Fig. 1.1

Schematic drawing of the arrangement of some of the chromophores in the RC of *R. viridis* (not to scale) showing the two-fold symmetry. The arrows show the strongly favoured path of electron transfer. P = primary donor bacteriochlorophyll pair, BC = accessory bacteriochlorophyll, BP = bacteriopheophytin (no Mg), and Q = quinone. Also shown is the non-heme iron. (From Ref. 8, used by permission of authors).

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Resonance Raman Study of
Nickel(II) pheophytin^a

by

Tony A. Mattioli, B.Sc., M.Sc.

A thesis submitted to
the Faculty of Graduate Studies and Research
in partial fulfilment of the requirements of
Doctor of Philosophy

Department of Chemistry

Carleton University

Ottawa, Ontario

Dec. 15, 1988

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submitted by

Tony A. Mattioli, B.Sc., M.Sc.

in partial fulfilment of the requirements
for the degree of Doctor of Philosophy

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ABSTRACT

The resonance Raman spectra of Ni(II) pheophytin *a* (Ni pheo *a*) excited in the Soret, Q_x , $Q_y(0-0)$, and $Q_y(0-1)$ absorption regions are reported. This non-luminescent Ni(II) analogue of chlorophyll *a* (Chl *a*) permits the recording of resonance Raman spectra excited in the Q_y absorption region, which is not possible for the highly fluorescent Q_y state of Chl *a*. The compound Ni pheo *a* was prepared by exchanging the Mg(II) central metal ion of Chl *a* for Ni(II).

Comparison of the relative intensities of various Raman bands excited in the Soret and in the Q_y regions indicates that different vibrational modes are coupled more effectively with different electronic transitions, thus revealing the presence of different vibronic coupling mechanisms.

Resonance Raman excitation profiles (REPs) of various Raman modes were measured in both the Soret and Q_y regions, and were analysed using a simple transform theory which accounted for non-Condon scattering effects. Within the simple theoretical framework, it was found that the REPs in the Soret region exhibited, primarily, Franck-Condon or A-term scattering with ~ 10% interfering contributions from non-Condon scattering. In contrast, the REPs in the Q_y region showed significant interference from non-Condon scattering, as revealed by the maximum of the REPs occurring in the 0-1 vibronic region. This was interpreted as arising from Herzberg-Teller scattering contributions (~ 40% and greater).

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1. INTRODUCTION.

In October of 1988, the Nobel Prize in chemistry was announced to be awarded to H. Michel¹ for his successful work in the growth of three-dimensional crystals of the photosynthetic reaction centre (RC) protein complex of the bacterium *Rhodospseudomonas viridis* (*R. viridis*), and to J. Deisenhofer and R. Huber² for their subsequent work on the X-ray diffraction studies of these crystals. The combination of these two efforts resulted in the first detailed three-dimensional structure ever obtained for an intact membrane protein (see Ref. 3 for a recent review on this subject). As its name implies, the photosynthetic reaction centre is the engine of the photosynthetic machine; it is where the fundamental light reactions of photosynthesis occur. When the RC is photoexcited, electron transfer occurs between pigments (chromophores) in the RC which establishes a membrane potential that drives the subsequent photosynthetic metabolism. The commonly accepted view⁴ is that the electron transport apparatus consists of a series of redox centres ordered in terms of decreasing redox potential where charge flows along the molecular chain in a directed manner by virtue of a "redox gradient".

Recent monographs^{5,6} review the techniques used to elucidate the relationships between structure and function of the photosynthetic apparatus; the work of Michel, Deisenhofer and Huber represents a major breakthrough in this area as they provide an atom-by-atom picture of the RC at about 3 Å resolution.

The RC protein consists of three protein subunits denoted as L, M, and H according to their electrophoretic mobilities^{3,7}. With a 3 Å resolution², 11 chromophores were recognised in the electron-density map of the RC of *R. viridis*. A schematic of the arrangement of some of these chromophores is shown in Fig. 1.1.

The primary electron donor of bacterial reaction centres is thought to consist of a pair of strongly interacting bacteriochlorophyll molecules (BChl) as shown at the top of Fig. 1.1⁸. X-ray work reveals that these two molecules are stacked over each other via their respective pyrrole rings I (about 3 Å apart) and are related by the 2-fold rotation axis. The tilt angle between the two BChl planes is about 15° and the Mg – Mg distance (Mg is the central metal ion in the BChl macrocycle) is about 7 Å. These two BChl molecules have been assigned as the special pair (P). The in-plane axes of these two BChl molecules are rotated approximately 35° from one another⁹. Associated with each BChl molecule in P is a BChl monomer (BC) (Mg – Mg distance about 11 Å) whose molecular planes exhibit a tilt angle of about 70°. Near each BChl monomer is a Bacteriopheophytin (BPPH) monomer (BP) about 11 Å apart (measured from their respective centres) with a 65° tilt angle. The orientation of these BChl and BPPH rings was determined from the electron densities of their respective phytol chains. The four BChl molecules were found to be bound to a histidine.

In general, it has become increasingly evident that all reaction centres are remarkably similar with respect to their initial electron transfer steps immediately following photoexcitation¹⁰. Absorption of a photon by the RC leads to an excited state in the primary donor P. Within a few picoseconds of excitation, an electron from P is transferred to an electron acceptor; in the case of Fig. 1.1 this would be BP_A (the bacteriopheophytin). Before the electron on BP_A[–] back-reacts with P⁺ (which would

Fig. 1.1

Schematic drawing of the arrangement of some of the chromophores in the RC of *R. viridis* (not to scale) showing the two-fold symmetry. The arrows show the strongly favoured path of electron transfer. P = primary donor bacteriochlorophyll pair, BC = accessory bacteriochlorophyll, BP = bacteriopheophytin (no Mg), and Q = quinone. Also shown is the non-heme iron. (From Ref. 8, used by permission of authors).

be inefficient), the electron is transferred to a second electron acceptor, a quinone Q_A . This quinone, in turn transfers the electron to the second quinone, Q_B . When this quinone is fully reduced, it probably picks up two protons, dissociates from the RC as a quinol and is replaced by a fresh quinone.

Most of the above information was obtained through spectroscopic techniques using information derived from circular dichroism^{9,11}, linear dichroism and photodichroism as well as picosecond spectral and kinetic measurements¹². A variety of spectroscopic techniques, including resonance Raman spectroscopy^{8,13}, have revealed a very high level of organisation in the *in vivo* chromophores.

Although X-ray crystallography is the only general technique available which provides the necessary information to construct an atom-by-atom model of complex biological molecules such as the photosynthetic RC complex, the primary processes of photosynthesis (photon absorption, charge separation and stabilisation, and even light harvesting) are not directly revealed. On the other hand, knowledge of the precise location and environment of important moieties in the RC aids enormously in the rationalisation of the energetics and kinetics of the light reactions. In fact, before the work of Deisenhofer *et al.*, a model for the arrangement of chromophores in the RC of *R. viridis* was proposed by Ke and Shuvalov¹² (based on spectroscopic evidence) which loosely resembles that of Deisenhofer *et al.*

Early electron resonance studies by Norris *et al.*¹⁴ first suggested that the oxidised BChl complex (P^+) is a π – radical cation in which the spin of the unpaired electron is delocalised over the π – electron systems of both BChl molecules. Other,

subsequent, EPR and ENDOR^{15,16} studies support this view. As Lutz⁸ suggests, the time-scale of these experiments ($\sim 10^{-7}$ s) is much too long to follow the fate of the unpaired electron. Using resonance Raman techniques, Lutz and Kleo¹⁷ were able to conclude that, in fact, a localised electron hole on a single BChl molecule of P870 (that is, the optical spectroscopic assignment of P which absorbs at about 870 nm) is created and that the unpaired electron did not occupy an orbital common to both BChl molecules in P870 as had been proposed¹⁸. The interpretation of the delocalised electron from EPR studies is now one of the electron "hopping" from one BChl molecule to the other on a time scale much faster than the EPR technique can follow.

The linear dichroism work of Breton¹⁹ supports the above view of the excitonic interaction of the paired BChl molecules in P. Furthermore, relative spatial arrangements of the pigments in the reaction centre of *R. viridis* predicted from this work agreed well with the data of Deisenhofer *et al.*

It is clear that the x-ray crystallographic work marked a breakthrough in the structural elucidation of photosynthetic reaction centres but it cannot directly reveal how the reaction centre functions. For this, we need techniques which directly probe the functional pigments (and associated proteins) and which exploit photon-electron interactions. The few spectroscopic techniques just mentioned have been proven to be good choices for this purpose. Even before the crystal structure was determined EPR studies suggested the close association of two BChl molecules, a picture which was refined and clarified by resonance Raman and linear dichroism (LD) spectroscopies. The molecular structure of the RC revealed by the X-ray studies provides a powerful and useful "molecular template" which can be used to correlate continuous and time-resolved optical and magnetic resonance spectroscopic observations by which

photosynthetic processes may be better rationalised (i.e. structure–function relationships). Optical measurements on single crystals of *R. viridis* reaction centres were first reported by Zinth *et al.*²⁰

Recent interest in photosynthetic reaction centres research has concentrated on electron transfer mechanisms^{21,22,23} and has given rise to application of electron transfer theories^{24,25,26} to bacterial reaction centres which is beyond the scope of this thesis. Despite this, however, it is useful to examine the common features of these electron transfer theories in order to ascertain how they may be evaluated, especially spectroscopically.

Electron transfer is influenced by electronic coulombic interactions as well as by the nuclear motions of the reactants and their surrounding medium. The electronic and vibrational contributions are usually separated using the Born–Oppenheimer approximation to give the electron transfer rate in the following general form^{27,28}

$$k = \frac{2\pi}{\hbar} V^2 F \quad (1.1)$$

where k is the rate constant, V is the electronic coupling interaction for the initial and final states, and F is the thermally averaged Franck–Condon nuclear overlap factor (this term has been treated more explicitly in Ref. 3). The V term is proportional to orbital overlap, and thus depends on the distance and relative orientation of the pigments. Both V and F are temperature dependent. It is clear that F is temperature dependent because of changes in the population of the vibrational modes which couple to the electron transfer. Perhaps less obvious is the temperature dependence of V which arises from protein contraction and concomitant changes in separation distances and

relative motions of the reactants^{21,29}.

