On the Aggregation of Meso-Substituted Water-Soluble Porphyrins

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Abstract: An investigation of the solution properties of several water-soluble porphyrins, tetracarboxyphenylporphine (TCPP), tetraphenylporphinesulfonate ($TPPS_3$), and tetra(*N*-methyltetrapyridyl)porphine (TMPyP), demonstrates that in an 80–90% ethylene glycol–water solvent system, the porphyrins all obey Beer's law at pH 7.5 and TMPyP obeys Beer's law in this solvent system at pH 0. Consistent with these findings is the absence of relaxation effects within the time range of the temperature-jump instrument available to us. In aqueous solution at pH 7.5, both TCPP and $TPPS_3$ dimerize, the extent of deviation from Beer's law being a function of porphyrin concentration and ionic strength. Under these same experimental conditions, TMPyP remains monomeric in solution. Values for the molar absorptivity of the dimers and the dimerization equilibrium constant have been determined for the former cases. Aqueous solutions of TCPP and $TPPS_3$ show relaxation effects from which the rate constants for the dimerization reaction were determined. For both porphyrins, as for the tetraethylenediamine derivative of protoporphyrin IX, the forward rate constants are near the diffusion controlled limit. Differences in properties between TCPP, $TPPS_3$, and TMPyP seem to be closely related to the charge type of the ionized groups at the periphery of the molecule.

The metal complexes of porphyrin compounds have extraordinary chemical reactivity when bound to specific proteins or included in organelle structures as found in nature. The porphyrin molecule itself is a fairly rigid chelate ligand having extensive electron delocalization in its nucleus, and these properties must be essentially involved in the chemical activity and biological function of the macromolecules in which they are found. The porphyrin function can be studied in the native macromolecules, which display all of their properties but in a very complex form, or by examining the chemistry of the prosthetic group alone in solution, expecting that some aspects of the protein-mediated reaction can be most easily examined in this model system. Unfortunately, most porphyrins, native and synthetic, are not sufficiently soluble in aqueous solution, particularly at or near neutral pH's, to permit their physicochemical characteristics to be extensively examined. Some water-soluble porphyrin derivatives have been prepared by attaching ionized groups to the porphine molecule.[3–6] We are reporting on the solution properties of several of these water-soluble porphyrins: tetraphenylporphinesulfonate ($TPPS_3$), tetracarboxyphenylporphine (TCPP), and tetra(*N*-methyltetrapyridyl)porphine (TMPyP). The structures of these substances are shown in Figure 1. Consistent with the established convention,[7] throughout this paper the symbol PH_2 is used for the monomeric porphyrin unit (*cf.* Figure 1). In a similar manner, PH_3^+ is used for the monomeric species in which an additional proton is added to the center of the molecule and PH_4^{2+} for the species containing four hydrogens in the central region. The terms "neutral porphyrin or free base" (PH_2), "monocation" (PH_3^+), and "dication" (PH_4^{2+}) are used although the formal charges of these species are other than 0, +1, and +2, respectively. More specifically, the TPPS derivative we have prepared contains three sulfonate groups ($TPPS_3$), so that the "neutral" porphyrin, PH_2, is actually an anion having a charge of −3.[8]

Experimental Section

Synthesis and Purification. $TPPS_3$. The synthesis of $TPPS_3$ is begun with the preparation of tetraphenylporphine (TPP) using a procedure developed by Adler and coworkers.[9–11] The synthesis consists of adding pyrrole and benzaldehyde to refluxing propionic acid. Dark blue TPP crystals form when the mixture is cooled in an ice bath. In some of the preparations, the crude TPP was purified by column chromatography on silica gel using chloroform as the eluent. The product TPP, obtained by the evaporation of the solvent, is sulfonated in concentrated sulfuric acid using a method devised by Menotti[12] and further developed by Winkelman and coworkers.[13] This technique leads to a mixture of TPPS derivatives differing in the number of sulfonate groups per molecule. Winkelman[14] describes a method for separating these isomers by means of

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(2) (a) Department of Chemistry, Ithaca College; (b) in partial fulfillment for the B.A. degree from Ithaca College; (c) University of Rome.

(3) E. B. Fleischer, *Inorg. Chem.*, **1**, 493 (1962).

(4) T. P. Stein and R. A. Plane, *J. Amer. Chem. Soc.*, **91**, 607 (1969).

(5) P. Hambright and E. B. Fleischer, *Inorg. Chem.*, **9**, 1757 (1970).

(6) E. B. Fleischer, J. M. Palmer, T. S. Srivastava, and A. Chatterjee, *J. Amer. Chem. Soc.*, **93**, 3162 (1971).

(7) J. E. Falk in "Porphyrins and Metalloporphyrins," Elsevier, New York, N. Y., 1964.

(8) Although the acid dissociation equilibrium constants have not been determined for the sulfonate groups, it is unlikely that the pK_a's for these groups are greater than 1 in aqueous solution. As reported in the "Handbook of Chemistry and Physics," Chemical Rubber Publishing Co., Cleveland, Ohio, 1960, the pK_a in water of benzosulfonic acid is 0.70 and for naphthalenesulfonic acid, it is 0.57.

(9) A. D. Adler, F. R. Longo, and W. Shergalis, *J. Amer. Chem. Soc.*, **86**, 3145 (1964).

(10) A. Adler, F. R. Longo, J. D. Finarelli, J. Goldmacher, J. Assour, and L. Korsakoff, *J. Org. Chem.*, **32**, 476 (1967).

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(12) A. R. Menotti, Doctoral Dissertation, The Ohio State University, Columbus, Ohio, 1941.

(13) J. Winkelman, G. Slater, and J. Grossman, *Can. Res.*, **27**, 2060 (1969).

(14) J. Winkelman, *ibid.*, **22**, 589 (1962).

