2\varepsilon_1 + 2\varepsilon_1\sqrt{8K_DC_T + 1} + 4K_DC_T\varepsilon_2 + \varepsilon_2 - \varepsilon_2\sqrt{8K_DC_T + 1})/8K_D$$

$$= (4K_DC_T\varepsilon_2/8K_D) + \{(\varepsilon_2 - 2\varepsilon_1) + (2\varepsilon_1 - \varepsilon_2)\sqrt{8K_DC_T + 1}\}/8K_D$$

$$A = (\varepsilon_2 C_T/2) + (2\varepsilon_1 - \varepsilon_2)(\sqrt{8K_DC_T + 1} - 1)/8K_D$$

This final equation corresponds to equation 3 shown in the RESULTS AND DISCUSSION.

(b) Derivation of Equations Used in NMR Studies

The simplest aggregation model involves an equilibrium between monomer and dimer species. If the exchange of molecules is at least moderately rapid, then a single signal will be observed for a given proton. The observed chemical shift, δ_{OBS}, will be the weighted mean of the monomer,

δ_M, and dimer, δ_D, chemical shifts:

$$\delta_{OBS} = (\delta_M[\text{MONOMER}] + 2\delta_D[\text{DIMER}])/C_0 \tag{76}$$

where C_0 is the analytical concentration of porphyrin. Therefore:

$$\delta'_{OBS} = (\delta'_M[\text{MONOMER}] + 2\delta'_D[\text{DIMER}])/C_0$$

$$\delta''_{OBS} = (\delta''_M[\text{MONOMER}] + 2\delta''_D[\text{DIMER}])/C_0$$

where the superscripts ' and " indicate signals arising from two different protons. Thus, the observed <u>difference</u> between the two signals will be given by:

$$\begin{aligned}\Delta\delta_{OBS} &= \delta'_{OBS} - \delta''_{OBS}\\ &= \{(\delta'_M - \delta''_M)[\text{MONOMER}] + 2(\delta'_D - \delta''_D)[\text{DIMER}]\}/C_0\end{aligned}$$

$$\Delta\delta_{OBS} = \{\Delta\delta_M[\text{MONOMER}] + 2\Delta\delta_D[\text{DIMER}]\}/C_0$$

Thus, the use of chemical shift <u>differences</u> is seen to yield an equivalent expression to that using the chemical shifts alone. This expression is readily extended to account for higher aggregates. The following expressions were used for models involving the species given:

(a) monomer, dimer and trimer

$$\Delta\delta_{OBS} = \{\Delta\delta_M[\text{MONOMER}] + 2\Delta\delta_D[\text{DIMER}] + 3\Delta\delta_{TR}[\text{TRIMER}]\}/C_0$$

(b) monomer, dimer and tetramer

$$\Delta\delta_{OBS} = \{\Delta\delta_M[\text{MONOMER}] + 2\Delta\delta_D[\text{DIMER}] + 4\Delta\delta_{TT}[\text{TETRAMER}]\}/C_0$$

(c) monomer, dimer, trimer and tetramer

$$\Delta\delta_{OBS} = \{\Delta\delta_M[\text{MONOMER}] + 2\Delta\delta_D[\text{DIMER}] + 3\Delta\delta_{TR}[\text{TRIMER}] + 4\Delta\delta_{TT}[\text{TETRAMER}]\}/C_0$$

The concentration of the various species were obtained by solution of the appropriate equilibrium and mass balance equations. Thus, for the model involving monomer and dimer only, the concentration of these species may be calculated as in APPENDIX II(a). Using the same symbols, one obtains:

$$\Delta\delta_{OBS} = \{\Delta\delta_M(-2 + 2\sqrt{8K_DC_0 + 1}) + 2\Delta\delta_D(4K_DC_0 + 1 - \sqrt{8K_DC_0 + 1})\}/8K_DC_0$$

$$= \Delta\delta_D + \{2(\Delta\delta_M - \Delta\delta_D)(\sqrt{8K_DC_0 + 1} - 1)\}/8K_DC_0$$

This equation is equivalent to that used by Abraham et. al. (76) with chemical shifts instead of chemical shift differences.