Recently, Fleming *et al.*²¹ have measured electron transfer rates in the reaction centre complexes of *R. viridis* and *Rb. sphaeroides* as a function of temperature and found the rates to increase as the temperature decreased. They concluded that the rate was influenced by a combination of the two temperature-dependent factors. Others have reported temperature-independent^{25,26} electron transfer rates for other RCs which were attributed to the role of higher frequency intramolecular vibrations being a major contribution to the transfer process. The reason for these seemingly contradictory results, as of yet, is not fully understood.

One part of the answer may lie in the assumptions imbedded in Eq. (1.1). A model proposed by Freisner *et al.*³⁰ has been recently used to explain photochemical hole-burning experiments³¹. This model abandons the usual equilibrium assumptions to include strong electronic and vibronic coupling between P and another nearby electronic state to account for the fast electron transfer.

The aim of this thesis is to use resonance Raman spectroscopy to probe the photochemically important first excited electronic state of a chlorophyll-like molecule *in vitro*. Resonance Raman spectroscopy is particularly useful in this situation because of the following factors:

- i) Raman spectra excited using the $S_0 \rightarrow S_1$ transition (the same transition involved for bacteriochlorophylls to initiate charge separation in the photosynthetic reaction centre) will reveal which vibrational modes in the ground state are

active during this electronic excitation and possibly the ones associated with the primary electron transfer process.

- ii) The resonance Raman excitation profiles (REPs), that is, the variation in Raman intensity as a function of exciting frequency within the absorption band, will reveal the nature of these modes, and in particular, will show whether or not any are vibronically coupled to another excited electronic state. The latter will suggest the validity or limitations of present electron transfer theories.
- iii) The results of these *in vitro* studies may be directly applied to *in vivo* resonance Raman studies of reaction centres, since much *in vivo* Raman data are available.

The comparison of REPs excited in various electronic transitions will provide better understanding of the electronic structure of chlorophyll molecules.

Resonance Raman spectroscopy has proven to be a valuable tool for studying photosynthetic pigments in their native protein membranes^{8,13,32,33}. Because it is a vibrational spectroscopy, it reveals structural information at the submolecular level (which is less than the 3 Å limit of the X-ray work). Because the incident radiation wavelength which excites the Raman spectrum is deliberately tuned into resonance with an absorption band of the chromophore being studied, the technique is highly selective with respect to other pigments or protein vibrations. It is also useful in elucidating specific interactions between the chlorophyll pigments and their host protein matrix^{32,34} whose vibrations and their coupling to the pigment vibrations are also factors contributing to electron transfer^{3,30,31,35}.

In addition to the above advantages, and in light of the above discussion on the electronic and vibrational contributions to the electron transfer rate, resonance Raman spectroscopy seems a logical choice of technique to characterise the possible role of each contribution for the various pigments thought to participate in the transfer. By the very nature of the resonance Raman effect, one may actually measure the amount of vibronic coupling associated with the electronic transition of the chlorophylls. By vibronic coupling^{36,37} we mean that the motions of electrons during an electronic transition and the nuclei of the molecule are somehow (usually weakly) correlated. The result is a coupling of two or more electronic states. The degree of this vibronic coupling has a direct consequence on the validity of Eq. (1.1) which has been assumed to be separable into electronic and vibrational factors.

Resonance Raman spectroscopy has been shown to be extremely useful in investigating vibronic coupling³⁶ (as will be discussed in Chapters 2 and 3). The very nature of the phenomenon involves this coupling; the electronic transition from a ground molecular state to an excited molecular state induces certain vibrational modes in the ground state which may or may not be coupled to another excited state. This will have definite consequences with regard to the validity of Eq. (1.1).

Much of the information concerning the resonance Raman spectra of chlorophylls has been obtained in *in vitro* studies using molecular spectroscopic methods; some reviews of the chlorophylls have appeared on various aspects such as their chemical^{38,39}, electronic^{40,41}, spectroscopic^{41,42} and functional properties⁴². Lutz *et al.*^{8,13} have reviewed the literature on the resonance Raman spectroscopy of chlorophylls both *in vivo* and *in vitro*. The main interest¹³ in studying the Raman scattering of chlorophylls *in vitro* is that it is one of the few techniques which can be

used *in vivo* and, hence, is used as reference for the analysis of *in vivo* data. Other spectroscopic techniques such as nuclear magnetic resonance⁴³, electron spin resonance^{14,15}, visible absorption⁴², circular dichroism and magnetic circular dichroism⁴⁴ have been used successfully, especially in revealing various coordinated chlorophyll species in various degrees of association.

The problem associated with studying resonance Raman scattering excited in the first excited state of unassociated chlorophylls *in vitro* is that the strong fluorescence of this state masks the Raman signal. To overcome this problem we have synthesised an analogue of chlorophyll *a* (Chl *a*) by replacing the central Mg(II) ion in Chl *a* with Ni(II). The resultant molecule is named nickel pheophytin *a* (Ni pheo *a*) and is identical in structure to that of Chl *a* except for the central metal ion.

To date, there has been no resonance Raman spectrum excited in the lowest excited state (giving rise to the Q_y absorption band) for isolated molecules of Chl *a*. However, a resonance Raman spectrum of copper pheophytin *a* (which is almost non-fluorescent) excited in the Q_y state has been recently reported⁴⁵. As well, a recent resonance Raman study of various nickel dihydroporphyrins in the solid state has been reported⁴⁶. Coherent anti-Stokes resonance Raman spectra of Chl *a* excited at 694.3 nm has also been recently reported.⁴⁷ No REPs were reported in the above studies.

In general, resonance Raman studies of chlorophylls have been limited to excitation in the Soret (blue band) or near the Q_x bands^{8,13} using continuous wave

radiation. Koningstein's group^{48,49,50,51} have used 3 ns laser pulses to excite Raman spectra of Chl *a* and its oligomers⁵² in the Soret band. REPs have been obtained in the Soret band but, to the best of our knowledge, not in the important Q_y band.

The work in this thesis presents the first REPs obtained for a Chl *a* analogue excited in the Q_y absorption band. This study will serve to correlate the general resonance Raman scattering characteristics of other metallochlorins, chlorophylls, and this Ni Chl *a* analogue (Ni pheo *a*), so that the specific results of Ni pheo *a* scattering may be extended to deduce properties of Chl *a* and bacteriochlorophylls *in vivo*. The data were analysed using newly developed transform methods^{76,82}. This theoretical framework is extremely well-suited to extracting excited state information directly from experimental REPs and circumvents time-consuming computational difficulties in the more traditional approaches to resonance Raman data analysis.

2. PROPERTIES OF METALLOPORPHYRINS AND METALLOCHLORINS

2.1 MOLECULAR STRUCTURE OF Ni(II) pheo *a*.

The structure of Ni(II) pheo *a* is the same as that of chlorophyll *a* except for the replacement of the central Mg(II) ion with Ni(II) ion. This structure is shown schematically in Fig. 2.1. The main characteristics of chlorophylls may be listed as:

- a) central metal ion (Mg for Chl *a*)
- b) reduction of a pyrrole ring in the tetrapyrrole macrocycle
- c) methyl, ethyl, vinyl, and propanoic acid groups as peripheral substituents
- d) a phytyl chain
- e) a pentanone ring.

The reduction of the ring IV pyrrole across the C₇ – C₈ bond indicates that Ni pheo *a* (as well as Chl *a*) is a chlorin. This, as we shall later see, imparts certain optical properties to the molecule. The phytyl chain gives the molecule its lipophylic properties. Probably the most important characteristics, in terms of electron acceptor–donor properties, are the central ion and the ring V carbonyl group.

Unlike Chl *a*, Ni pheo *a* does not have strong coordination properties between the ring V carbonyl group and the central metal ion⁵³. This results in poor self–aggregation properties for Ni pheo *a*. As well, the metal–ligand interaction

Fig. 2.1.

Molecular structure of Ni(II) pheophytin *a*. Chlorophyll *a* has the same structure except that the central metal ion is Mg(II).

(ligand = porphyrin macrocycle) is much stronger for Ni(II) than it is for Mg(II).

The most important property of Ni pheo *a* of relevance to this work is the non-fluorescent Q_y state. The complexation of the pheophytin ligand with open-shell Ni(II) ion completely quenches the fluorescence from the photophysically important Q state. This allows one to obtain red-excited rR spectra for chlorophylls which would be otherwise completely masked by the intense fluorescence from the Q_y state.

2.2. OPTICAL SPECTROSCOPY OF METALLOPORPHYRINS.

We begin this section by briefly describing the absorption spectra of metalloporphyrins in general. We do this because of the strong interdependence between the resonance Raman effect and optical absorption. Although Chl *a* and Ni pheo *a* are chlorins, it is instructive to describe the general features of metalloporphyrins, the parent complexes, and note the effects of descending symmetry.

2.2.1 Metalloporphyrins.

The electronic states and spectra of porphyrins are fairly well described by Gouterman's four-orbital model^{54,55}. Transitions occur between the two highest occupied molecular orbitals (HOMOs), $a_{1u}(\pi)$ and $a_{2u}(\pi)$, and the two lowest unoccupied molecular orbitals (LUMOs) $e_g(\pi^*)$. The absorption spectrum thus arises from dipole $\pi \rightarrow \pi^*$ transitions connected with the transitions of electrons localised, mainly, in the conjugation pathway of the ligand porphyrin.