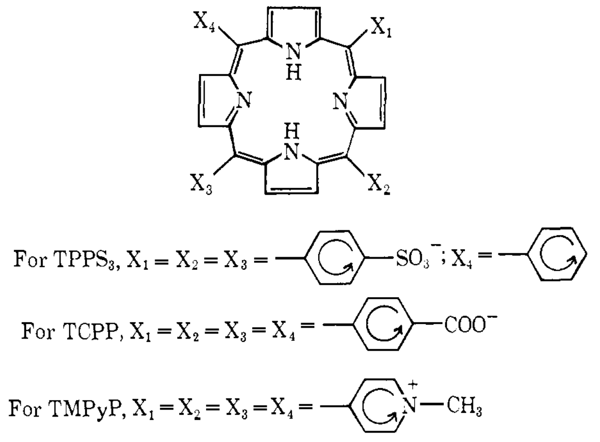

Figure 1. Structures of porphyrins. This form of the porphyrin in which two of the pyrrole nitrogens are protonated is referred to as the neutral porphyrin or free base form (PH_2).

column chromatography on Celite using a pyridine–chloroform–water solvent system. Our attempts at separating isomers using this method gave uniformly poor results, always leading to a product which on elemental analysis showed low values for C, N, and S and a large percentage of an incombustible residue. The method was modified using the same solvent system on paper previously treated with EDTA. The band pattern was precisely that described by Winkelman[14] but the elemental analysis again yielded poor results. Finally, the porphyrin was successfully purified by column chromatography on Dowex 50-WX8 cation exchange resin (H form). The TPPS mixture, which had previously been repeatedly washed with acetone to remove excess sulfate, was placed on the column and eluted with water. Porphyrin bands developed which were not completely resolvable as some species were only slightly retarded by the column. On the basis of the intensity of the green color, a minor component was the first to come off the column followed by a very intense band which did not spread with elution. This band was collected and lyophilized to dryness and used for the following experiments. Immediately behind the major component was another component which tailed considerably but which could be completely eluted with water. Remaining on the column was what appeared to be the second-most major component which could not be removed from the Dowex even with strong acid. The major component gave an elemental analysis consistent with it being the trisulfonated product, in agreement with previous work.[14] *Anal.* Calcd for $C_{44}H_{30}N_4S_3O_9 \cdot 4H_2O$: C, 57.01; H, 4.13; N, 6.05; S, 10.43. Found: C, 57.09; H, 3.80; N, 6.05; S, 10.38; residue 0.03.

TCPP. This porphyrin was synthesized and purified by a method described in the literature.[15] *Anal.* Calcd for $C_{48}H_{30}N_4O_8$: C, 72.91; H, 3.82; N, 7.09. Found: C, 72.77; H, 3.99; N, 6.94.

TMPyP. This porphyrin was synthesized using a variation[16] of a method reported in the literature.[5] The synthesis involves forming the quaternary salt of tetrapyridylporphine (TPyP) which was either prepared by literature methods[15] or purchased from the Mad River Chemical Co., Yellow Springs, Ohio. The TPyP is refluxed overnight with methyl *p*-toluenesulfonate in DMF. The reaction mixture is cooled in an ice bath and the product is removed by filtration. The collected violet crystals are washed with acetone and dried under high vacuum. *Anal.* Calcd for $C_{72}H_{66}N_8S_4O_{12}$: C, 63.42; H, 4.88; N, 8.22; S, 9.41. Found: C, 63.25; H, 5.07; N, 8.36; S, 9.21.

Spectral Studies. The infrared spectra of these compounds were determined on a Perkin-Elmer 237B spectrophotometer in the solid state using KBr pellets. For every case the spectra resembled those characteristic of para-substituted *meso*-tetraphenylporphines.[17] The infrared spectrum obtained by us for TCPP was nearly identical with that reported in the literature, except for the absence of the 700-cm^{-1} peak in our study. A nuclear magnetic resonance study[6] reported on the tetrasulfonated isomer of TPPS prepared in a manner similar to the way we prepared our product disclosed that the sulfonations occur in the para positions only. An nmr study[16] of TMPyP showed that all the methyl protons in the molecule are identical and that, therefore, the pyrrole-type nitrogens do not add methyl groups under the conditions of this synthesis. A sample of our TMPyP was shown to add two protons to form a PH_4^{2+} species (*vide infra*) which further corroborates the specificity of the methyl group addition.

All visible spectra were determined on a Cary 14. The solvent systems used were (1) 80–90% ethylene glycol–water which was, in most experiments, buffered with 0.01 *M* Tris at pH ~7.5 as measured with a glass electrode; (2) 80–90% ethylene glycol–water containing varying concentrations of HNO_3 (here pH readings could be compared directly with concentrations of hydrogen ion added to the solvent system. The measured values agreed with the calculated values to within 0.1 pH unit); (3) various aqueous solutions ranging in pH from about 1–10 and for a few experiments above. Both $TPPS_3$ and TMPyP are soluble in water and in water–ethylene glycol solutions over a very extended pH range. However, TCPP is soluble in polar solvents only when the carboxylate groups are ionized and, therefore, our studies with this porphyrin are limited to pH 5 and above.

Solutions were freshly prepared before spectral analysis and were protected from direct sunlight and fluorescent light until they were inserted into the cell compartments Several solutions were allowed to stand for varying times in direct light and showed a spectral change with time. The effect was more severe for dilute solutions than for concentrated ones. The spectral results obtained in this study agree quite well with those available in the literature.[5,6,17] The visible spectrum of TCPP had been investigated in pyridine[17] and our spectrum showed nearly identical peak positions but somewhat larger molar absorptivities. The spectral properties of these species in the visible range is a major subject of this paper and will be expanded upon in succeeding sections.

Relaxation experiments were carried out using a temperature-jump apparatus described elsewhere.[18] Here again all solutions were freshly prepared and protected from light until the temperature jump experiment. The equilibrium temperature of these studies, like those of the spectral studies, was 25°. Each relaxation time represents an average of at least three photographic determinations, with the relative error of these measurements at ±10%.