Higher order equations are obtained for the more complex models involving trimers and/or tetramers. Their development is illustrated in APPENDIX II(c).

(c) Solution of Higher-Order Equations

The model involving the species monomer, dimer and tetramer is used here to illustrate the calculations involved in testing the various models.

$$2\ \text{MONOMER} \rightleftarrows \text{DIMER}$$

$$K_D = D/M^2$$

$$2\ \text{DIMER} \rightleftarrows \text{TETRAMER}$$

$$K_{TT} = TT/D^2$$

$$= TT/(K_DM^2)^2$$

where K_{TT} = constant for tetramer formation

TT = tetramer concentration

The remaining symbols are the same as those used in APPENDICES II(a) and II(b).

The mass balance equation for $TPPS_4$ for this model is:

$$C_0 = 4TT + 2D + M$$
$$= 4K_{TT}K_D^2M^4 + 2K_DM^2 + M$$

A numerical solution of the above equation may be found for a given C_0 if K_{TT} and K_D are known using the Newton-Raphson iterative method. For a function F(x), successive iterations are obtained using the formula

$$x_{n+1} = x_n - F(x_n)/F'(x_n) \qquad (158)$$

where $F'(x)$ is the derivative of the function $F(x)$ and x_n is the n^{th} approximation of the root. For the model above,

$$F(x) = F(M)$$
$$= 4K_{TT}K_D^2M^4 + 2K_DM^2 + M - C_0$$

$$F'(x) = F'(M)$$
$$= 16K_{TT}K_D^2M^3 + 4K_DM + 1$$

An initial approximation, M_0, was obtained by assuming the presence of only monomer and dimer species and solving the resulting quadratic equation with K_D = 15800 M^{-1} as in APPENDIX II(a). The value of M_0 obtained in this manner proved to be a reasonable approximation since the monomer and dimer were major species over the concentration range examined. Using an estimate for K_{TT}, values for M, D and TT were readily obtained.

e.g.,

$$K_D = 15800$$

$$K_{TT} = 10$$

$$C_0 = 10^{-2}$$

$$M_0 = (-2 + 2\sqrt{8K_DC_0 + 1})/8K_D$$

$$= 5.4694363 \times 10^{-4} \text{ *}$$

$$M_1 = M_0 - F(M_0)/F'(M_0)$$

$$= (5.4694363 - 0.2122463) \times 10^{-4}$$

$$= 5.2571900 \times 10^{-4}$$

$$M_2 = M_1 - F(M_1)/F'(M_1)$$

$$= (5.2571900 - 0.0055214) \times 10^{-4}$$

$$= 5.2516686 \times 10^{-4}$$

$$M_3 = M_2 - F(M_2)/F'(M_2)$$

$$= (5.2516686 - 0.0000041) \times 10^{-4}$$

$$= 5.2516645 \times 10^{-4}$$

The iterations were continued until the change in successive approximations was less than 10^{-5} of the value (i.e., $F(M_n)/F'(M_n) < M_n \cdot 10^{-5}$).

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* Values were not rounded off to the appropriate number of significant figures until all calculations were completed.

This value of M was then used to calculate D and TT ($4.35763680 \times 10^{-3}$ and 1.8988998×10^{-4}, respectively, in the example above) and the values substituted into the equation developed in APPENDIX II(b).

$$\Delta\delta_{OBS} = \{\Delta\delta_M \cdot M + 2\Delta\delta_D \cdot D + 4\Delta\delta_{TT} \cdot TT\}/C_O$$

The final values for $\Delta\delta_M$, $\Delta\delta_D$, $\Delta\delta_{TT}$ and K_{TT} were obtained by non-linear least-squares fitting of the equation above to the observed ^{1}Hmr data.

Similar procedures were used for the other models.

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