For a metalloporphyrin of symmetry D_{4h} , the $e_g(\pi^*)$ orbitals are degenerate while the HOMOs are not. The electronic configuration of the ground singlet state, 1A_g , is $(a_{1u})^2 (a_{2u})^2$. The excited configurations will be $(a_{1u} e_g)$ or $(a_{2u} e_g)$. Strong configuration interaction between these excited configurations⁵⁵ gives one symmetric and one antisymmetric combination (in a 50/50 mixing scheme). The symmetric combination corresponds to the intense Soret, or B, transition in the blue region of the visible spectrum, while the antisymmetric combination corresponds to the weaker Q band. For the symmetric combination, the transition dipoles of the original two $\pi \rightarrow \pi^*$ transitions (which are similar) add to give a large resultant dipole, while the subtractive component in the antisymmetric combination yields a small transition dipole and, thus, results in weak absorption.

In metalloprophyrins of D_{4h} symmetry, the very intense Soret ($\epsilon \sim 10^5 \text{ M cm}^{-1}$) or B or γ band is seen at $\sim 400 \text{ nm}$. The lower energy transition, called the Q(0) or α band, is an order of magnitude weaker than the Soret band. This lower energy transition can "borrow" intensity (via the Herzberg-Teller mechanism^{56,57}) from the higher energy transitions through appropriate vibrations. These are of relatively high frequency and produce a vibronic side band, called the Q(1) or β band, which peaks at an energy $\sim 1250 \text{ cm}^{-1}$ higher than the Q(0) band. The ratio of these intensities is due to vibronic interaction with the Soret band and is highly sensitive to the nature of the substituents in the porphyrin ligand.

The above picture may be complicated when one considers a metal complex with a vacant d-electron shell.⁵⁸ In general, the absorption spectrum may contain the

following transitions:

i)	$\pi \rightarrow \pi^*$	transitions (of the porphyrin)
ii)	$d \rightarrow d$	transitions (of the metal)
iii)	$\pi \rightarrow d$	transitions (charge transfer) CT
	$d \rightarrow \pi^*$	transitions (charge transfer) CT.

The central question of molecular spectroscopy is to determine the relative positions of these levels (transitions). Conventional absorption spectroscopy is not well-suited to tackle this problem since dd and CT bands are of low intensity and are masked by the strong $\pi \rightarrow \pi^*$ absorption of the porphyrin.

Ni(II) porphyrin complexes.

In most solvents⁵⁸, the central metal ion of Ni(II) porphyrin complexes is diamagnetic, meaning that all of the d electrons (d^8) are spin-paired, leaving the high $d_{x^2-y^2}$ orbital vacant. For the $d_{z^2} \rightarrow d_{x^2-y^2}$ transition (denoted as $S_d(^1B_{1g})$), the exact position of these levels is not known, since they have never been observed; it could lie either in the IR region or on the slope of the intense porphyrin absorption band. It is estimated⁵⁹ that the $S_d(^1B_{1g})$ level is 9000 cm^{-1} above the ground state, as shown in Fig. 2.2.

Fig. 2.2.

Schematic diagram of a Ni(II) porphyrin energy levels. Arrows show the most probable channels of energy degradation.

Also shown in Fig. 2.2 are the probable channels of energy degradation which account for the non-fluorescent nature of Ni-porphyrins. Upon photoexcitation of the Ni-porphyrin to the $S_1(\pi\pi^*)$ state, extremely efficient intersystem crossing (via heavy atom effect), $S_1(\pi\pi^*) \rightarrow T_1(\pi\pi^*)$, takes place; this effectively quenches any fluorescence from the $S_1(\pi\pi^*)$ state. Picosecond absorption experiments⁵⁸ reveal that the $T_1(\pi\pi^*)$ state is populated with a rate constant of 10^{11} s^{-1} . Such a large rate constant was interpreted as arising from $d - \pi^*$ interactions owing to the influence of the S_d and T_d states.

It was also found that, within experimental error, the above results depend on the Ni(II) ion and not on different substituents on the porphyrin ring. Also, due to the presence of a low lying $T_d(^3B_{1g})$ level in Ni porphyrins, the triplet state $T_1(\pi\pi^*)$ is quenched as well, which explains the absence of any phosphorescence. It is expected that Ni porphyrins are totally non-luminescent.

On the basis of the above properties, we note that Ni pheo *a* is an appropriate choice of molecule to study in lieu of Chl *a* to probe the Q_y band. Firstly, all fluorescence and phosphorescence are quenched. Secondly, any hidden CT states are believed to be out of the visible region of the spectrum, and thus, no interferences should be seen.

2.2.2 Metallochlorins.

Metallochlorins differ from metalloporphyrins by the reduction of one of the porphyrin pyrrole rings. This change has a dramatic effect on the electronic absorption spectrum; the visible Q band (α , β) of metalloporphyrins which is ~ 10 -fold less

intense than the Soret band, is more intense for metallochlorins (~ 3 – 5-fold less intense than the Soret band).^{40,41} Metallochlorins also show a loss in planarity^{60,61}.

The π – conjugation pathways of metalloporphyrins and metallochlorins are fundamentally different⁶². This is shown in Fig 2.3 where the metalloporphyrin shows x, y equivalence and, thus, leading to degenerate x and y electronic transitions (in D_{4h} symmetry). For the metallochlorin, there is an inherent x, y inequivalence in the π – conjugation pathways⁴¹, regardless of substituents, resulting in an absorption spectrum with different Q_x and Q_y visible transitions and, occasionally, two transitions in the Soret^{62,63} region. Where accidental degeneracy occurs, a single Soret band may result. The lower energy Q_y transition is more strongly allowed relative to the degenerate Q bands of the metalloporphyrin parent molecule. It is this band which is responsible for the intense "chlorin" transition. The other, Q_x , transition is almost parity forbidden and thus much weaker⁴¹. The absorption spectra of Ni(II)–tetramethylporphyrin and Ni(II)–tetramethylchlorin illustrate these points.⁶⁴

Fig. 2.3.

Inner π – conjugation pathways for a Ni metalloporphyrin (top) and a Ni metallochlorin (bottom). The porphyrin (top) shows x,y equivalence whereas the chlorin (bottom) does not.

2.2.3 Chlorophylls.

Chlorophylls are Mg(II) – chlorins with several peripheral substituents along the π – conjugated macrocycle. These substituents were discussed in Section 2.1. In general, external substitution of the macrocycle which does not significantly perturb the π – conjugation pathway will not have much effect on the optical absorption spectrum. A good indication of this is given when one studies the absorption spectrum of protochlorophyll⁴⁰. This molecule possesses the same peripheral substituents as Chl *a* but ring IV of protochlorophyll is not reduced. Strictly speaking, this would make it a metalloporphyrin structure and, as expected, the Soret band is much more intense than the Q bands (see Fig. 2.4). One will note, however, that the presence of the ring V decreases the symmetry of the π – conjugation by the presence of the carbonyl group thus making the x and y polarised transitions non-degenerate; the carbonyl group of the ring V substituent actually extends the π – conjugation as, is shown in Fig. 2.5. The result of this extended conjugation in the y-direction of the molecule is a red-shift in the Q_y band and breaking of the strict D_{4h} symmetry.

For Chl *a*, the intensity of the Q_y band is increased relative to that of the Soret band and red-shifted. Based on the molecular orbital theory of Gouterman and co-workers^{54,55}, Weiss^{40,41} has provided a theoretical analysis of the absorption, fluorescence polarisation, and circular dichroism for chlorophylls and related structures. More recently, Christoffersen *et al.*^{65,66} and Petke *et al.*^{67,68,69} have performed *ab initio* CI calculations for ethyl chlorophyllide *a* (phytyl chain replaced by an ethyl group). Their results indicated that for Chl *a* there were several transitions other than single Q_x and Q_y transitions which were predicted in the absorption spectrum. Some

of the calculated transitions had mixed x and y character. Experimentally, this was found to be the case in a linear dichroism study of oriented Chl *a* films conducted by Fragata *et al.*⁷⁰ These workers concluded that the simplifying assumption that Chl *a* possessed two mutually perpendicular Q_x and Q_y transitions was not totally correct, as would be sustained in the strict four-orbital model. In fact, they concluded there were nine electronic origins between 670 and 346 nm. The Q_y band at 670 nm is actually thought to be x-y mixed but predominantly y (90%). The Soret band is somewhat more complicated, with a mixed x-y transition (40% x) at 429 nm and two other pure x transitions at 436 nm at 389 nm.

Fig. 2.4.

Absorption spectra of (A) protochlorophyll *a*, (B) chlorophyll *a*, and their corresponding metal-free pheophytins.

Fig. 2.5.

Molecular structure of chlorophyll *a* indicating the π – conjugation pathway which extends into the ring V keto group at C_9 .

The position of the Q_x bands of chlorophylls has been suggested by Katz *et al.*^{42,71,72} to be particularly sensitive to the coordination of the central Mg(II) ion. For a hexa-coordinated Chl *a* complex, these workers assigned the Q_x band to be between the $Q_y(0-0)$ and $Q_y(0-1)$ transitions, while in a penta-coordinated Chl *a* complex, the Q_x band is shifted to the blue and lies underneath the $Q_y(0-1)$ transition.

Contrary to the assignment of Katz *et al.*^{42,71}, Weiss^{40,41} has assigned the Q_x band to be much more blue-shifted (~ 575 nm for Chl *a* in ether) based on MCD⁴⁴ (magnetic circular dichroism) data which Katz would interpret as the $Q_y(0-2)$ band for penta-coordinated Chl *a*.

It is clear that this controversy is/was the result of assuming that only one " Q_x " and only one " Q_y " transition were present. In fact, Fragata *et al.*⁷⁰ have demonstrated that there are several such transitions, some pure *x* and *y*, and some of mixed polarisation (i.e. degenerate *x* and *y* transitions). The apparent shift of the " Q_x " band with coordination may now be interpreted as changes in intensity of one or several of these resolved origins. For pheophytin *a* (Chl *a* free-base form, de-metallated), the origins are approximately in the same spectral position but of very different relative intensities. Because pheophytin *a* has no central metal ion, this molecule has no coordinated complexes.