Calculations of the various equilibrium and kinetic parameters were carried out on the Ithaca College RCA 70/35 computer. For the equilibrium calculations a general minimization routine, SIMPLEX, was used.[19]

Results and Discussion

I. Ethylene Glycol Solutions. The spectral characteristics of the three porphyrins in the visible range were studied in ethylene glycol solutions containing 10–20% water. The species PH_2 is red in solution whereas the acidic forms, where soluble, are green. The visible spectra in basic solution (pH ~7.5) are of the aetio type as is shown for TCPP in Figure 2. Beer's law experiments were carried out in both acidic and basic regions for TMPyP and in the basic region for TCPP and $TPPS_3$. Whereas TCPP is insoluble under acidic conditions in this solvent system, $TPPS_3$ is soluble and shows maxima in the visible region at 489, 598, 649, and 707 mμ. However, the apparent molar absorptivities [ϵ_{app} = absorbance/(path length) × (total concentration of porphyrin)] of these peaks, and especially the 489 mμ peak, are dependent upon the ionic strength, on the amount of water present in the solvent mixture, and on the nature of the electrolyte used to maintain the ionic strength. For example, a solution which is 0.1 *M* in HNO_3 shows the following

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[15] F. R. Longo, M. G. Finarelli, and J. B. Kim, *J. Heterocycl. Chem.*, 6, 927 (1969).

[16] D. Bennett and R. A. Plane, private correspondence.

[17] N. Datta-Gupta and T. J. Bardos, *J. Heterocycl. Chem.*, 3, 495 (1966).

[18] R. F. Pasternack, K. Kustin, L. A. Hughes, and E. Gibbs, *J. Amer. Chem. Soc.*, **91**, 4401 (1969).

[19] Copyrighted by J. P. Chandler, Department of Physics, University of Indiana, Bloomington, Ind., 1965. The routine was further expanded by T. Needham, Department of Chemistry, University of Illinois, Urbana, Ill.

Table I. Visible Absorption Spectra of Porphyrins at 25°

Porphyrin	Solvent	μ	pH	λ_{max}, mμ ($\epsilon \times 10^4$) Soret	Visible bands				
TPPS$_3$	Ethylene glycol–H$_2$O	0.1	7.5	418 (42.9)	514 (1.67)	550 (0.71)	587 (0.54)	642 (0.35)	
	Ethylene glycol–H$_2$O	0.1	1.2			Ionic strength dependent			
	H$_2$O					Concentration dependent			
TCPP	Ethylene glycol–H$_2$O	0.1	7.5	419 (45.4)	517 (1.57)	552 (0.79)	588 (0.52)	643 (0.44)	
	H$_2$O					Concentration dependent			
	Pyridine[a]			422 (16.1)	486 (0.31)	517 (1.44)	552 (0.70)	591 (0.43)	649 (0.29)
	Pyridine[b,c]			423 (34.0)	sh	517 (1.93)	552 (0.96)	592 (0.63)	648 (0.50)
TMPyP	Ethylene glycol–H$_2$O	0.1	7.6	426 (23.9)	518 (1.85)	553 (0.68)	590 (0.68)	648 (0.20)	
	Ethylene glycol–H$_2$O	1.0	0	457 (25.0)	597 (1.38)	638 (1.99)			
	H$_2$O[d]		4–7	422 (14.9)	518 (0.918)	551 (0.679)	585 (0.372)	641 (0.118)	
	H$_2$O[b]	1.0	7.2	424 (22.6)	520 (1.45)	559 (0.92)	587 (0.75)	643 (0.25)	
	H$_2$O[d,e]	1.0	0	446 (19.3)	590 (1.13)	643 (1.47)			
	H$_2$O[b]	1.0	0	446 (31.9)	592 (1.40)	643 (1.90)			
	H$_2$O[d,f]	1.0	14	~455	578 (1.0)	630 (8.91)			
	H$_2$O[b]	1.0	14	450 (12.3)	577 (1.25)	623 (0.833)			

[a] Reference 17. [b] Present work. [c] Beer's law experiments were not performed in pyridine. The extinction coefficients we are reporting were determined for concentrations of 6.2×10^{-5} M for the visible bands and 2.3×10^{-6} M for the Soret band. [d] Reference 5. [e] Figure 1 of ref 5 clearly shows that the peak referred to as λ 534 mμ in the text is actually closer to λ 643. [f] Figure 4 of ref 5 shows that the λ 630 peak is actually less intense than the λ 578 band for the PH$^-$ species.

maxima: 489 ($\epsilon_{app} = 3.9 \times 10^4$ M^{-1} cm^{-1}), 598 ($\epsilon_{app} = 7.6 \times 10^3$ M^{-1} cm^{-1}), 648 ($\epsilon_{app} = 3.7 \times 10^4$ M^{-1} cm^{-1}), and 707 ($\epsilon_{app} = 1.1 \times 10^3$ M^{-1} cm^{-1}). However, a solution which is 0.07 M in KNO$_3$ at pH 1.5 with HNO$_3$ shows the following peaks: 489 ($\epsilon_{app} = 8.3 \times 10^3$ M^{-1} cm^{-1}), 598 ($\epsilon_{app} = 8.3 \times 10^3$ M^{-1} cm^{-1}), 648 ($\epsilon_{app} = 4.0 \times 10^4$ M^{-1} cm^{-1}), and 707 (shoulder). Apparently this porphyrin behaves in a complex manner under these conditions probably involving aggregation, electrolyte–porphyrin interaction, or both. Similar behavior was found for TPPS$_3$ in aqueous solution (*vide infra*).

