High-Resolution Spectroscopy of Porphyrins

Porphyrins are a class of molecules that not only have many interesting properties themselves but are constituents of some larger, biologically important molecules such as hemoglobin. A detailed knowledge of the electronic structure of porphyrins will contribute to understanding the physical basis of the biological functions of molecules containing porphyrins. Spectroscopic studies at APL involving both conventional and laser optical spectroscopy and electron-spin-resonance spectroscopy are described.

B. F. Kim
and
J. Bohandy

Introduction

Porphyrins form a class of compounds that are among the most ubiquitous in nature and that display a variety of chemical and physical properties. They absorb light in the visible and ultraviolet regions of the spectrum; some exhibit luminescence; some are catalysts or photosensitizers; and some exhibit paramagnetism, photoconduction, and semiconduction. Thus, it is not surprising that they are vital components of and play many diverse roles in biochemical systems.

The parent compound of all porphyrins is called porphin. The basic configuration is an aromatic structure that can have either two hydrogen atoms (free base porphin) or one metal atom (metalloporphin) in the center of the structure. The chemical structure of a metalloporphin is shown in Fig. 1a, where M represents one of many possible metals. An example of the spectrum of a metalloporphin is given in Fig. 1b. Different compounds in this class of molecule are distinguished by different metal ions and by molecular hydrocarbon chains or other chemical subspecies that may be attached to the periphery of the basic porphyrin structure at the 1 to 8 and α, β, γ, δ hydrogen positions. In some cases, the hydrocarbon chains or other chemical subspecies play a role in the function of the porphyrin. In other cases, the side chains serve only to attach the porphyrin structure to a chemically inert substrate such as a large protein molecule. In some highly sophisticated biochemical systems, the large substrate serves to place the chemically active porphyrin into the proper spatial position for the porphyrin to interact with another chemically active species.

The metal ion can greatly influence the chemical activity of the porphyrin species. A familiar biochemical process in which a metalloporphyrin plays a major role is photosynthesis, which converts solar energy into chemical energy for plants. Here, the principal active chemical species is the chlorophyll molecule, which contains porphyrin rings with a magnesium ion in the center. Figure 2a shows the structure of chlorophyll *a*, one type of chlorophyll.

Vitamin B_{12} is another example of a metallo-

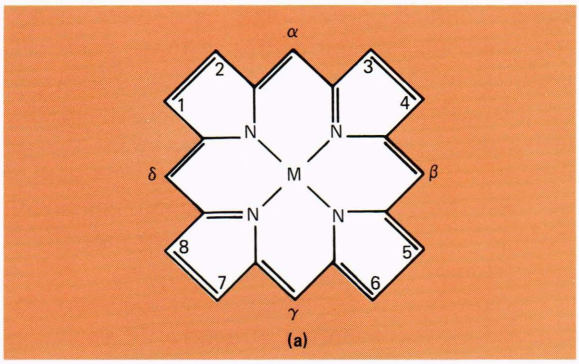

Fig. 1 (a)—The structure of a metalloporphin molecule. A carbon and a hydrogen atom are understood to be at each apex not attached to a nitrogen atom.

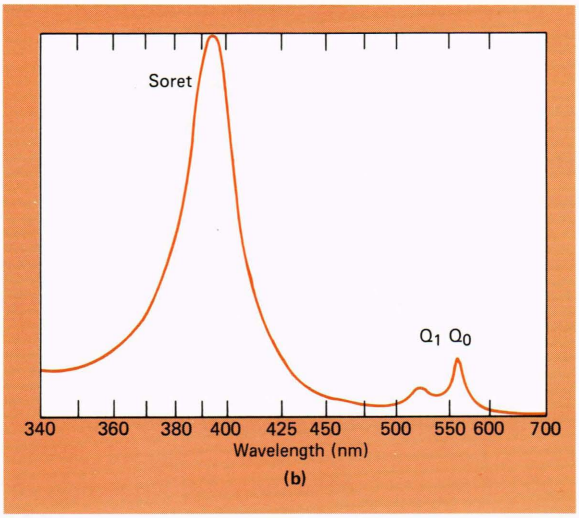

Fig. 1 (b)—A typical broadband absorption spectrum of a metalloporphin.

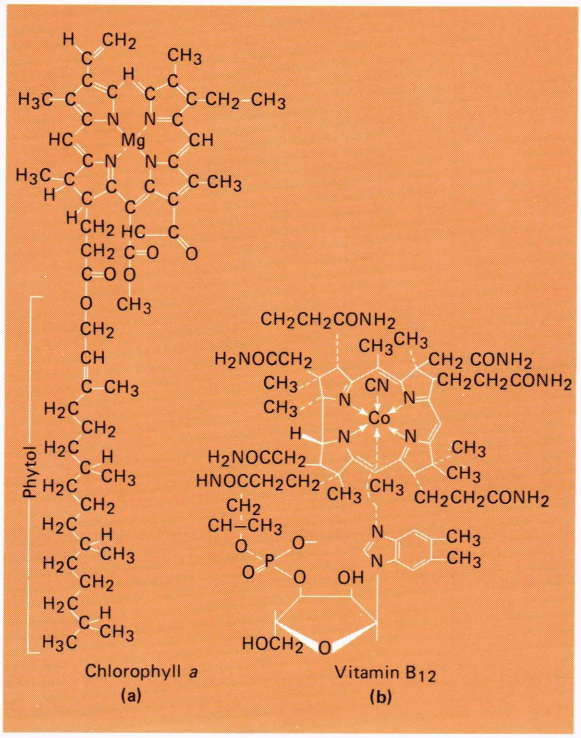

Fig. 2—The structure of two important molecules that contain a metalloporphyrin.