Realising the complex nature of the absorption spectrum of metalloporphyrins, in this work we will adopt the convention of Boldt *et al.*⁴⁶ (as well as that of Weiss) for the assignment of the conventional Q_x and Q_y bands of Ni pheo *a*. We shall see in the resonance Raman studies why these choices are justified, although certainly not

definitive.

2.3 REPs OF METALLOPORPHYRINS AND CHLORINS.

The resonance Raman excitation profile (REP) is the variation in intensity of a Raman mode as a function of excitation energy in the absorption region. Although this will be discussed later, we may state at this point that REPs can be useful in assigning features in an absorption band and provide information about electronic and vibrational structure which may not be resolved in an absorption spectrum^{36,73,74,75}. They also reveal information about the structure of the excited state⁷⁶ such as symmetry, geometry, and dynamics. The Raman spectrum itself gives vibrational information about the ground state, and thus its geometry and electronic structure.

As in the previous section, we will first discuss the REPs expected from metalloporphyrins and progress down in symmetry to the chlorophylls.

2.3.1 Metalloporphyrins.

Traditionally, metalloporphyrins have provided many resonance phenomena for Raman spectroscopists to study⁷⁷. Porphyrins are attractive compounds in the study of resonance Raman phenomena because of their intense absorption bands in the visible region. They serve as invaluable model compounds in the structural elucidation of heme proteins^{78,79} and other biologically important macromolecules⁸⁰ such as certain cytochromes^{81,82} and prosthetic groups in photosynthetic reaction centres^{8,13}, to name but a few. In a recent review, Spiro *et al.*⁷⁷ list several factors which have made

metalloporphyrins one of the most studied classes of compounds in resonance Raman spectroscopy.

1. Strong, visible $\pi \rightarrow \pi^*$ transitions make the porphyrin ring convenient to excite with available lasers.
2. Configuration interaction^{77,99} and vibronic mixing between these transitions results in many interesting resonance phenomena.
3. Conjugation pathways may be altered and studied by introducing a wide variety of possible peripheral substituents, central metal ions, and axial ligands.
4. Vibrational frequencies have been found to be sensitive to porphyrin geometry and electronic structure.
5. They are model compounds for the prosthetic groups of many biologically important complex proteins (heme proteins, vitamin B₁₂, chlorophylls).

The above factors have contributed to the development of resonance Raman theory and its relation to electronic optical transitions.

When Raman spectra are excited in the region of allowed electronic transitions, the vibrational modes which are expected to be enhanced are those which lend intensity to the electronic spectrum; these are the vibronically active modes. These may be grouped into two categories⁵⁷:

- A. Modes which connect the ground state to the excited state involved in resonance through the Franck–Condon overlap, or which change the

energy of the resonant state.

- B. Modes which mix the resonant electronic state with another electronic state.

Type A vibrational modes (as we shall see in Chapter 3) are expected to be totally symmetric. Type B modes, however, may possess any symmetry contained in the direct product of the two mixed electronic transitions.

It was mentioned in the previous section that the absorption spectrum of metalloporphyrins is dominated by an intense Soret or B band and a much weaker α band which "borrows" intensity from the Soret band.

Excitation near the intense B band enhances only totally symmetric modes, indicative of A-term scattering, as expected. The resonance Raman (rR) spectra in this region are dominated by modes corresponding to the stretching of the porphyrin ring π - bonds; this is expected for enhancement via $\pi - \pi^*$ transitions. These modes are, typically, between $1100 - 1650 \text{ cm}^{-1}$ and are almost always polarised⁸³. The strongest mode in this region is a 1360 cm^{-1} band which corresponds to the breathing mode⁸⁴ which exhibits a small origin shift in the excited state⁸⁵. This is to be expected from the large size of the porphyrin π - system which should keep the excited state distortion relatively small.

For A type modes, in the limit of a small origin shift, theory predicts that the measured REP will exhibit equal enhancement at the electronic origin (0-0) and at the first vibronic level (0-1). This applies to simple scattering situations. Champion and

Albrecht⁸⁶ have observed deviations from this simple case in the Soret-excited REPs of Fe(II) cytochrome *c* (an iron porphyrin), which was explained as being due to the influence of many low-frequency modes which produce a strong intensification of the REP at the 0-1 region. Later, the same observations could be explained by the inclusion of non-Condon scattering⁸⁷ (scattering via vibronic coupling), which would also produce the same observed results. An interesting point is that a small contribution⁸⁸ (~ 10%) of non-Condon scattering could reproduce the apparent large deviation from simple scattering theory.

These and other observations brought to attention the importance^{88,89,90,91,92} of multimode and non-Condon effects in the rR description of metalloporphyrins in the Soret region. Warshel *et al.*⁹³ suggested that there can be present Raman transition pathways in which modes other than the one being monitored are also promoted in the resonant state. If the Franck-Condon overlap for this helping mode is large, then it is possible to observe a second resonance in the REP of the monitored mode. Usually, these subsidiary mode terms are small but they may affect the shape of the profile.

Despite all of the above interfering factors, in general, A-type scattering is observed in the Soret region of metalloporphyrins.

For excitation in the Q bands, A-term enhancements are smaller due to the weaker electronic transition. In general, one finds that rR spectra of Q-band excited modes are dominated by the non-totally symmetric modes responsible for the Q - B states mixing which gives rise to the Q band absorption in the first place. The symmetry of the possible modes contributing to this mixing must be contained in the direct product of the two transition moments. For example,

$$E_u \times E_u = A_{1g} + A_{2g} + B_{1g} + B_{2g} \quad (2.1).$$

This is known as B-term scattering.

For B-term scattering⁹⁴ theory predicts equal scattering intensities in the REP at the 0-0 and 0-1 regions, the same as in the A-term scattering case.

In actual fact, the 0-0 and 0-1 maxima do not always exhibit the same intensities in the REP. Non-adiabatic effects⁹⁵ where extra enhancement is observed at higher vibrational levels are attributed to the higher 0-1 maxima observed. This is predicted via electron-nuclear coupling with higher electronic states^{96,97}. Higher 0-0 maxima have been attributed to Jahn-Teller activity in the degenerate Q-state.⁹⁸

In a recent rR study of metallooctaethyl porphyrins by Schick and Bocian⁹⁹, most of the above cases were observed in the measured detailed REPs of several modes of various symmetry. It is evident that simple scattering theory alone cannot account for the observed rR scattering of metalloporphyrins.

2.3.2 Metallochlorins.

Some interesting resonance phenomena occur when the symmetry of metalloporphyrins is reduced to that of metallochlorins. In metallochlorins, one of the four C_bC_b bonds (see Fig. 2.1) is saturated; metallochlorins have been considered as

models for chlorophylls and heme *d*.¹⁰⁰ The two important changes are the change in π – conjugation resulting in x , y inequivalence, and the loss of planarity. The infrared and rR spectral properties of metallochlorins are considerably different from those of the corresponding metalloporphyrins. Andersson *et al.*^{62,64,101,102} have listed a generalised set of vibrational spectroscopic properties for metallochlorins compared to the parent metalloporphyrins:

1. an increase in the total number of IR and Raman bands,
2. an increase in the number of polarised rR bands,
3. a strong, polarised $C_a - C_m$ mode at $> 1600\text{ cm}^{-1}$ (depending on the molecule and the resonant electronic state),
4. altered polarisation for porphyrin A_{2g} and B_{1g} modes,
5. rR-active split porphyrin E_u modes,
6. predominance of polarised rR modes with both Soret and visible excitation,
7. close correspondence in frequency for IR and Raman features, and
8. the notable presence of two or more polarised bands in the region of $C_a - N$ stretch (the so-called oxidation state marker).

Some of the above points are made more clear by correlating the symmetry properties of the in-plane vibrations of simple metallochlorins (symmetry C_2 , in general) with those of the parent metalloporphyrin (symmetry D_{4h})^{62,100}, as shown in Table 2.1:

TABLE 2.1: Symmetry Correlation Between D_{4h} and C_2 groups for in-plane Vibrations.

D_{4h}			C_2	
A_{1g}	polarised		A_1	polarised
B_{1g}	depolarised			
E_u	(Raman forbidden)		B_2	depolarised or anomalously polarised
A_{2g}	anomalously polarised			
B_{2g}	depolarised			

The A_{1g} and B_{1g} species of the D_{4h} group combine to give the A_1 species in C_2 symmetry. The A_{2g} and B_{2g} species of D_{4h} group are combined into the B_2 species of the C_2 group. From the above Table it is anticipated that the number of bands and their polarisation properties will be changed or altered in the rR spectra of metallochlorins from those of metalloporphyrins; in fact, more Raman bands are observed as symmetry is broken.

From the work of Kitagawa *et al.*¹⁰⁰, it is apparent that many depolarised (dp) and anomalously polarised (ap) bands of NiOEC (Ni(II)-octaethylchlorin) are strongly enhanced in the vibronic coupling between the Q and B states, since dp and ap bands are non-totally symmetric. It is also noteworthy that rR bands in the $1000 - 1300 \text{ cm}^{-1}$ region are strongly enhanced in the Q band region of metallochlorins. This is distinctly different from metalloporphyrins for which rR bands

above 1500 cm^{-1} are intensified in the Q band region. Such differences can be considered to be characteristic of metallochlorins¹⁰⁰. This suggests that electronic excitation in the Q band region involves a change of the $C_a C_b$ bond for metallochlorins but not so much so for metalloporphyrins.

Because of the relatively stronger Q_y transition of metallochlorins, it has been suggested^{62,64,101} that only Franck-Condon (or A-term) scattering mechanisms are operative in the visible region of excitation, and thus there should be a predominance of polarised, totally symmetric Raman modes in both the Soret and Q spectral regions. In metalloporphyrins, recall that depolarised Raman bands dominate the spectrum in the Q region owing to vibronically mixed Herzberg-Teller scattering. The Soret region, on the other hand is dominated by polarised Raman bands arising from Franck-Condon scattering.