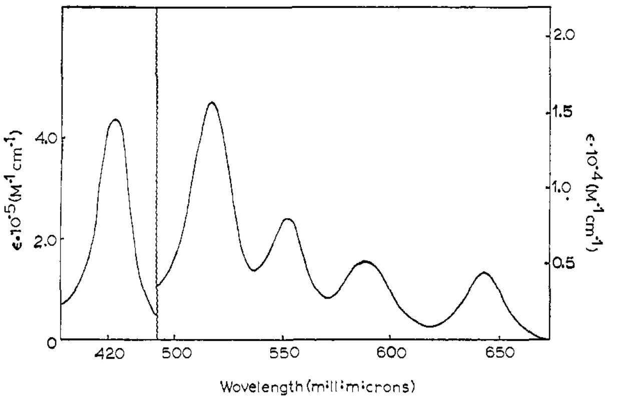

Figure 2. The visible spectrum of TCPP in ethylene glycol–water, 0.1 M KNO$_3$, pH 7.5. This spectrum, like the ones for TPPS$_3$ and TMPyP, is of the aetio type.

For the three porphyrins at pH ~7.5 and for TMPyP at pH ~0, the absorbance was found to be directly proportional to concentration (Figures 3, 4) over the concentration range investigated (10^{-7}–2×10^{-5} M for TCPP and TPPS$_3$ and 10^{-7}–6×10^{-5} M for TMPyP).

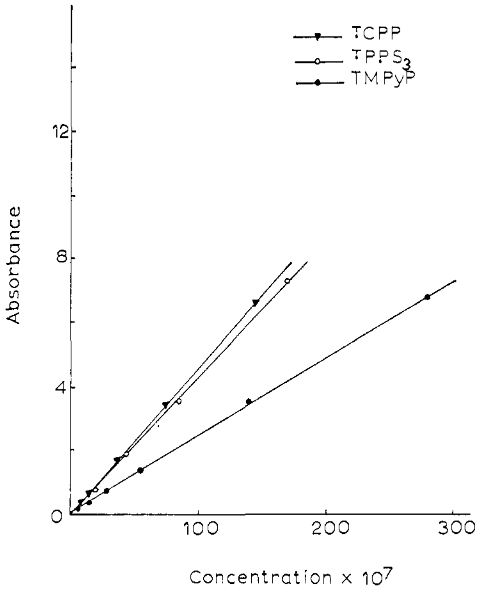

Figure 3. Beer's law experiments for TCPP, TPPS$_3$, and TMPyP in ethylene glycol–water at pH ~7.5, 0.1 M KNO$_3$.

The molar absorptivities were determined for each peak maximum and the results are summarized in Table I. A number of experiments were run for these systems in which the concentration and/or identity of electrolyte was varied. The spectra proved to be independent of

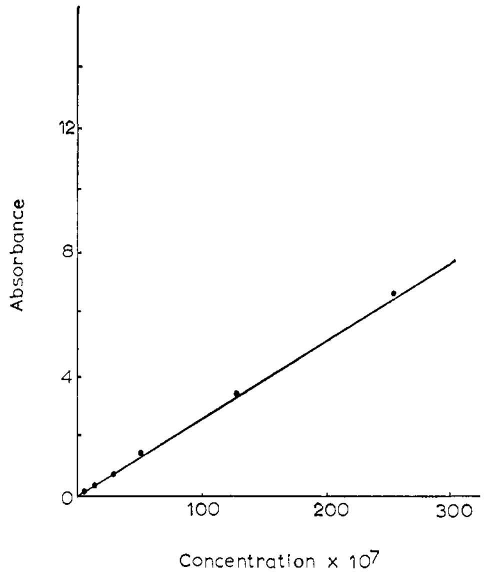

Figure 4. Beer's law experiments for TMPyP in ethylene glycol-water at 1 M HCl.

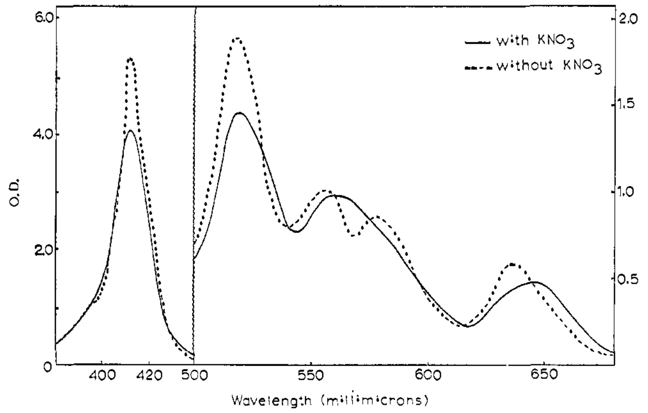

Figure 5. Spectra of TCPP at constant concentration in aqueous solution as a function of ionic strength, pH 7.5.

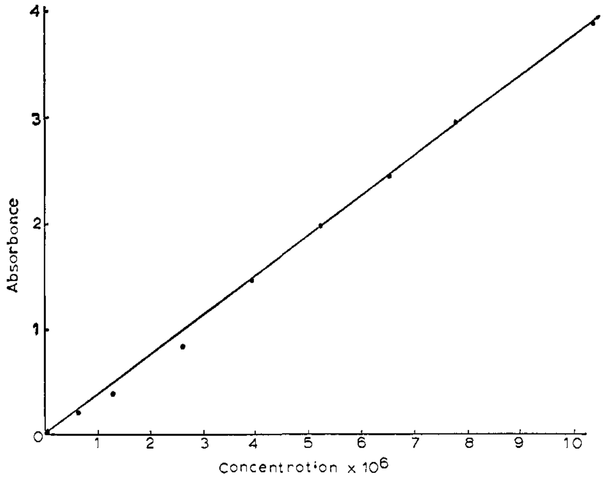

Figure 6. Beer's law experiments at 415 mμ for TCPP in aqueous solution, pH 7.5, without added KNO₃.