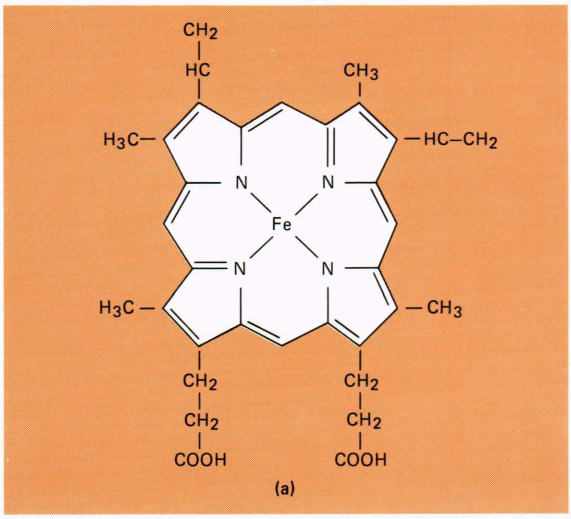

Fig. 3 (a)—The structure of heme (iron protoporphyrin), which picks up oxygen in the lungs and releases it in the capillaries.

porphyrin that occurs in enzymic catalysis in the human body. Its structure, shown in Fig. 2b, is a cobalt porphyrin complex. It is essential for normal growth in humans and is used to treat pernicious anemia.

One of the most important and widely studied metalloporphyrins is iron porphyrin. The complex shown in Fig. 3a is iron protoporphyrin, or heme; it forms the chemically active group of the heme proteins hemoglobin, myoglobin, and the cytochromes. Hemoglobin, of which there are about 280 million molecules in one red cell, carries oxygen, in the blood, from the lungs to tissues. Myoglobin stores oxygen in muscle, particularly in marine animals. Cytochromes are found mainly in the mitochondria of cells; they play an important role in the process called oxidative phosphorylation in which foodstuffs are converted into stored biochemical energy. The heme group is attached to the rest of the protein by a bond between the iron atom and a nitrogen atom of a

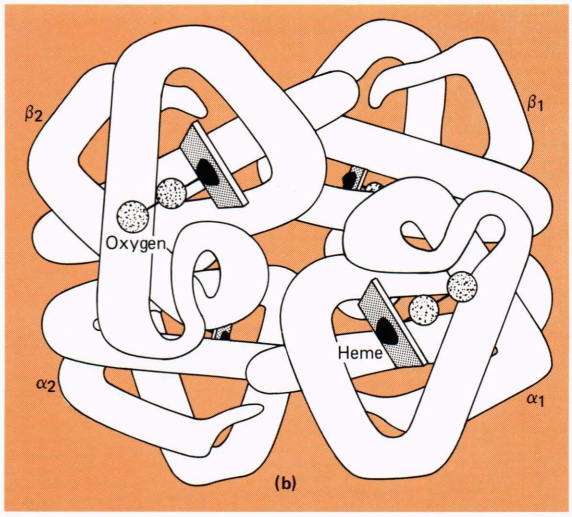

Fig. 3 (b)—A representation of an entire hemoglobin molecule consisting of four protein chains: two identical alpha chains and two identical beta chains.

histidine amino acid residue. In each case, however, the chemical activity takes place in the heme group. Figure 3b shows schematically an entire hemoglobin molecule with its four protein chains.

The variety of chemical properties that porphyrins exhibit correlates with the details of their structure. Thus, porphyrins with different metal ions or different side groups will generally exhibit chemical and physical properties peculiar to their chemical structures. Needless to say, their structures are tailored to optimize their performance in biochemical systems. For example, two hydrogen atoms on the periphery of the porphyrin structure in chlorophyll change the electronic structure so that the molecule absorbs more light in the red-green region of the spectrum. This allows chlorophyll to absorb more solar energy than unreduced porphyrins do and makes the molecule more efficient in its photosynthesis role.

Thus, this class of porphyrin compounds is of great interest as a subject of scientific research not only to increase our knowledge of biochemical systems, but also because of the opportunity it provides to study relations between chemical structure and chemical function.

Of particular interest in the Applied Physics Laboratory (APL) of The Johns Hopkins University are spectroscopic studies of the quantum structure of molecules. We know from the principles of quantum mechanics that govern such microscopic species as molecules that species consisting of bound particles exist in discrete energy states, and that the properties of these states are described by standing wave functions. The states are characterized with respect to modes of the nuclear vibrations, modes that describe the charge distribution of the electrons, and magnetic modes for those states that have a magnetic moment. ("Mode" as used here refers to the customary description of a standing wave structure.)

Spectroscopy is an experimental methodology that probes the quantum structure by detecting changes in the quantum states when the molecule absorbs energy to achieve a higher energy state or when it loses energy when it decays into a lower energy state. The energy absorbed or lost is in the form of electromagnetic radiation. The energy is related to the frequency of the radiation by the Einstein relation

$$E = h\nu$$

where h is Planck's constant and ν is the frequency.

Spectroscopies differ principally in the magnitude of the energies observed. One might classify spectroscopies as gamma ray, X ray, ultraviolet, visible, infrared, microwave, and radio-frequency. Studies conducted at APL have used visible (optical) spectroscopy and microwave spectroscopy. The latter technique is called electron spin resonance (ESR). Energies in the range of 200 to 1200 nm are involved in optical spectroscopy. In this range, transitions between the electronic states can be observed.

Figure 4 shows a diagram of typical energy levels. In absorption, one observes transitions from the ground state to the various excited states of the molecule. Normally, a molecule in an excited state decays to the first or lowest excited state, with the energy loss in the molecule being spent in the form of heat. The subsequent decay from this state to the ground state is often accompanied by the emission of electromagnetic radiation, referred to as luminescence. In this way, it is possible to observe vibrational energy levels in both the excited electronic and ground states, as indicated in Fig. 4.