Indeed, the work of Andersson *et al.*^{62,64,101,102} on the rR scattering of substitutionally symmetric metallochlorins did show a predominance of polarised Raman bands with excitation in the visible regions. The low resolution REPs from Ni(II) tetramethylchlorin (NiTMC) indicated that most modes followed the absorption spectrum where the maximum in the REPs corresponded to the 0-0 transition. Contrary to this, the work of Kitagawa *et al.*¹⁰⁰ indicates that most of the measured REPs for Ni(OEC) peak at the 0-1 region of the Q_y band. This apparent conflict is due to the lack of available laser excitation lines and poor resolution of the measured REPs. To date, no such high resolution REP in the Q_y band of a metallochlorin has been found in the literature. It is the purpose of this thesis to measure such a REP.

2.3.3 Chlorophylls.

There are several reports in the literature concerning the resonance Raman spectra of chlorophylls, which have been recently reviewed by Tasumi and Fujiwara¹⁰³, and Lutz and Robert⁸. In general, the rR spectroscopy of chlorophylls is still more complex than those of metallochlorins due to non-symmetric peripheral substitutions about the macrocyclic ring but primarily due to the presence of the isocyclic ring V which is added to the basic chlorin structure. In addition, the presence of the carbonyl group (see Fig. 2.1) on the ring V extends the conjugation of the π system in the y direction, and thus the observed red-shift in the Q_y band.

The resonance Raman spectrum of Chl *a* in acetone was first published by Lutz¹⁰⁴. This spectrum, as in all subsequent studies, was excited in the Soret region with CW lasers, where 55 active modes are observed in the $50 - 1750 \text{ cm}^{-1}$ region. The REPs of both Chl *a* and Chl *b* modes excited in the Soret region all showed the same maxima in a very small range, indicating an electronic 0-0 origin blue-shifted by $\sim 392 \text{ cm}^{-1}$ from the Soret absorption maximum¹³. Furthermore, these profiles agreed qualitatively with a simple Albrecht A-term in the pre-resonance region, indicating Franck-Condon scattering. Also indicative of strong Franck-Condon scattering is the predominance of polarised Raman bands. Lutz^{13,104} reported that with the exception of an anomalously polarised 1265 cm^{-1} Raman band, all of the observed rR modes excited in the Soret band were polarised. (Note: this is probably a C-N stretch, oxidation state marker which Andersson *et al.*⁶² claim is a complicated band with other underlying modes). Lutz suggests that this anomalously polarised band may be explained by a dominant contribution of a B-type resonance involving one of the Q bands and the Soret band.

No excitation profiles have been reported for Chl *a* excited in the radiative Q_y band. However, a luminescent (almost non-fluorescent) Cu(II) analogue of Chl *a* (Cu(II) pheophytin *a*) has yielded a rR spectrum excited in the Q_y region^{2,60}. It was observed that all rR bands appeared polarised with the possible exception of a weak 220 cm^{-1} mode. The fluorescent background may have obscured any of the weaker bands. This prevalence of polarised bands is anticipated with the reduction in symmetry. The formal symmetry of Chl *a* is C_1 , which contains only A_1 species that are expected to be polarised. In general, it was also observed that the out-of-plane modes in the region of $700 - 970\text{ cm}^{-1}$ appeared more intense than the same modes of Chl *a* excited in the Soret region. The authors suggested that this was due to the difference in vibronic coupling in the Q_y resonance Raman excitation compared to that in the Soret.

Another interesting rR study of Ni(II) chlorins excited in their Q_y bands was reported by Boldt *et al.*⁴⁶ In this work, the authors report the Q_y -excited resonance Raman spectrum of Ni(II) methylpyropheophorbide *a*, a molecule that differs from Chl *a* as follows:

1. Mg(II) replaced by Ni(II),
2. phytyl chain replaced by H,
3. carbomethoxy group at C_{10} replaced by H.

All other peripheral substituents are the same. Their work indicated something quite unexpected in that the addition of the isocyclic ring V to the basic chlorin structure results in the localisation of certain vibrational modes, rather than being

delocalised over the entire conjugated ring. However, no Raman profiles were recorded.

At present there are no reported REPs excited in the Q_y region for any chlorophyll. It is the purpose of this work to obtain detailed REPs of several chlorophyll modes when excited in the Q_y band and the associated vibronic band; this will provide further insight into the scattering mechanisms for metallochlorins in general, and more importantly will allow us to probe the vibronic coupling mechanisms of this photochemically important state of the chlorophylls.

3. THEORY

3.1 INTRODUCTION.

There are various ways one may envision the Raman effect. For non-resonance Raman scattering, the classical picture is conceptually useful in relating the physical concepts which give rise to the vibrational Raman effect. The basic physical principles of vibrational Raman scattering may be listed as^{105,106}:

1. Radiation is scattered from a molecule due to an oscillating dipole induced by the incident radiation.
2. For visible and ultraviolet radiation, the scattering is due to electrons and not directly by the nuclei, which is negligible.
3. Vibrational Raman scattering is due to the parametric dependence of the molecular electronic polarisability upon the nuclear positions.

For a vibrating molecule, the electron cloud will distribute itself in a manner following the nuclear oscillations with a frequency characteristic of the vibration. The scattered radiation will contain this vibrational information in the form of a beat pattern. The Raman vibrational spectrum is obtained by analysing the spectral content of the scattered radiation which contains the elastically scattered light (Rayleigh) and the inelastically scattered light (Raman).

3.2. RESONANCE RAMAN EFFECT.

Description of the resonance Raman (rR) effect requires a quantum mechanical approach since the incident radiation is now coincident or in resonance with an allowed electronic transition. At present, there are three popular theoretical frameworks which describe rR intensities. A general overview of these has been recently reviewed by Myers and Mathies⁷⁶.

A. Sum-Over-States Picture.

The traditional approach to quantum mechanically describe rR intensities begins with the Kramers-Heisenberg-Dirac (KHD) dispersion formula, which originates from second-order time-dependent perturbation theory. This is well treated in the monograph by Placzek¹⁰⁵ and the book by Koningstein¹⁰⁶.

In this formalism, the Raman cross-section is given as^{76,107}

$$\sigma_{I \rightarrow F}(E_L) = \frac{8\pi e^4 E_S^3 E_L}{9h^4 c^4} \sum_{\rho, \lambda} |(\alpha_{\rho\lambda})_{I \rightarrow F}|^2 \quad (3.1)$$

The polarisability, α , is given from KHD theory as

$$(\alpha_{\rho\lambda})_{I \rightarrow F} = \sum_V \frac{\langle F | m_\rho | V \rangle \langle V | m_\lambda | I \rangle}{E_V - E_I - E_L - i\Gamma} + \frac{\langle F | m_\lambda | V \rangle \langle V | m_\rho | I \rangle}{E_V - E_F + E_L - i\Gamma} \quad (3.2)$$

where

E_L = incident photon energy

E_S = scattered (Raman) photon energy

$|I\rangle$ = initial vibronic molecular state (wavefunction)

$|F\rangle$ = final vibronic molecular state

$|V\rangle$ = intermediate (virtual) vibronic molecular state

E_I = initial state energy

E_F = final state energy

Γ = homogeneous linewidth

$m_{\rho,\lambda} = \rho^{\text{th}}$ or λ^{th} vector component of the transition dipole length (moment).

From the above equations, the Raman effect is interpreted as a two-photon process where a virtual absorption from an initial state to an intermediate (virtual for the non-resonant case) state is promptly followed by a virtual emission to the final state. This process is represented by the sum of all possible excited vibronic states, which may all act as intermediate states. The magnitude of the contribution from each state depends on both the energy denominator and the transition dipole element. As the energy of the incident radiation approaches resonance with one of the states, the vibronic levels of that state begin to dominate the sum.

When E_L is in strict resonance with a strongly allowed absorption band, the transitions which dominate the sum become real transitions and are no longer virtual. The result (in the absence of interferences) is a tremendous increase in the scattering intensity. In this case, several approximations are usually invoked to simplify the above equations. As a first approximation, the wavefunctions are traditionally separated into component electronic and vibrational parts via the Born-Oppenheimer

approximation. This gives

$$\langle F | \mathbf{m}_\rho | V \rangle = \langle i | \mathbf{M} | v \rangle \quad \text{and} \quad (3.3a)$$

$$\langle V | \mathbf{m}_\lambda | I \rangle = \langle v | \mathbf{M} | i \rangle \quad (3.3b)$$

where $|i\rangle$ and $|f\rangle$ are the initial and final vibrational wavefunctions, respectively, in the ground electronic state, and $|v\rangle$ is the vibrational wavefunction in the excited electronic state, e . $\mathbf{M}$ denotes the *pure* electronic transition moment between the ground electronic state and the resonant excited state.

In general, $\mathbf{M}$ is a weakly varying function of the nuclear coordinates. To explicitly take into account changes in $\mathbf{M}$ as the nuclear coordinates vary, $\mathbf{M}$ is expanded in a Taylor series about the equilibrium molecular geometry¹⁰⁸:

$$\mathbf{M} = \mathbf{M}^0 + (\partial \mathbf{M} / \partial Q)^0 Q + \dots \quad (3.4)$$

where Q is a normal mode of the molecule; there is an equivalent expression for every normal mode. Considering only the first two dominating terms in Eq. (3.4), the polarisability may now be written as

$$\alpha_{i \rightarrow f}(E_L) = A + B \quad (3.5)$$

where

$$A = (M^0)^2 \sum_v \frac{\langle f|v\rangle \langle v|i\rangle}{\epsilon_v - \epsilon_i + E_0 - E_L - i\Gamma} \quad (3.6)$$

$$B = M^0 (\partial M / \partial Q)^0 \sum_v \frac{\langle f|Q|v\rangle \langle v|i\rangle + \langle f|v\rangle \langle v|Q|i\rangle}{\epsilon_v - \epsilon_i + E_0 - E_L - i\Gamma} \quad (3.7)$$

Note that we have neglected the non-resonant part of Eq. (3.2) and have dropped the polarisation subscripts. We also note that because of the resonance condition, the summation is over all possible vibrational states in the resonant excited state, rather than over all possible vibronic levels of the singlet manifold.