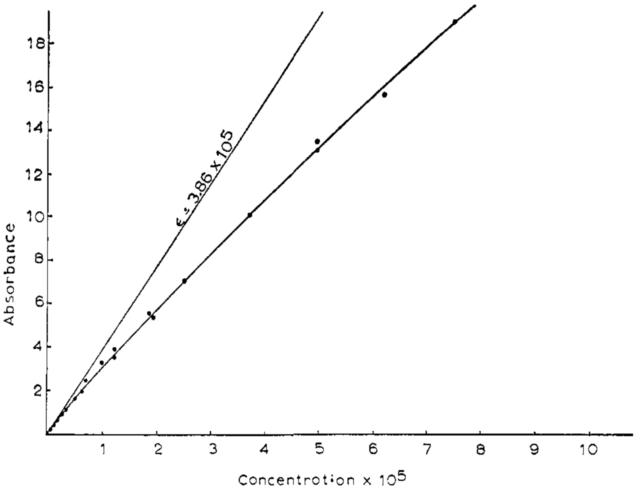

Figure 7. Beer's law experiments for TCPP in aqueous solution, pH 7.5, with added KNO₃. The line marked $\epsilon = 3.86 \times 10^5$ defines Beer's law behavior for TCPP. The filled circles represent the experimental data and the line passing through the points is the theoretical curve obtained for the dimerization model with $\epsilon_D = 3.84 \times 10^5\ M^{-1}\ \text{cm}^{-1}$ and $K = 4.55 \times 10^4\ M^{-1}$.

ionic strength in the range investigated, $0 \leq \mu \leq 1\ M$ using Tris, LiCl, NaCl, KNO_3, and HCl as electrolytes. Temperature-jump experiments were attempted for all three porphyrins at pH ~7.5 and for TMPyP at pH ~0 and no relaxation effects were found within the time range of the instrument for this solvent system.

The spectral results lead us to conclude that $TPPS_3$, TCPP, and TMPyP exist as monomers at pH ~7.5 in this ethylene glycol-water solvent system as does TMPyP at pH ~0. That no relaxation effects were found is consistent with the conclusion that there are no aggregation equilibria of these porphyrins under these conditions.[20]

II. Water Solutions. (a) TCPP. Because of solubility considerations the properties of this porphyrin were studied only at pH >5. It was found that in aqueous solution, the positions of TCPP peaks as well as their apparent molar absorptivities depend on concentration and ionic strength. For example, for a solution of fixed concentration buffered with 0.01 M Tris to a pH of 7.5, the intensities of the various bands depend on the ionic strength (Figure 5). Beer's law experiments were carried out at 415 mμ in 0.01 M Tris, pH ~7.5, both with added KNO_3 and in the absence of KNO_3. For the TCPP solutions in which no KNO_3 was added, an extended concentration range over which Beer's law was obeyed resulted (Figure 6). From these data we obtain a value of the molar absorptivity of the monomeric form of TCPP at 415 mμ. This value is $\epsilon_M = 3.86 \times 10^5\ M^{-1}\ \text{cm}^{-1}$.

A plot of absorbance *vs.* total concentration of porphyrin at pH ~7.5 in the presence of 0.1 M KNO_3 showed considerable deviation from linearity (Figure 7). Solutions which were made 0.1 M in $NaClO_4$ showed identical peak heights to those of the same porphyrin concentration and pH but which were 0.1 M in KNO_3. The curvature in the Beer's law plots is interpreted as being due to aggregation of TCPP in aqueous solutions. The linear region in the presence of KNO_3 at relatively low concentrations is assumed to be due to the presence of monomeric TCPP only. We have analyzed the data by assuming that as the concentration is increased both monomer and dimer are stable. Using

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[20] R. R. Das, R. F. Pasternack, and R. A. Plane, *J. Amer. Chem. Soc.*, **92**, 3312 (1970).

the value of ϵ_M obtained from solutions in which no KNO_3 was added in the curve fitting program,[19] we determined values for the molar absorptivity of the dimer, ϵ_D, at 415 mμ and the equilibrium constant $K = [P_2H_4]/[PH_2]^2$, at 25°. Here $[P_2H_4]$ = concentration of the dimer at equilibrium and $[PH_2]$ = concentration of the monomer. The equation used for the curve fitting, derived from $A = \epsilon_M[PH_2] + \epsilon_D[P_2H_4]$ and $C_0 = [PH_2] + 2[P_2H_4]$, is

$$A_M - A = ((2\epsilon_M - \epsilon_D)(4KC_0 + 1 - \sqrt{1 + 8KC_0}))/8K$$

where C_0 is the total concentration of the porphyrin and $A_M = \epsilon_M C_0$. In Figure 7, a line corresponding to $\epsilon_M = 3.86 \times 10^5\ M^{-1}\ \mathrm{cm}^{-1}$ has been drawn; if there were no aggregation equilibrium, all of the data points, shown as circles in Figure 7, would be expected to lie on this line. Shown also is the theoretical curve obtained for $\epsilon_D = 3.84 \times 10^5\ M^{-1}\ \mathrm{cm}^{-1}$ and $K = 4.55 \times 10^4\ M^{-1}$.