ESR is a measurement technique that is used when the ground state contains a magnetic moment. Here, the ground state consists of a pair of states, each with a magnetic moment oriented in opposition to the other. In the absence of a magnetic field, the two states have the same

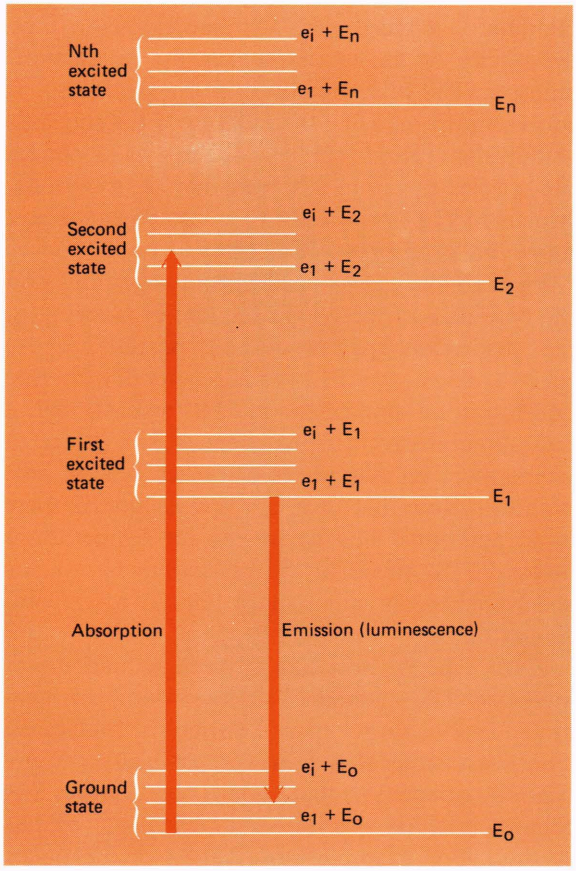

Fig. 4—Absorption and emission processes. The E's are electronic energy levels, typically greater than 16 000 cm^{-1} for porphyrins. The e's are vibrational energy levels superimposed on the E's. For porphyrins, the e's are less than 1700 cm^{-1}.

energy. However, in the presence of a magnetic field, the energies of the two states are different; if microwave energy of the proper frequency is imposed on the sample, energy will be absorbed by the molecules, causing a transition from the lower component of the doublet to the higher energy component. The application of these general techniques to the study of porphyrin molecules will be described in more detail later.

Much work has been done in the field of porphyrin spectroscopy. The spectrum shown in Fig. 1b is typical of most metalloporphyrin spectra. The strong band at approximately 400 nm, called the Soret band, is common to all porphyrins. The weaker bands in the region of 500 to 600 nm are called "Q bands." The structure of this region is similar for most metalloporphyrins although the reduced porphyrins, such as chlorophyll, exhibit the departures from this structure described previously. The region of the Q bands has been the principal focus of spectroscopic study in this and other laboratories.

The apparent simplicity of the spectrum shown in Fig. 1b is not appropriate for a molecule with the structural complexity of porphyrin. The spectra should be rich in spectral components (lines) due to energy transitions between vibrational states. In fact, there are many components in porphyrin spectra, but they are masked or hidden beneath the broad spectral structure shown in Fig. 1b. Thus, the broad character of such spectra is undesirable because it obscures the details of the quantum structure of the molecules. APL has directed its recent efforts in this field toward obtaining detailed porphyrin spectra by eliminating or reducing the factors that are responsible for the broadband character of the spectra.

Sharp-Line Spectra

Two principal mechanisms cause the broad bands in the recordings of many porphyrin spectra. One is thermal broadening, which results when the spectra are observed with the sample at high (room) temperature. Thermal broadening is easily eliminated by subjecting the sample to the temperature of liquid helium (4.2 K) or lower. The second is nonuniform solvent-molecule interactions. The solvent molecules exert forces on the guest porphyrin molecules that cause small changes in the porphyrin energy structure. Since the porphyrins are in a random environment in a liquid solvent, each porphyrin will be in a different "solvent site" and experience different forces from the solvent molecules at any given time. Thus, the energy structure will be slightly different for each porphyrin. This broadening effect may be thought of as being due to a continuous distribution of inequivalent porphyrin site species in the host solvent. The corresponding distribution of sharp-line spectra is then recorded as a broadband spectrum. This process of inhomogeneous broadening is depicted in Fig. 5a.

The problem of inhomogeneous broadening requires the proper choice of a solvent matrix. There are two principal classes of hosts for this purpose: frozen inert gas matrices[1] and crystalline matrices.

[1] L. L. Bajema, M. Gouterman, and B. Meyer, "Absorption and Fluorescence Spectra of Matrix Isolated Phthalocyanines," *J. Mol. Spectros.* **27**, 225–235 (1968).

In the first case (sometimes referred to as the method of matrix isolation), the solvent interactions are reduced in magnitude; thus, shifts in the spectra due to such interactions are correspondingly reduced. In the second case, the periodicity of the matrix makes the interactions uniform. The latter method, in an ideal case, is equivalent to reducing the number of the solvent's inequivalent porphyrin site species to a single-site species by using a solvent having a periodic structure. The spectra are then equivalent, and a single line is observed (Fig. 5b).

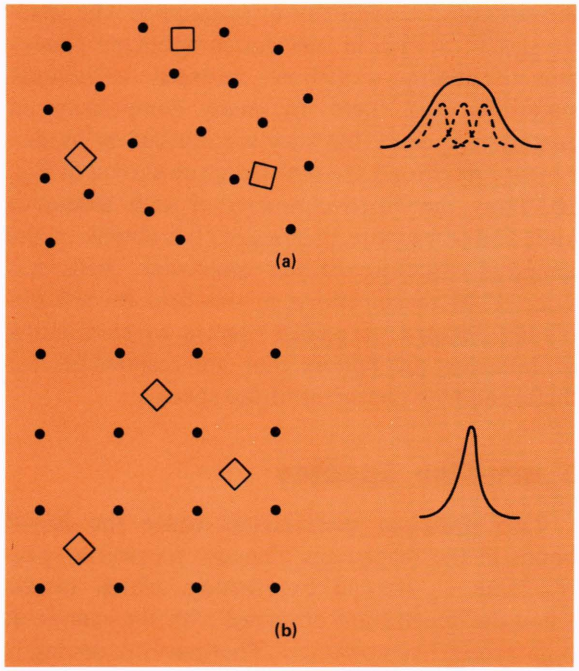

Fig. 5—Inhomogeneous broadening.
(a) The observed spectrum is a superposition of slightly displaced individual lines.
(b) A periodic matrix or lattice makes each site equivalent and one sharp line is observed.