For comparison, at the same level of approximation the optical absorption cross-section is given by¹⁰⁹

$$\sigma_A(E_L) = \frac{4\pi^2 e^2 M^2 E_L}{3\hbar c n} \sum_v \frac{\Gamma}{\pi} \frac{|\langle v|i\rangle|^2}{(\epsilon_v - \epsilon_i + E_0 - E_L)^2 + \Gamma^2} \quad (3.8)$$

where n is the solution refractive index. The absorption cross-section depends on the same excited state parameters as does the Raman cross-section. Note that the absorption and Raman cross-sections depend differently on Γ . In absorption, increasing Γ broadens the spectrum but *does not* change the integrated intensity (each term in the sum over states involves a normalised Lorentzian lineshape). In contrast, increasing Γ always results in a lower Raman cross-section because of the resulting smaller real part of the Raman scattering amplitude.

Usually, the A term is the dominant contributor to rR intensity ($M \gg \partial M / \partial Q$ for allowed transitions). As seen in Eq. (3.6), A enhancement is directly proportional to the electronic transition moment and inversely proportional to the homogeneous linewidth, Γ . Also, the A term depends on the Franck–Condon factors $\langle f | v \rangle \langle v | i \rangle$, which increase with the extent of the displacement of the excited state potential along the normal coordinate (i.e. the origin shift). Note also that we are in the Condon approximation where, for an allowed transition, M is large and is assumed to have negligible nuclear dependence, and is thus considered constant (adiabatic approximation). Non-adiabatic effects occur when nuclear motions actually change M and it can no longer be expanded in Q (i.e. Q changes).

Only totally symmetric modes are enhanced via the A term since only totally symmetric modes cause an origin shift. For non-totally symmetric modes, the origin shift and the Franck–Condon factors are both zero (in the absence of an origin shift, either $\langle f | v \rangle$ or $\langle v | i \rangle$ must be zero since i and f must differ for a Raman transition). Non-totally symmetric modes are intensified via the B term because of the Q -dependent vibrational integrals contained in the B expression. The normal coordinate connects vibrational levels differing by one quantum so that $\langle 1 | Q | 0 \rangle \langle 0 | 0 \rangle$ and $\langle 1 | 1 \rangle \langle 1 | Q | 0 \rangle$ both contribute to the B term.

The transition moment derivative in Eq. (3.7) is evaluated⁹⁴ using the Herzberg–Teller approach¹¹⁰ where it is considered to arise from the mixing of the resonant excited state, e , with a nearby state, s . The expression now becomes

$$B = \frac{(M_s^0)(M_e^0) h_{es}}{E_s - E_e} \sum_v \frac{\langle f | Q | v \rangle \langle v | i \rangle + \langle f | v \rangle \langle v | Q | i \rangle}{\epsilon_v - \epsilon_i + E_0 - E_L - i\Gamma} \quad (3.9)$$

where $h_{es} = \langle s | \partial H / \partial Q | e \rangle$. This integral mixes the two states via a given normal coordinate. The B term becomes important in the case where a forbidden or weakly allowed transition gains intensity from vibronic mixing with a strong allowed transition, as is the case in metalloporphyrins. When in resonance with such a weak transition, Raman vibrations that are effective in "borrowing" intensity from the strong band to the weak band will be observed. This enhancement depends on the size of the mixing integral and the energy difference of the two mixing states. (A limit to this case is the Jahn–Teller mechanism where the mixing states are degenerate.) In the quasi-allowed Q bands of metalloporphyrins, this Herzberg–Teller intensity borrowing mechanism from the strongly allowed Soret transition is in effect.

As will be discussed in Section C, recent work has indicated that this type of non–Condon scattering makes a small but important contribution to the Soret–excited REPs of metalloporphyrins, where the excited state displacements are much smaller than in polyenes despite the fact that both have strongly allowed electronic transitions. It has been pointed out⁷⁶ that while the A term is dominant on resonance with strongly allowed transitions, non–Condon effects are not always negligible. In the case of symmetry–forbidden or weakly allowed transitions the coordinate dependence of M and, thus, non–Condon effects, are clearly important.

The polarisation properties of rR scattered modes provides information about the symmetry of the mode. This polarisation is measured as a *depolarisation ratio*, ρ , which is the ratio of the measured Raman intensity of perpendicular polarisation to that of parallel polarisation with the incident radiation. The depolarisation ratio may be

categorised as follows:

$\rho < 0.75$	polarised (p)
$\rho = 0.75$	depolarised (dp)
$\rho = \infty$	inversely polarised (ip)
$0.75 < \rho < \infty$	anomalously polarised (ap)

For totally symmetric modes, the Raman bands will be polarised ($\rho < 0.75$). The reader is referred to Spiro^{77,94} for a discussion of the depolarisation ratios of metalloporphyrin rR bands.

B. Time-Dependent Picture.

In 1979, Lee and Heller¹¹¹ adopted a time-dependent formalism to derive alternative but equivalent expressions to describe the rR effect. In the Condon approximation, the time-dependent theory expresses the denominator of Eqs. (3.6) and (3.7) as a half-Fourier transform.^{76,112} The Raman polarisability is now written as

$$\alpha_{i \rightarrow f} = \frac{1}{\hbar} \int_0^\infty \sum_v \langle f|v\rangle \langle v|i\rangle \cdot \exp \left[\frac{-i(E_v - E_i + E_0 - E_L - i\Gamma)t}{\hbar} \right] dt \quad (3.10)$$

The expression for the rR cross-section can be shown to be^{76,111}

$$\alpha_{i \rightarrow f}(E_L) = \frac{8\pi E_S^3 E_L e^4 M^4}{9\hbar^6 c^4} \left| \int_0^\infty \langle f|i\rangle \exp \left[\frac{i(E_L + \epsilon_i)t}{\hbar} \right] e^{-\Gamma t/\hbar} dt \right|^2 \quad (3.11)$$

The corresponding absorption cross-section is written as a full rather than a half-Fourier Transform of the energy denominator.^{76,113}

$$\sigma_A(E_L) = \frac{4\pi e^2 M^2 E_L}{6\hbar^2 c n} \int_{-\infty}^{\infty} \langle i|i(t) \rangle \cdot \exp\left[\frac{i(E_L + \epsilon_I)t}{\hbar}\right] e^{-\Gamma|t|/\hbar} dt \quad (3.12)$$

In the above expressions, $i(t)$ represents the time-evolving excited state vibrational wavepacket of the initial state $|i\rangle$ under the influence of the excited state vibrational Hamiltonian which propagates it.

It is instructive at this point to discuss the physical interpretation of the above expressions, which is shown pictorially in Fig. 3.1. Initially, the system is in state $|i\rangle$, a vibrational eigenstate of the ground electronic surface. Incident radiation via the electronic transition moment causes a vertical transition to the corresponding excited state surface, where the wavefunction $|i\rangle$ is now under the influence of a new Hamiltonian of which $|i\rangle$ is not an eigenstate, and thus begins to propagate (i.e. it is no longer a stationary state). The time-dependent overlap of the wavepacket with the initial state, $\langle i|i(t) \rangle$, determines the absorption. At time zero, $\langle i|i(0) \rangle = \langle i|i \rangle$, which is unity. At time t , and if there is a significant shift between ground and excited surfaces, the wavepacket moves away from its initial position on the excited state surface. As a result, the overlap value drops but then reaches another maximum as the wavepacket completes one full cycle of harmonic motion and returns to its original position in the excited state. The shape of the absorption spectrum is determined by

Fig. 3.1. Schematic representation of the time-dependent picture of resonance Raman scattering (adapted from Ref. 76). The excited state wavepacket $|i(t)\rangle$ is the initial ground state vibrational wavefunction propagated on the excited state potential surface. E_L and E_S are the incident and scattered photon energies, respectively. E_0 is the energy gap between the potential minima. Δ is the equilibrium geometry change upon excitation. The initial and final vibrational wavefunctions in the Raman process are denoted as $|i\rangle$ and $|f\rangle$, respectively.

$\langle i|i(t) \rangle$ and its time dependence. The broadest feature in the absorption spectrum is governed by the shortest time event. If the displacement between ground and excited states is large, the initial decay of $\langle i|i(t) \rangle$ is rapid and the absorption spectrum envelope is broad. In the sum-over-states picture, this corresponds to extended vibrational progressions.

For resonance Raman, the amplitude is given by the half-Fourier transform of the overlap between this same wavepacket and the final state, $\langle f|i(t) \rangle$. At time zero, this Raman envelope is zero until after a short time, t , it approaches a maximum when the wavepacket is positioned to obtain sufficient overlap with a lobe in $|f\rangle$, then decreases to a minimum and then again to a maximum on the return trip. At the end of one full cycle, the overlap again goes to zero. The shape of the Raman excitation profile (the variation of Raman intensity with incident excitation frequency) is determined by $\langle f|i(t) \rangle$ and its time dependence. The intensity depends on the integrated area of $\langle f|i(t) \rangle$. We note that if the ground and excited state potentials are not significantly displaced, then the wavepacket does not travel enough to gain sufficient overlap with $|f\rangle$ and thus the Raman intensity is low. Therefore, only those vibrations which cause significant distortion upon electronic excitation are strongly enhanced. This is the A-term scattering of totally symmetric modes which is also obtained in the sum-over-states picture.

Both the sum-over-states and time-dependent expressions are mathematically equivalent, and both are equivalent descriptions at the same level of approximation. The choice of formalism depends on the problem under investigation. For multimode effects and the influence of a dissociative state on the rR intensities, it is easier to work

in the time-dependent picture. This formalism has been extensively used by Mathies and co-workers in their studies of rhodopsins^{76,114,115}.