Further corroboration of the aggregation model for TCPP comes from relaxation experiments. Temperature-jump studies were carried out on porphyrin solutions varying in concentration from 10^{-6} to $>10^{-4}\ M$. All the solutions were 0.1 M in KNO_3 and 0.01 M in Tris, pH 7.5. The analysis of the relaxation data involved an extension of an equation published earlier[20]

$$\tau^{-1} = 4k_{22}[PH_2] + k_{-22}$$

where k_{22} and k_{-22} are defined for the reaction

$$PH_2 + PH_2 \underset{k_{-22}}{\overset{k_{22}}{\rightleftharpoons}} P_2H_4 \quad K = k_{22}/k_{-22}$$

However

$$[PH_2] = (-1 + \sqrt{1 + 8KC_0})/4K$$

Then

$$\tau^{-2} = (8k_{22}{}^2/K)C_0 + k_{22}{}^2/K^2$$

Figure 8 shows a plot of τ^{-2} *vs.* C_0. The slope is $7.50 \times 10^{11}\ M^{-1}\ \mathrm{sec}^{-1}$ while the intercept $\sim 2 \times 10^6\ \mathrm{sec}^{-2}$. The slope to intercept ratio is equal to $8K$ which leads to an independent determination of K. Although this procedure leads to an imprecise determination of K,[21] it is useful for comparison with the more precise spectral value. We obtain $K \sim 5 \times 10^4\ M^{-1}$ which is in excellent agreement with the value obtained from spectral measurements. Using $K = 4.55 \times 10^4\ M^{-1}$, we obtain from the slope of Figure 8 that $k_{22} = 6.4 \times 10^7\ M^{-1}\ \mathrm{sec}^{-1}$ and $k_{-22} = 1.4 \times 10^3\ \mathrm{sec}^{-1}$.

These results may be compared with those obtained for the dimerization of the tetraethylenediamine derivative of protoporphyrin IX which is also water soluble.[20] As found for the protoporphyrin derivative, the monomer and dimer of TCPP have Soret bands at almost the same wavelength but of different molar absorptivities (per monomer unit). The equilibrium constant for dimerization found here is about an order of magnitude smaller than that obtained in the earlier study indicating that the presence of the phenyl rings, which are considerably out of the plane of the porphyrin nucleus,[22] interferes with dimerization. The forward rate constant obtained for the protoporphyrin derivative is $7.8 \times 10^7\ M^{-1}\ \mathrm{sec}^{-1}$ and the value for k_{-22} is $1.1 \times 10^2\ \mathrm{sec}^{-1}$. Therefore, the dimerization rate is near diffusion controlled for both porphyrins and differences in stability show up primarily in the dissociation rate constants.[20,23,24]

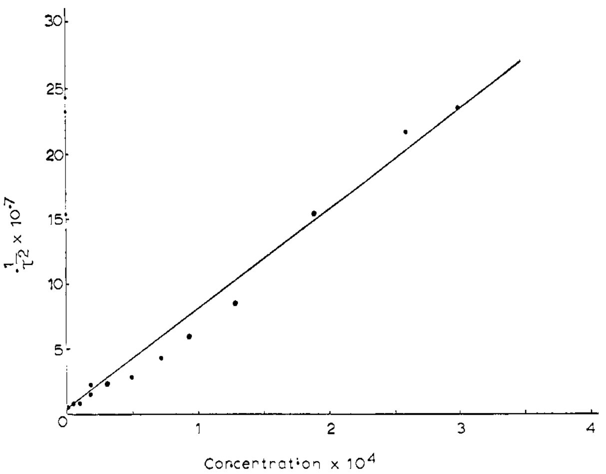

Figure 8. A plot of $1/\tau^2$ *vs.* total concentration of porphyrin for TCPP, pH 7.5, 0.1 M KNO_3.

(b) TPPS$_3$. Similar studies to those described for TCPP were carried out for $TPPS_3$ as well. In addition, because this porphyrin is soluble in acidic solutions, a number of experiments were carried out at pH <5. However, the properties of this porphyrin in acidic media prove to be very complicated as will be discussed later.[6]

At a pH of 7.5 as for TCPP, it was found that the spectral characteristics of $TPPS_3$ depend on concentration of porphyrin and ionic strength but not on the identity of the electrolyte. Beer's law experiments were run in 0.01 M Tris both with and without added KNO_3 and the results are shown in Figures 9 and 10. From the results of Figure 9, we obtain that $\epsilon_M = 4.00 \times 10^5\ M^{-1}\ \mathrm{cm}^{-1}$ at 413 mμ. Using this value of ϵ_M for solutions containing 0.1 M KNO_3, we obtained that $\epsilon_D = 2.46 \times 10^5\ M^{-1}\ \mathrm{cm}^{-1}$ and $K = 4.82 \times 10^4\ M^{-1}$. Relaxation experiments were conducted for $TPPS_3$ at pH 7.5 and the results are shown in Figure 11. The slope of the line is $8.10 \times 10^{12}\ M^{-1}\ \mathrm{sec}^{-2}$ and the intercept $\sim 3 \times 10^7\ \mathrm{sec}^{-2}$ leading to an approximate value of $K \sim 3 \times 10^4\ M^{-1}$ which is in good agreement with the more precise value obtained from spectral studies. Using $K = 4.82 \times 10^4\ M^{-1}$, we obtain $k_{22} = 2.2 \times 10^8\ \mathrm{M}^{-1}\ \mathrm{sec}^{-1}$ and $k_{-22} = 4.6 \times 10^3\ \mathrm{sec}^{-1}$. Again, the forward rate constant is near the diffusion-controlled limit and that the equilibrium constant for dimerization for $TPPS_3$ is less than that of the protoporphyrin IX derivative is manifested in the dissociation rate constant.

As the pH of $TPPS_3$ solutions is lowered from 7.5 to about 1, the detailed spectral changes are dependent on the total $TPPS_3$ concentration. Specifically, for very dilute solutions, there is a decrease in intensity of the Soret at 413 mμ with a concomitant absorbance increase at 435 mμ as the porphyrin converts from the PH_2 form to $PH_4{}^{2+}$. However, for more concentrated $TPPS_3$ solutions, not only does a band of Soret intensity develop at 435 mμ but one at 489 mμ as well.