Figure 6 illustrates the effects of temperature and the type of host matrix on the line broadening of the absorption spectra of zinc porphin. These spectra, taken at APL, were recorded with zinc porphin in an amorphous matrix (stycast) and a crystalline matrix (triphenylene). The effect of temperature on zinc porphin in each type of host is shown. Note that in an amorphous matrix there is no additional line narrowing for temperatures below 77 K, while in the crystalline matrix the spectrum reveals many sharp lines at a temperature of 4.2 K.

Each type of host has advantages and disadvantages. The method of matrix isolation is generally applicable to a large variety of molecules. The guest molecules, however, are generally not spatially oriented so that certain observations that require oriented species cannot be made by this method. For example, observation of the polarization of absorption spectra requires samples with oriented molecules. For this reason, work in APL has used crystalline host materials.

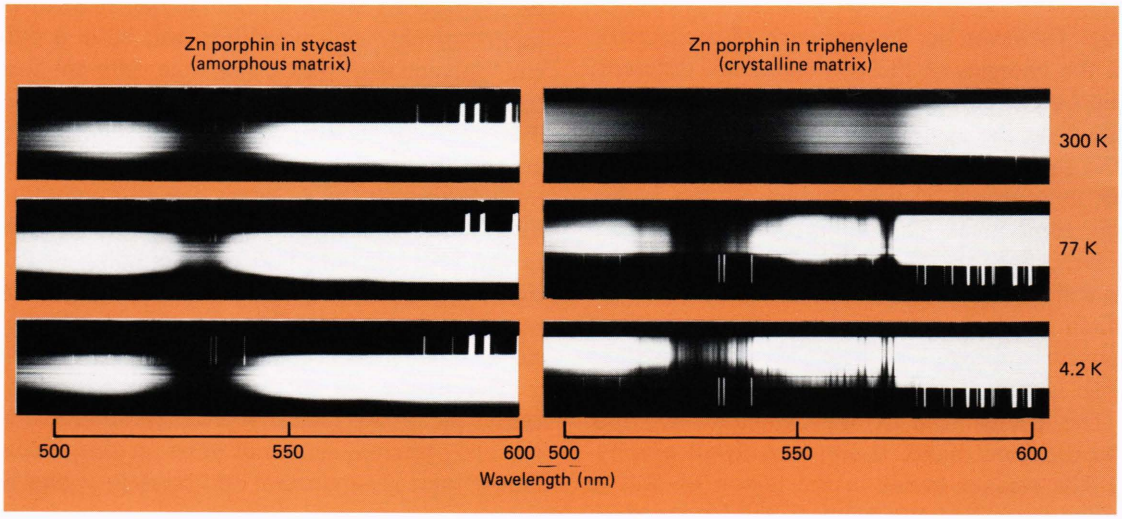

Fig. 6—A comparison of the optical absorption spectra of zinc porphin in an amorphous and crystalline host at room temperature, at 77 K, and at 4.2 K. Note that there is no appreciable line narrowing of the spectrum in the amorphous host in going from 77 K to 4.2 K.

Historically, the crystalline host materials used in porphyrin spectroscopy have been principally the n-paraffins, particularly n-octane. The use of these materials, which were first advocated and employed extensively by Soviet workers[2,3], is sometimes referred to as the Shpol'skii method. The materials are not crystalline at room temperature; thus there are some problems in handling samples of them, which are typically prepared by dissolving the porphyrins in n-octane and lowering the temperature to 77 K or 4.2 K (the temperatures of liquid nitrogen and liquid helium, respectively). In most cases, the samples are polycrystalline and thus yield nonoriented guest molecules. In some cases, single-crystal samples have been prepared[4] and Zeeman effect studies made. However, polarized absorption spectra have not been reported with these samples.

APL has recently found aromatic crystalline host materials suitable for spectroscopic studies of porphyrins. Most of the work to date has been done with triphenylene. Single-crystal specimens are prepared by slowly evaporating solutions. Triphenylene is dissolved in the appropriate solvent with a small amount of the required porphin, e.g., zinc porphin. After a period ranging from a few days to two weeks, suitable crystals are obtained—in this particular case, triphenylene doped with zinc porphin. The use of lightly doped crystals not only eliminates nonuniform solvent-molecule interactions but reduces dipolar interactions between adjacent porphin molecules by increasing the distance between them. These materials yield sharp-line optical spectra of the guest porphyrin molecules.

This is the first use for porphyrin spectroscopy of crystalline materials that are not in the class of normal paraffins. Such materials have an important advantage over n-paraffins in that they are crystalline at room temperature. This allows relative ease in orienting and manipulating the samples for study and, possibly, more controlled crystal growth than is possible for the normal paraffins.

Figure 7 shows spectra of zinc porphin, copper porphin, and vanadyl porphin in single crystals of triphenylene at 4.2 K[5,6]. Note that the spectra are polarized, indicating the presence of oriented molecules. The polarization refers to the orientation of the electric vector of the absorbed light radiation with respect to the crystal optic axis.