A point which will be mentioned here and discussed later is the effect of the homogeneous linewidth, Γ , on the observed Raman intensities. From Eq. (3.11) we see that the Raman cross-section is obtained by taking the overlap of the propagating wavepacket with the final state, $|f\rangle$, multiplying it by the damping factor, $e^{-\Gamma t/\hbar}$, and then taking the half-Fourier transform with respect to the energy $E_L + \epsilon_i$, and finally squaring. In the time-dependent picture, Γ represents the lifetime of the excited state potential, which can be affected by many mechanisms¹¹⁶. The rR effect is a coherent two-photon process which is only in effect when the ground and excited potentials are superimposed; the time duration of this superposition is directly determined by Γ . The longer the lifetime of the excited state potential surface, the more oscillations the wavepacket undergoes and therefore the greater will be the rR cross-section. In the sum-over-states picture this manifests itself as a decrease in α because of the increase in the value of Γ in the denominator of Eq. (3.2). (Recall that a short lifetime is represented by large Γ values.)

C. Transform Methods.

In direct contrast to the previous two formalisms, which express Raman cross-sections in terms of molecular parameters, transform methods use multimode information contained in the absorption spectrum to generate the Raman excitation profile (REP), which yields information of the excited state displacement. Although some potentially interesting information is not explicitly revealed in this procedure, it is a very useful approach in analysing experimental Raman intensities, particularly where

limited data are available. In many cases, the use of a transform method enables one to easily obtain a simple REP from knowledge of only the absorption spectrum, and then to explore origins of deviations from the simple scattering theory.

Transform approaches to rR scattering may be formulated in either the sum-over-states¹¹⁷ picture or the time-dependent picture.^{118,119} The transform theory exploits the Kramers-Kronig relationship between the optical absorption cross-section and the rR amplitude.

The optical theorem¹²⁰ provides equivalent relations for the complex index of refraction, which is defined as

$$N(\nu) = n(\nu) - \frac{i}{4\pi\nu} \alpha(\nu) \quad (3.7)$$

where $n(\nu)$ is the real (or ordinary) index of refraction and α is the absorption coefficient (imaginary part). We see that the imaginary part of the complex index of refraction gives rise to the absorption spectrum, while the real part is known as the "dispersion curve". The relation between $n(\nu)$ and $\alpha(\nu)/4\pi\nu$ is causal and they are related via the Kramers-Kronig relations¹²¹; once one is known, the other may be determined.

The deep theoretical relationship between light scattering and optical absorption was first described by Hizhnyakov and Tehver¹²² in a time-correlator formalism but was only realised later by Tonks and Page¹¹⁸ as a potential tool in the analysis of rR intensities. The imaginary part of the amplitude for Rayleigh scattering is proportional

to the absorption cross-section. Furthermore, the real and imaginary parts of the scattering amplitude (which is, strictly speaking, still determined by the complex molecular polarisability) are related via the Kramers-Kronig relations^{109,121}. For Raman scattering, the above direct relationship does not strictly hold but a similar one has been derived.^{123,124}

The basic transform relation, at the simplest level of approximation, may be expressed as (see Ref. 123 for a concise derivation as well as Ref. 76):

$$\alpha_{0-1}(E_L) \propto S_1^{1/2} [A_0(E_L) - A_0(E_L - \epsilon_1)] \quad (3.14)$$

where α_{0-1} = rR amplitude (polarisability) for the 0-1 vibrational transition

E_L = the laser energy

ϵ_1 = the energy of the fundamental Raman mode

$S_1 = \Delta^2/2$ (essentially, a scaling factor in the transform method)

Δ = dimensionless displacement parameter between the ground and excited state.

The function $A_0(E_L)$ is defined by

$$A_0(E_L) = P \int_0^\infty \frac{\sigma(E)}{E(E-E_L)} dE + i\pi \frac{\sigma(E_L)}{E_L} \quad (3.15)$$

where $\sigma(E)$ is the optical absorption cross-section at energy E , and P denotes the principal part of the integral. The function $A_0(E_L - \epsilon_1)$ is the same function displaced by the energy of the vibrational quantum of interest.

We note here that the first term in Eq. (3.15) represents one of the Kramers-Kronig relations between $A_0(E_L)$ and $\sigma(E)$, thus establishing their causal relationship. Strictly speaking, $\text{Re}\{A_0(E_L)\}$ is the Hilbert transform¹²⁵ of $\sigma(E)$. The complete expression for the fundamental Raman cross-section is written as

$$\sigma_{0 \rightarrow 1}(E_L) = \frac{E_L E_S^3 n^2}{2 \hbar^2 c^2 \pi^3} S_1 |A_0(E_L) - A_0(E_L - \epsilon_1)|^2 \quad (3.16)$$

All energies are in cm^{-1} and σ is in $\text{\AA}^2/\text{molecule}$.

From Eq. (3.16), it is obvious that the REP generated depends only on the shape of the absorption band and the mode frequency. This is to be expected when the Kramers-Kronig relationships are involved, because it is then assumed that the absorption and the REP functions are causal. Also, the absolute scaling of the REP depends on S , which itself depends on the excited state displacement parameter, Δ .

The above, simple transform method provides a basic means for calculating

"first-order" REPs. The inherent assumptions of the simple transform are listed below:

- i) no inhomogeneous broadening
- ii) a single electronic state
- iii) constant transition length (adiabatic in the Condon approximation)
- iv) no frequency changes or Duschinsky rotation
- v) no Herzberg–Teller or Jahn–Teller activity.

The attractive feature of the transform method is that once the REP is generated, any of the above restrictions may be relaxed in order to obtain the best fit with the experimental REP. Modifications can be made to suit the above situations. This provides clues to the molecular dynamics which are occurring during excitation, which otherwise could only be probed with much more complicated apparatus.

In this work, REPs will be measured for Ni pheo *a*, a metallochlorin. Parent metalloporphyrins are notorious for Herzberg–Teller and Jahn–Teller activity in the Q states. This will cause deviations from simple A–term (or Franck–Condon) scattering, which is the limit of the working transform technique just described. The inclusion of non–Condon and nonadiabatic effects into the transform theory have been developed by Stallard and Schomacker *et al.*^{126,127,128} Their procedure introduces a new parameter, C_r , to the basic transform equation.⁸² In our notation, Eq. (3.16) is now (simplified)

$$\sigma_{0-1}(E_L) \propto S_1 |(1+C_r) A_0(E_L) - (1-C_r) A_0(E_L - \epsilon_1)|^2 \quad (3.17)$$

The parameter C_r is a measure (non–Condon to Condon ratio at the amplitude level) of

the amount of vibronically (non—Condon or B—term) induced transition moment which contributes to the scattering amplitude. It also includes nonadiabatic effects

$$C_r^{\pm} = C_r \pm \frac{C_r \epsilon_1}{\Delta_0} \quad (3.18)$$

where Δ_0 is the energy gap between the resonant and the coupled state. The inclusion of C_r , although apparently trivial, has been shown to be theoretically valid and physically significant¹²⁶.

For the work presented in this thesis, the inclusion of C_r to account for vibronic coupling was the only modification made to the basic transform theory.

Implementation.

REPs were generated according to the prescription of Chan and Page.¹²⁴ The main technical point of the procedure is the evaluation of the principal—value integral of Eq. (3.15). This can be done analytically from the digitised absorption spectrum⁷⁶, as was done by Chan and Page.¹²⁴ In this work, a more elegant and computationally faster method is used which employs fast Fourier transform techniques¹²⁵ that significantly reduce noise in the generated REPs.

We have previously stated that $\text{Re}\{A_0(E_L)\}$ and $\sigma(E)$ are related via the Kramers—Kronig relations. This means that one can obtain either function by performing a Hilbert transformation¹²⁵ on the other function. By definition, the

Hilbert transform of a function $f(x)$ (i.e. F_{Hi}) is

$$F_{Hi} = f(x) \otimes (-)\frac{1}{\pi x} \quad (3.19).$$

The above equation is interpreted as convoluting the function $f(x)$ with $-1/\pi x$. This transformation does not affect the amplitude of the spectral components but merely alters their phases by $\pi/2$. Convolution is accomplished by multiplying the Fourier transforms of each function and then inverse Fourier transforming the resultant product. In the case of Eq. (3.19) this is particularly straightforward because the Fourier transform of $-1/\pi x$ is simply $i \operatorname{sgn} s$, which is equal to $+i$ for positive s and $-i$ for negative s values.

The problem now becomes Hilbert transforming the digitised absorption spectrum, $\sigma_A(E)$ (which is the imaginary part of $A_0(E_L)$) to obtain the real part of $A_0(E_L)$. Figure 3.2 illustrates the intermediate steps involved with the transform method used to generate the REPs from the absorption spectrum.

3.3 HOMOGENEOUS LINEWIDTH

The most fundamental limitation of the transform technique is its inadequacy in compensating for any inhomogeneous broadening which may be present in the observed absorption band. All the expressions given so far for rR descriptions apply only to the single-molecule case. Since the homogeneous linewidth, Γ , is a single-molecule parameter, it is important to use this value when the correct shape of the REP is desired, and especially for accurate Raman cross-sections.

Fig. 3.2.

Intermediate steps in the transform method. The absorption spectrum is the Soret band of a Chl - like species. a) The absorption spectrum (the imaginary part of $A_0(E_L)$) and its translation of 1190 cm^{-1} , the vibrational quantum of interest. Below is the square of the difference. b) The Hilbert transform of a) which represents the real part of $A_0(E_L)$. Below is the square of the difference. c) The sum of the real and imaginary parts contributing to the REP. d) The resulting REP in relation to the absorption spectrum.