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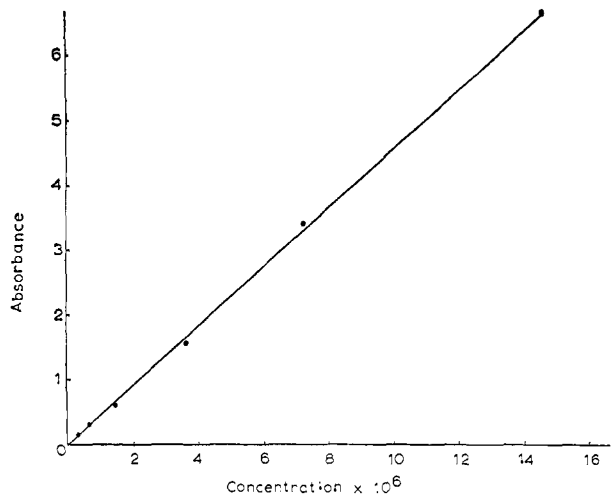

Figure 9. Beer's law experiments at 413 mμ for $TPPS_3$ in aqueous solution, pH 7.5, without added KNO_3.

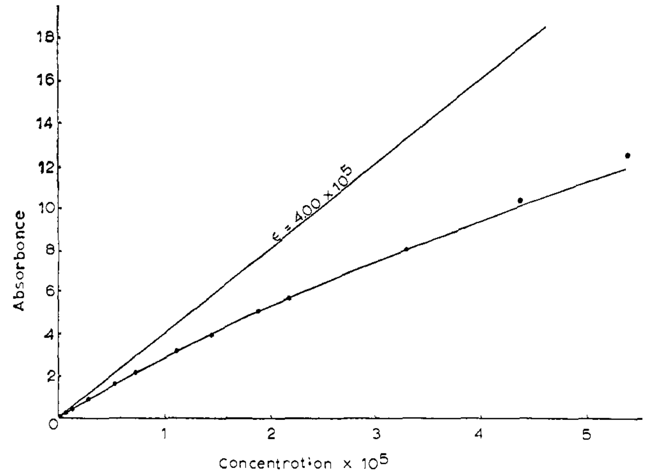

Figure 10. Beer's law experiments for $TPPS_3$ in aqueous solution, pH 7.5, with added KNO_3. The line marked ϵ = 4.00 × 10^5 defines Beer's law behavior for $TPPS_3$. The filled circles represent the experimental data and the line passing through these points is the theoretical curve obtained for the dimerization model with $\epsilon_D = 2.46 \times 10^5\ M^{-1}\,cm^{-1}$ and $K = 4.82 \times 10^4\ M^{-1}$.

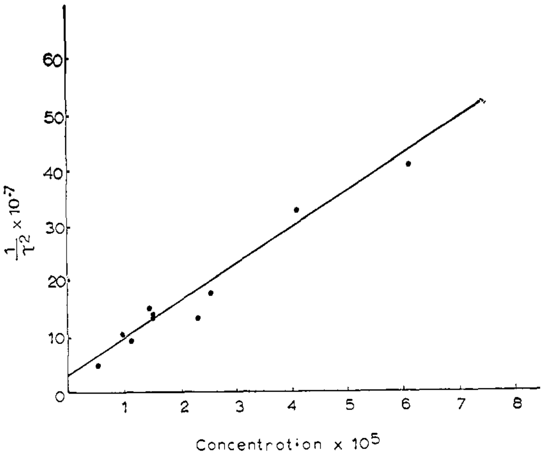

Figure 11. A plot of $1/\tau^2$ *vs.* total concentration of porphyrin for $TPPS_3$, pH 7.5, 0.1 *M* KNO_3.

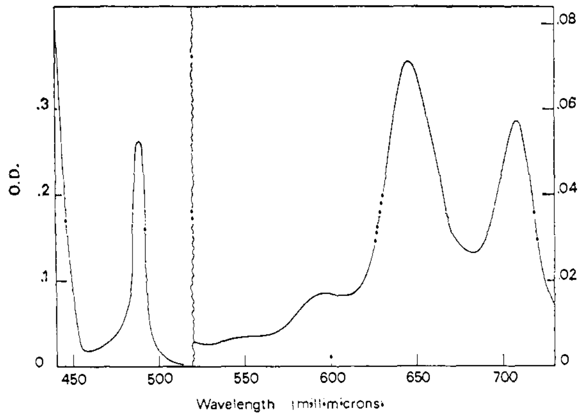

Figure 12. The visible spectrum of $TPPS_3$ in water, total concentration 1.8 × 10^{-6} *M*, pH 3.3, 0.1 *M* KNO_3. Not shown is the Soret band at 435, absorbance 0.69.

This situation is quite similar to that obtained in ethylene glycol for the PH_4^{2+} species. When the total concentration of the porphyrin is raised to above 10^{-6} *M* keeping other conditions identical, peaks at 489 and 710 mμ become quite prominent (Figure 12). Unlike the situation for the free base TPPS form, the intensities of the various peaks at pH ~1–3 depend not only on concentration and ionic strength but on the identity of the electrolyte with the 489-mμ peak being especially sensitive to the nature of the dissolved electrolyte. For a solution in which the porphyrin concentration is 2.0 × 10^{-5} *M*, pH 2.8, and ionic strength 0.1 *M*, the intensity of the 489-mμ peak decreases in the order $LiCl > KCl > KNO_3 > Ca(NO_3)_2$. Furthermore, the intensity of the 489-mμ peak decreases with increasing concentration of a given electrolyte above $\mu \sim 0.1$ *M*. We have found that the 489-mμ peak disappears entirely in 3 *M* $NaClO_4$.

At concentrations below 10^{-6} *M*, $TPPS_3$ appears monomeric in acidic solutions on the basis of Beer's law experiments. We attempted a spectrophotometric titration in this concentration range to determine the p*K* of the porphyrin and the number of protons, *n*, added by the porphyrin PH_2 species.[6] We obtained a p*K* ~ 4.8 for $TPPS_3$ which is the value obtained for the tetrasulfonated species but we found that *n* had nonintegral values, usually between 1.0 and 1.5, and that the apparent p*K* had a wavelength dependence. These results will be discussed in greater detail in the next section.