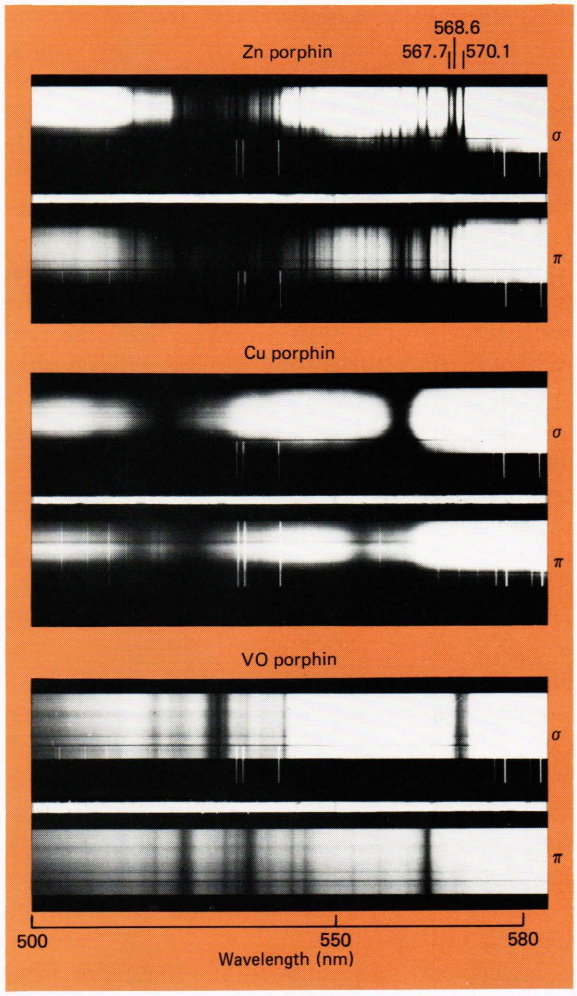

Fig. 7—A comparison of the polarized optical absorption spectra of zinc, copper, and vanadyl porphin in single crystal triphenylene at 4.2 K. (π and σ indicate that the electric vector of the incident light is parallel and perpendicular, respectively, to the crystallographic c axis.)

[2] E. V. Shpol'skii, A. A. Il'ina, and L. A. Klimova, "Fluorescence Spectrum of Coronene in Frozen Solutions," *Dokl. Akad. Nauk. SSSR* **87,** 935 (1952).

[3] A. T. Gradyushko, V. A. Mashenkov, and K. N. Solov'ev, "Study of Porphin Metal Complexes by the Method of Quasi-Line Spectra," *Biofizika* **14,** 827–835 (1969).

[4] I. Y. Chan, W. G. Van Dorp, T. J. Schaafsma, and J. H. van der Waals, "The Lowest Triplet State of Zn Porphin," *Mol. Phys.* **22,** 741–751 (1971).

[5] B. F. Kim, J. Bohandy, and C. K. Jen, "Polarized Sharp Line Absorption Spectra of Zn Porphin," *J. Chem. Phys.* **59,** 213–224 (1973).

[6] J. Bohandy, B. F. Kim, and C. K. Jen, "Optical Spectra of Cu Porphin and VO Porphin in Single Crystal Triphenylene," *J. Mol. Spectros.* **49,** 365–376 (1974).

This was the first observation of the polarization of sharp absorption spectra for a porphyrin molecule. The polarization of spectral transitions is of great importance in classifying transitions. It is a parameter in the selection rules of spectral transitions of particular symmetry types. Selection rules determine whether or not a transition can occur between two states with the absorption or emission of electromagnetic radiation. This information, therefore, provides a crucial test for confirming the classifications of the energy states. Another noteworthy observation on Fig. 7 is that the metal in these porphyrins has a significant effect upon the spectra, although these effects are not resolved in the usual broadband spectra. This illustrates the importance of eliminating the factors that cause spectral broadening.

An important disadvantage in using crystalline host materials for studying porphyrin spectra has been the occurrence of more than one type of porphyrin site species in the host crystal. Although ideally a single type of guest porphyrin site species would exist in the host crystal, in actual practice more than one guest site species may be present. The number of different types of site species is small, however, and does not result in broadband spectra. The consequences of this are important, nevertheless, because the presence of several guest site species affects the ability to make valid interpretations of the spectra. The perturbations of the host lattice on the guest porphyrin molecules cause the resulting spectra from different site species to be slightly different, as discussed above. This introduces difficulty in interpretation since the spectra from different site species may overlap in some regions and be indistinguishable from one another. The problem is common to any of the crystalline hosts, including the normal paraffins.

It has been determined that there are three principal site species of zinc porphin in triphenylene (each of the three lines indicated in Fig. 7 at 570.1, 568.6, and 567.7 nm belongs to a different site species). Thus, there are approximately three times as many spectral lines in this spectral recording as should be present for a single site species. A similar condition exists for the corresponding luminescence spectra. The difficulty in making detailed spectral assignments from such multiple site spectra has generally prevented full exploitation of the use of crystalline hosts to obtain sharp-line porphyrin spectra.

In addition to the confusion that results from trying to assign spectral components in multiple site spectra, a more fundamental difficulty in the interpretation of sharp-line spectra has existed that is intimately related to the problem of multiple-site spectra. Although several inequivalent types of site species will result in several closely spaced spectral lines in a given spectral region, it is possible that such a grouping of lines, called a multiplet, may not be due to the existence of several different types of site species. Some workers[7], in fact, have expressed the opinion that multiplet structure may be intrinsic to a single site species. Others[8] have done theoretical studies that attribute multiplet structure to a single site species. Thus, the interpretation of multiplet structure is of fundamental importance in the theory of porphyrin spectra. The resolution of this problem requires the ability to observe the spectra of a single type of site species.

Single-Site Spectra

Techniques for laser excitation of luminescence have been used at APL to investigate the problem of multiple site spectra. There are two specific procedures in which these techniques are employed. In one, a single sharp absorption line is excited, and the corresponding luminescence spectrum is recorded by photoelectric detection. This spectrum corresponds to the particular porphin species that is excited. The process is repeated for other absorption lines within a multiplet. In this way, the luminescence spectra for the various species of porphin sites that might exist in the sample are recorded separately. In the second procedure, a single sharp luminescence line is used as a signature for a particular species of porphin site. The line is isolated from the luminescence spectra by a spectrograph and detected photoelectrically. The excitation source scans in wavelength the region of the absorption spectra. When the excitation wavelength coincides with an absorption line of a porphin site species corresponding to the luminescence signature, the

[7] R. I. Personov and O. N. Korotoev, "The Nature of Multiplets in the Quasiline Spectra of Organic Molecules," *Sov. Phys. Dokl.* **13,** 1033–1036 (1969).

[8] L. V. Iogansen, "Lower Electronic Levels of Porphyrins and Phthalocyanines in a Model of Collective Excitation," *Dokl. Akad. Nauk. SSSR* **207,** 605–607 (1972).

luminescence is detected. Hence, the absorption spectra of a particular porphin site species are recorded. By using different luminescence lines in a multiplet for detection signatures, the absorption spectra for the various species of porphin sites are recorded separately. The two procedures assure unambiguous correlation between the absorption and luminescence spectra for a site species.

The method requires an excitation source of high intensity, narrow spectral bandwidth, and variable wavelength output. A scanning tunable dye laser meets these requirements. Figure 8 shows the use of such a laser in exciting and detecting luminescence. An AVCO nitrogen ultraviolet pulsed laser operating at 337.1 nm is used to pump the dye laser. The peak power for the nitrogen laser is 100 kW with a maximum repetition rate of 100 pulses/s. Wavelength tuning of the dye laser is accomplished by using an optical grating as one of the reflectors in the laser cavity. A broadband dielectric reflecting mirror with 50% reflectivity from 400 to 700 nm is used as the output reflector of the laser cavity. Wavelength scanning is provided by a precision sine grating drive. The proper combination of dyes allows an output than can be tuned from approximately 400 to 680 nm. The bandwidth of the dye laser's output in its present configuration is less than 0.1 nm and can be made smaller. The wavelength coverage and bandwidth of the laser are adequate for these studies of porphin.

The peak intensity of the dye laser (several kilowatts) is sufficient for this work, although the average intensity (several milliwatts) is too low for excitation in luminescence spectroscopy. The problem is overcome by suitable signal-acquisition techniques, principally by using a boxcar integrator. The instrument, which is essentially a gated integrator, can be used to enhance the signal-to-noise ratio by gating the recorded signal to coincide in time with the luminescence signal (thereby eliminating the noise that occurs between signal pulses) and by signal averaging with suitable time constants and scan rates. In conjunction with the appropriate phototube, a dual-channel PAR boxcar integrator is used to detect luminescence. The luminescence is detected with one channel while the laser output is monitored with the other. The ratio of the two signals can be taken automatically in real time, allowing greater accuracies than would otherwise be possible and

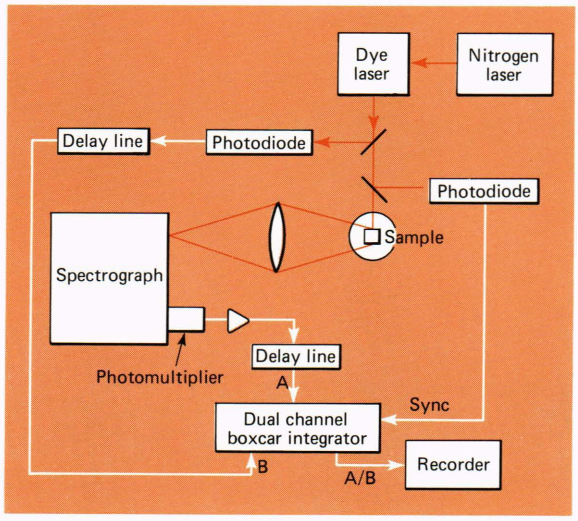

Fig. 8—A block diagram of the apparatus used for laser excitation of luminescence.

eliminating the effects of wavelength-independent pulse fluctuations in the excitation source.

The spectra of zinc porphin have been reexamined using the site-selection spectroscopic techniques described above[9]. Each of the three components of the triplet structure has been shown by single-site spectroscopy to originate from a different site species. Figure 9a shows the luminescence spectrum of zinc porphin in triphenylene obtained with conventional broadband excitation; Figs. 9b, 9c, and 9d show the results obtained with narrowband excitation at 570.1, 568.6, and 567.7 nm. One sees that the sum of the lower three spectra is equal to the spectrum recorded with broadband excitation. Excitation spectra of each of the three sites were also recorded. Thus, the multiplet structure of zinc porphin was accounted for by the existence of three inequivalent site species, and no multiplet structure was observed that was intrinsic to a single site species. For zinc porphin, this result refutes the theoretical work of Iogansen[8] and Personov and Korotoev[7], which attributes multiplet structure to equivalent porphin molecules. A determination of the origin of multiplet structure in high-resolution spectra of porphyrins was one of the principal goals of the work.

The elimination of confusion in the spectra, caused by the presence of spectral lines from dif-

[9] B. F. Kim and J. Bohandy, "Single Site Spectra of Zn Porphin in Triphenylene," *J. Mol. Spectros.* **65,** 413 (1977).

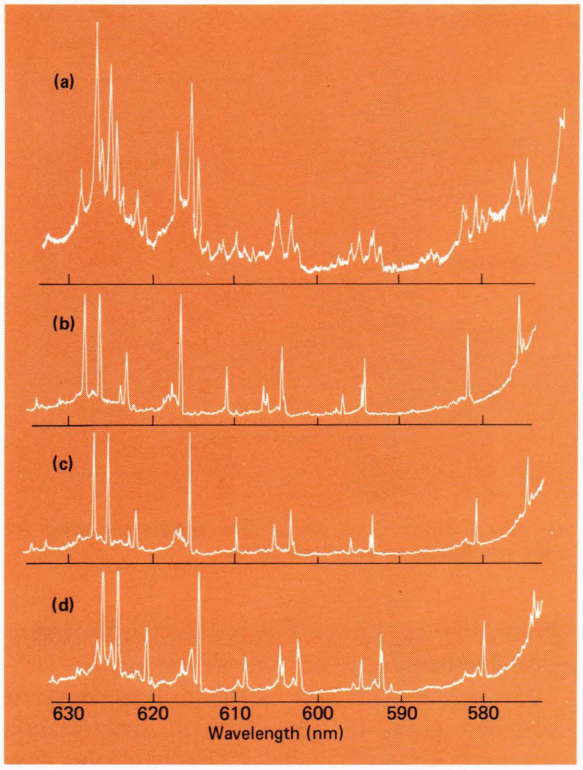

Fig. 9—Luminescence spectra of zinc porphin in triphenylene at 4.2 K: (a) shows broadband excitation; (b), (c), and (d) show excitation at 570.1, 568.6, and 567.7 nm, respectively.

ferent site species, made possible unambiguous classifications of electronic and vibrational (vibronic) absorption and luminescence spectra. The results were compared with the available theory of vibronic spectra of porphins and were found to agree within the limits imposed by approximations in the theory. Although significant physical insight into the vibronic structure of zinc porphin resulted from the study, the experimental data were not fully exploited because the state of the art of experimental studies, at present, exceeds that of theoretical studies. More detailed theoretical descriptions of vibronic spectra are possible and, with the advent of these advances in experimental methodology, will undoubtedly be forthcoming.