We conclude that the trisulfonated TPPS in aqueous solution dimerizes as does tetracarboxylated TCPP but unlike the tetrasulfonated TPPS which has been reported as being monomeric.[6] The equilibrium constant for dimerization for $TPPS_3$ is almost identical with TCPP and the forward rate constants in both cases are near the diffusion-controlled limit. In the acidic region, the behavior of TPPS is very complicated and there appears to be specific interactions between dissolved electrolytes and the porphyrin. The p*K* for TPPS is about 5 and an approximate value of 5 was obtained for TCPP before it precipitated. For TCPP, as for TPPS, a Soret band at about 435 mμ began to appear as the pH approached 5. This is in contrast to TPyP and TMPyP which have p*K* values of about 2.[5,25] The formal charge type of the meso-substituted porphyrin appears to have a pronounced effect upon the

[25] E. B. Fleischer and L. E. Webb, *J. Phys. Chem.*, 67, 1131 (1963).

pK of the porphyrin and, as will be discussed later, on other solution properties as well.

(c) TMPyP. Beer's law experiments were carried out at pH 0 and 7.2 where the predominant species are PH_4^{2+} and PH_2, respectively. Consistent with an earlier report,[5] both species obey Beer's law over an extended concentration range (Figure 13) indicating that TMPyP exists in aqueous solution as a monomer throughout an extensive pH and concentration range. Temperature-jump studies were attempted for TMPyP at pH 0 and 7.5 and no relaxation effects were obtained. This result further corroborates the spectral finding that TMPyP exists in solution as a monomer with no aggregation equilibrium to perturb with a sudden temperature rise.

Spectrophotometric titrations were attempted to determine the pK and the number of protons added to PH_2 to form the acid form of TMPyP. We carried out the experiment several times and never obtained n = 2.0 as had been reported earlier.[5] Similar to the results obtained for $TPPS_3$, we found nonintegral values for n, varying from 1.1 to 1.5 and showing a wavelength dependence. Furthermore, as for $TPPS_3$, we found that instead of an isosbestic point, there is a narrow region of intersection for the Soret bands of the acid and basic forms. We are led to conclude that while the stable acidic form of this porphyrin is PH_4^{2+}, the two protons are not added simultaneously.[26]

TMPyP undergoes a color change[5] at pH 14 forming the species PH^- having a Soret band at 450 mμ and visible bands at 577 and 623 mμ. However, for TCPP and $TPPS_3$ the four-banded spectrum characteristic of the PH_2 species was obtained in strongly alkaline solution. For $TPPS_3$ the bands shifted from 413 to 412, 518 to 520, 554 to 558, 580 to 589, and 637 to 648 mμ when the pH of the solution was raised from 7.5 to 14. For TCPP, peak shifts of less than 5 mμ were observed in comparing a solution of pH 7.5 to one which was 6 M in NaOH. Beer's law experiments were conducted for TMPyP at a pH of 14 at 450 mμ over a concentration range of 6.0×10^{-7}–9.0×10^{-6}. Over this range, Beer's law is obeyed.

In summary, we conclude that whereas none of the porphyrins studied aggregate at pH ~7.5 in 80% ethylene glycol–water mixtures, TCPP and $TPPS_3$ dimerize in water while TMPyP does not. Both TCPP and $TPPS_3$ contain negatively charged groups on their periphery while TMPyP contains positive groups at these positions. The charge type of the peripheral groups has been shown to have a very large effect on the acidity and basicity of the porphyrin and, more particularly, of the pyrrole nitrogens. The cationic porphyrins (TMPyP and TPyP) are considerably less basic than the anionic porphyrins and, where evidence exists, considerably more acidic. TMPyP and TPyP add protons only some three pH units below the region in which $TPPS_3$ and TCPP add protons; TMPyP forms a PH^- species whereas $TPPS_3$ and TCPP do not. Now an additional property correlates with charge type for these meso-substituted porphyrins; the anionic porphyrins aggregate in water while the cationic porphyrins do not. It may be that the positive centers on the periphery cause the delocalized π-electron cloud to be more diffuse over the surface of the molecule while negative centers lead to a partial localization of electron density near the center. This localized electron density would make the center a more attractive site for protons thereby increasing the basicity and decreasing the acidity of the species and would lead to stronger van der Waal's interactions for a stacking-type dimer.

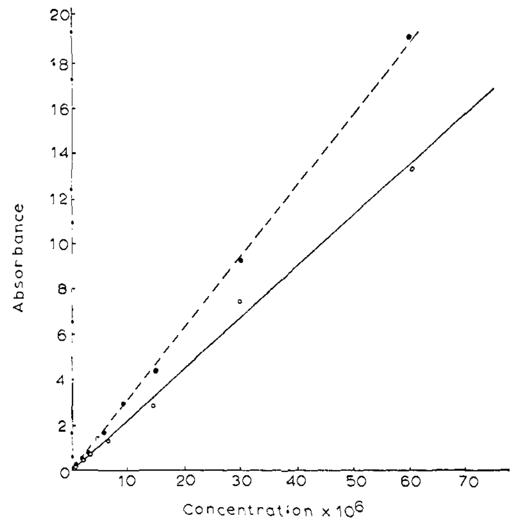

Figure 13. Beer's law experiments for TMPyP in aqueous solution, 1 M ionic strength: (- - -) pH 0, 446 mμ; (——) pH 7.2, 424 mμ.

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(26) A very detailed study of the acid–base equilibrium involving a sample of TMPyP supplied by us has led to the same conclusion. Two pK values have been determined: 1.51 for the ionization of PH_3^+ (pK_3) and 0.62 for the ionization of PH_4^{2+} (pK_4). The sum of these values pK_3 + pK_4 = 2.13 in excellent agreement to the value found for this sum earlier[5] but by using a model of a one-step, two-proton addition: T. Wirth and R. A. Plane, private communication.