The method for obtaining single-site spectra is independent of the structural details that distinguish inequivalent porphyrin site species. Thus, no direct information was provided on the structure of a particular site species. In order to obtain some measure of the nature of the different site species in zinc porphin, a series of samples was prepared, each using a different solvent. It was found that the number and wavelengths of lines in a principal spectral multiplet were different for samples prepared with different solvents. Figure 10 illustrates this, showing the multiplet corresponding to the pure electronic transition in luminescence. Each line of the multiplet belongs to a different zinc porphin site species. Since the host material, triphenylene, was the same for each sample, one concludes that the different site species are the result of chemical ligands (coordinate group bonds) introduced by the solvents used in crystal growth. The line at 567.7 nm, which occurs with the sample prepared without solvents (from a melt), also occurs with most of the other samples. The line apparently belongs to a site species that has no chemical ligand. The identification and spatial orientation of the chemical ligands were not determined in these preliminary results. The results are nevertheless

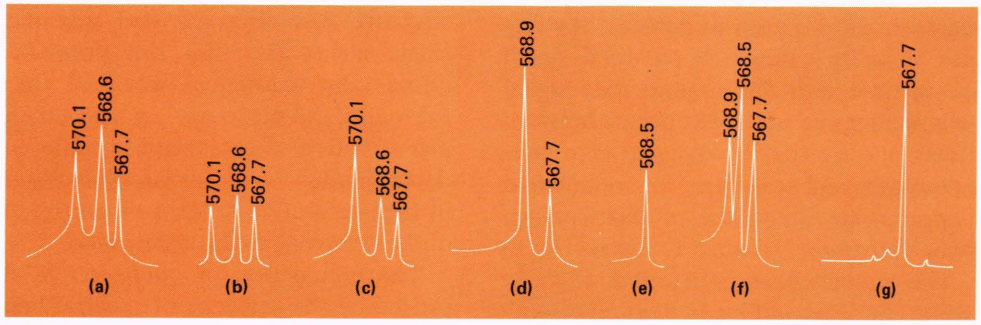

Fig. 10—Fluorescence spectra of the 0–0 multiplet of zinc porphin in triphenylene at 4.2 K for samples with different solvent preparations: (a) iso-octane, methanol, and dichloro-ethane; (b) iso-octane and dichloro-ethane; (c) dichloro-ethane; (d) n-octane; (e) benzene; (f) n-octane and benzene; and (g) no solvent (prepared from melt).

significant because they indicate an avenue for further research that can provide basic knowledge of the physical basis for the chemical behavior of porphyrins.

Electron Spin Resonance

A complete interpretation of the optical spectra requires a knowledge of the orientation of the porphyrin molecules in the host crystals. Iron, copper, and vanadium are transition metals that have unpaired electrons in their ground-state configuration and, as such, are paramagnetic. ESR provides a sensitive technique for probing the local environment of paramagnetic species in crystals. In its simplest form, ESR can be described as follows (see Fig. 11): If an electron is situated in an external magnetic field, H, the interaction energy between the electron magnetic moment and the applied field is given by

$$E = g\beta HS_z$$

where S_z is the component of the spin angular momentum of the electron along H, β is a constant called the Bohr magneton, and g is a dimensionless constant termed the electron g factor. S_z can have the values $\pm\frac{1}{2}$, corresponding to allowed orientations of the electron spin in directions parallel or antiparallel to H. The separation between these two energy states is $\Delta E = g\beta H$, so the resonance condition for observing transitions between these two states becomes

$$h\nu = \Delta E = g\beta H.$$

The frequency of the radiation involved falls in the microwave region of the spectrum. For frequencies of 10 GHz, magnetic fields up to 10 kG are required.

For a completely free electron, g has a value of 2.0023. However, its value in the solid state can be markedly different; in particular, the value depends upon the angle between the applied field and the molecular symmetry axis. The spatial variation of the g factor for an iron porphyrin, such as exists in a hemoglobin molecule, is shown schematically in Fig. 12, where $g = g_\perp$ when H is anywhere in the heme plane (i.e., the system has axial symmetry) and $g = g_{||}$ when H is parallel to the normal to the heme plane. Thus, one can locate the normal to the heme plane by rotating the crystal and plotting the resultant g values. Such rotational ESR studies of single crystals of hemoglobin determined the orientation of the heme planes before X-ray studies were completed; the information aided in determining the structure of the rest of the molecule.

Similar studies have been done here to determine the orientation of the porphin planes in triphenylene. Triphenylene has four molecules per unit cell; if the paramagnetic porphin molecules enter the lattice substitutionally, ESR spectra due to each of the four nonequivalent sites would be observed. It has been shown at APL that copper porphin[10], vanadyl porphin[11], and iron porphin indeed occupy substitutional triphenylene sites, with the normals to the porphin planes making an angle of 51° with the crystallographic c axis. Copper porphin and vanadyl

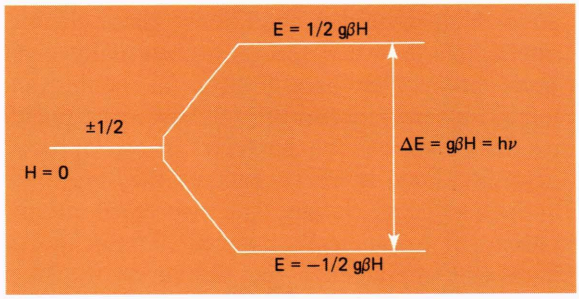

Fig. 11—The basic transition involved in ESR spectroscopy.

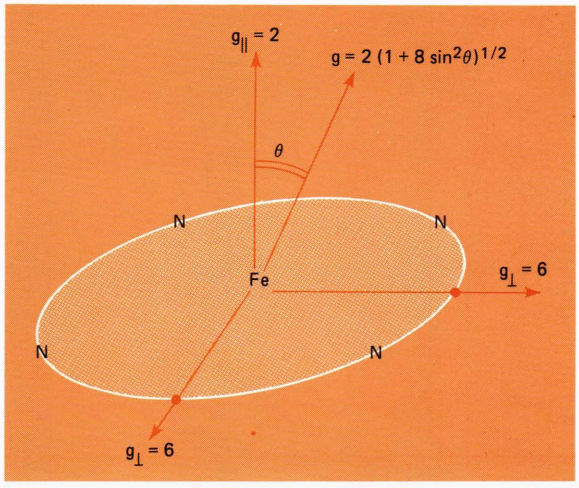

Fig. 12—The variation of g for heme as a function of the angle away from the heme normal.

[10] J. Bohandy and B. F. Kim, "An Electron Spin Resonance Study of Copper Porphin," *J. Magn. Reson.* **26,** 341 (1977).
[11] J. Bohandy, B. F. Kim, and C. K. Jen, "An ESR Study of Vanadyl Porphin," *J. Magn. Reson.* **15,** 420–426 (1974).

porphin were shown to be axially symmetric. However, deviations from axial symmetery were observed in iron porphin.

Figure 13 shows a typical plot of the angular variation of the ESR spectrum of iron porphin in triphenylene at 8 K. The crystal is mounted in such a way that the normal labeled "1" is perpendicular to H. For an axially symmetric situation, as described previously, there would be no angular variation for the site, and a straight horizontal line would be observed. The small deviation from axial symmetry observed for this site indicates a nonaxial distortion either from the local surroundings of the iron porphin or from asymmetric axial ligands on the iron. In principle, one can relate the anisotropy in the plane to the directions of the nitrogen atoms, so that not only the orientation of the heme normal but also the azimuthal orientation of the heme plane can be determined relative to the crystal axes. However, this requires more detailed information on excited states than is presently available. The utility of the ESR technique is nevertheless apparent.

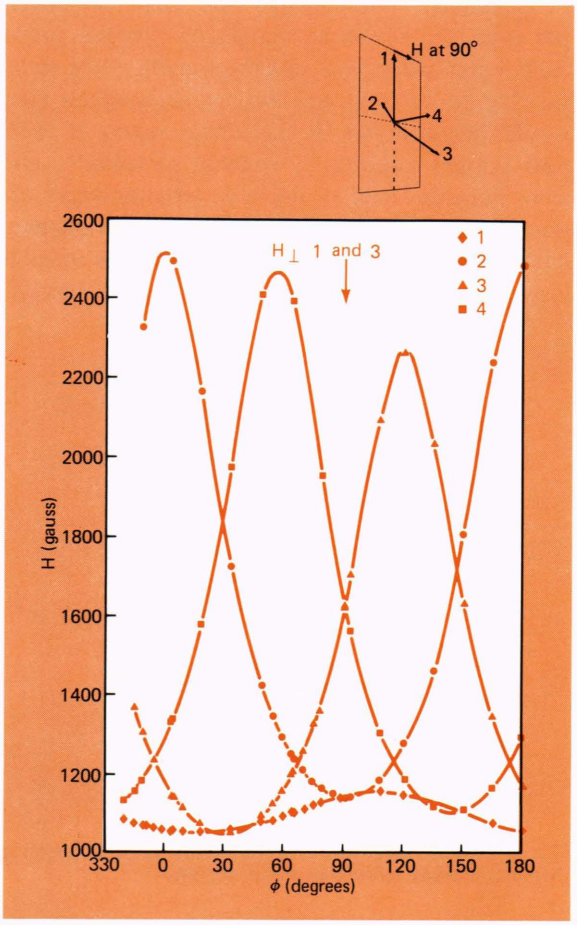

Fig. 13—Angular variation of the ESR spectrum of iron porphin in single-crystal triphenylene at 8 K. (The crystal is mounted so that normal "1" is perpendicular to the plane of the magnetic field. ϕ is the azimuthal angle in the plane of site 1.)

Future Plans

Recent advances in experimental porphyrin spectroscopy should allow corresponding advances in the theoretical characterization of the quantum structure of porphyrins. Determination of the structure cooperatively by theoretical and experimental efforts is a classical problem in optical spectroscopy. Application of this approach to a number of porphyrin species, an immediate goal of our work, will enable us to infer variations in the quantum structure of porphyrins with different chemical structures. Such basic information relates indirectly to the dependence of chemical function on the quantum structure of the molecules. This way, one hopes to correlate subtle differences in quantum structure with specific chemical or photochemical properties.

However, our results concerning the nature of inequivalent site species in triphenylene indicate an avenue of study more directly related to chemical behavior. In this approach, such chemical species as oxygen and carbon dioxide which are known to interact with porphyrins, will be introduced into crystals during their preparation along with the guest porphyrin molecules. The molecules that interact with those chemical species will exhibit shifts in their spectral line positions that are caused by the interactions with the attacking chemical species (as described previously). Although the spectral shifts may be small, the effect of the ligands can be determined by comparing the spectra to those of ligand-free porphyrin under high-resolution conditions. These chemical interactions between species are normally highly transient in such natural hosts as liquids and, therefore, are inaccessible to most experimental probes. They can be observed in the approach described here, however, because they are frozen in the host crystal lattice. The theoretical interpretation of the chemically induced shifts in spectral lines will characterize the nature of the interactions between the porphyrin molecules and the attacking chemical species.