Consider an isolated molecule in an excited state. If the only way it can relax is radiatively, then in this case the homogeneous linewidth, Γ , represents the natural radiative width which is, typically, a fraction of a wavenumber.⁷⁶ Now consider a complication by allowing neighbouring molecules to collide with the excited molecule. These collisions destroy the coherence of the molecule–radiation interaction by affecting the phase factor in the molecular wavefunction. This causes dephasing of the coupled molecule–radiation state via radiationless relaxation. These processes broaden the spectral line without actually shortening the radiative lifetime, since they leave the molecule still excited. In solution environments, even faster radiationless relaxation is possible because of solvent–solute interactions. Recent cross–section measurements of I_2 in hexane¹²⁹ imply that Γ is of the order $10 - 20 \text{ cm}^{-1}$. The lineshape due to Γ is Lorentzian, the Fourier transform of a decaying exponential in time.

For an excited state, the homogeneous linewidth, Γ , is usually written as the sum of two contributions^{130,131}

$$\Gamma = \frac{\gamma}{2} + \hat{\Gamma} \quad (3.20)$$

The linewidths can be written as $\Gamma = \hbar/t$ ¹³² so that the above equation is equivalent to

$$\frac{1}{T_2} = \frac{1}{2T_1} + \frac{1}{T_2^*} \quad (3.21)$$

where T_2 (associated with the homogeneous linewidth, Γ) is the total dephasing decay constant due to all processes which contribute to the diminution of coherence, T_1 (associated with linewidth, γ) is the population decay constant due to radiative and

nonradiative processes inherent to the molecule itself, and T_2^* (associated with the linewidth, $\hat{\Gamma}$) is the *pure* dephasing decay constant resulting from fluctuations in the environment to which the molecule is coupled. The $1/T_1$ or γ term can be explicitly written as the sum of several components¹³⁶

$$\frac{1}{T_1} = \frac{1}{T_e} + \frac{1}{T_v} + \frac{1}{T_r} \quad (3.22)$$

where T_r = decay time due to radiative decay (fluorescence)
 T_v = decay time due to intramanifold vibrational decay (vibrational relaxation)
 T_e = decay time due to extramanifold nonradiative decay

Of course, for each of these decay times there exists their corresponding effective linewidths

$$\gamma = \gamma_e + \gamma_v + \gamma_r \quad (3.23)$$

We have established that the Raman cross-section is proportional to T_2 or $1/\Gamma$. The dependence of the Raman scattering cross-section with the resonant excited state¹²⁹ is the probability for absorption to that state followed by the probability for spontaneous emission to the desired final state *during* the lifetime of the excited state.

When a photon from the incident radiation field is absorbed by the molecule,

several events may occur:

1. The molecule can lose energy to the solvent bath and return to the ground state (γ_e).
2. The molecule can relax in the excited state and emit a photon ($\gamma_v + \gamma_r$).
3. The molecule can spontaneously emit a photon before the field-molecule coherence is interrupted by the solvent bath ($\hat{\Gamma}$).

When a molecule can spontaneously emit a photon before any of the above dephasing processes effectively destroy the coherently prepared excited state, resonance Raman scattering will occur.

The $\hat{\Gamma}$ or pure dephasing term warrants some discussion at this point. It is the medium or environment which introduces this dephasing¹¹⁶ by modulating the electronic energy gap of the molecule, and thus destroys the well-defined phase relationship between the photon field and the molecule (and the electronic states that are resonating due to the field). It is these dephasing events which actually cause the formation of real excited state populations.^{133,134} The emission from this incoherently prepared population of excited state molecules is fluorescence. In the event (or time-scale) that coherence is maintained, the emitted radiation is Raman or Rayleigh scattering.

For the case where there is no pure dephasing, we have $T_2 = 2T_1$, and thus the only dephasing is due to the decay of the population. The observed linewidth would be due to the lifetime of the state. In this coherent limit, the radiation emitted would be Raman or Rayleigh scattering. When $T_2 < 2T_1$, then T_2^* is non-zero and an incoherent

population of excited states is formed. This results in the emission of both fluorescence and scattering.

Examining Eq. (3.21), we see that the population decay directly affects the total dephasing time, and thus the Raman cross-section. A decrease in the population decay time, T_1 , will decrease the total dephasing time, resulting in an increase in the homogeneous linewidth of the excited state. In our work, exchanging Mg(II) with Ni(II) in the chlorophyll macrocycle results in a drastic decrease in the radiative lifetime of the Q_y state. Ordinarily, Chl *a* has a radiative lifetime of about 6 ns⁵⁰, but the Q_y radiative lifetime of Ni pheo *a* is probably less than 0.6 ps.⁵⁴ This represents a change of at least four orders of magnitude in T_1 . Furthermore, if this 0.6 ps lifetime is comparable in magnitude to T_2^* , the total dephasing time is expected to significantly decrease, which results in a lowered scattering cross-section.

For the case of the ferrocytochrome *c* Soret band, it is expected that pure dephasing contributions to Γ are small^{127,135}, and that γ is the dominating factor, in particular γ_e . This seems logical in the sense that very little, if any, incoherent emission (fluorescence) is observed from this state. Also, since the Q_y or S_1 state of Ni porphyrins (and in particular Ni pheo *a*) are not luminescent, then we would expect γ to dominate the total dephasing time of this state. In a sense, the problem becomes less complicated since Γ is primarily dominated by one term, γ .

Based on the above discussion, we expect Ni pheo *a* to exhibit broader spectral absorption features and a lowered resonance Raman cross-section as compared to chlorophyll *a* in the case where γ significantly contributes to Γ .

Real molecular samples consist of very large molecules which will possess other intrinsic dephasing mechanisms that result in broader Γ values. Also, if the distribution of molecular velocities, or (of more importance to this work) the distribution of molecular environments in the condensed phase is statistical (i.e. Gaussian), the absorption line will acquire a Gaussian profile. This is the origin of inhomogeneous broadening and is known as the *site-effect*. Unlike homogeneous broadening, this is a many-molecule (environment) effect and does not contribute to the Raman cross-section at the amplitude level.

There are mechanisms responsible for Gaussian-broadening Γ at the single-molecule level that directly affect the calculated rR cross-section. Damping owing to dynamics along unseen low-frequency vibrations¹³⁷ or to a motion which causes upper state dissociation¹¹⁴ causes the decaying in time to be more Gaussian in nature. The Fourier transform of a Gaussian decay gives a Gaussian lineshape. It is thus possible to observe a predominantly Gaussian absorption band¹³⁸ of $\sim 300 \text{ cm}^{-1}$ which is not significantly inhomogeneously broadened.

The resonance Raman effect may distinguish between homogeneous and inhomogeneous broadening if absolute cross-sections are known. This is due to the fact that homogeneous broadening appears as damping at the amplitude level, while inhomogeneous broadening produces a distribution of transition energies at the probability level.

To illustrate the points mentioned, we consider a gedanken molecule whose absorption spectrum shows a strong 0-0 transition and a weaker 0-1 (represented as only one mode for simplicity) transition (see Fig. 3.3). It is stressed that this

single-molecule absorption is not realistic, but it facilitates the clarification of several points of the transform method. This absorption spectrum is constructed of two Lorentzians, each with a bandwidth of 50 cm^{-1} (HWHM). The REPs of several modes ($50, 100, 500, 1000$, and 1500 cm^{-1}) generated with this absorption spectrum are shown in Fig. 3.4. We note that for relatively high-frequency Raman modes (vibrational quanta $\gg \Gamma$) such as the $1500, 1000$, and even 500 cm^{-1} modes, the expected equal enhancements in the 0-0 and 0-1 regions are observed. Only for those modes whose vibrational energy are comparable to Γ do interference effects begin to appear (the 100 and 500 cm^{-1} modes).

Let us now consider the case where Γ is increased via another mechanism. This is represented by another set of Lorentzians 320 cm^{-1} wide (HWHM) as shown in Fig. 3.3 as well. The REPs generated from this absorption spectrum are shown in Fig. 3.5. In general we see that the cross-sections are lower than those of Fig. 3.4, especially for the lower frequency modes. This is expected in light of the discussion in Section 3.2C. We also see interference effects in the higher frequency modes such as the 1000 cm^{-1} mode. These interferences effectively draw the 0-0 and the 0-1 maxima closer together by blue-shifting and red-shifting them, respectively.

To study the effects of erroneously transforming an inhomogeneously broadened absorption spectrum, a 300 cm^{-1} (HWHM) Gaussian band was convoluted onto the 50 cm^{-1} Lorentzian (see Fig. 3.3). The REPs generated from this absorption band are shown in Fig. 3.6. The REPs from the 50 cm^{-1} Lorentzian that have been Gaussian broadened to 300 cm^{-1} , are shown in Fig. 3.7. Comparison of these two sets of REPs indicate the type of errors caused by transforming an absorption band which exhibits significant inhomogeneous broadening. In most cases, the shape of the experimental

a *b* *c*

Fig. 3.3. Absorption spectra of the gedanken molecule. All lineshapes are expressed as half-widths at half-maximum.

- a) 50 cm^{-1} Lorentzian lineshape
- b) 320 cm^{-1} Lorentzian lineshape X 6
- c) 50 cm^{-1} Lorentzian convoluted with a 300 cm^{-1} Gaussian lineshape.

Fig. 3.4.

REPs generated using lineshape in Fig. 3.3a. The particular Raman modes (in cm^{-1}) are indicated in the Legend. The bottom figure is an expansion of the above figure over the region 14400 cm^{-1} to 15800 cm^{-1} .

Fig. 3.5.

REPs generated using lineshape in Fig. 3.3b. The particular Raman modes (in cm^{-1}) are indicated in the Legend. The bottom figure shows the 50 cm^{-1} and 100 cm^{-1} modes in an expanded intensity scale. Both are X 6.

Fig. 3.6.

REPs generated using lineshape in Fig. 3.3c. The particular Raman modes (in cm^{-1}) are indicated in the Legend.

Fig. 3.7.

REPs generated using lineshape in Fig. 3.3a then convoluted with a 300 cm⁻¹ Gaussian lineshape.

REP (if well-resolved and reasonably complete) will indicate the proper Γ ; one may deconvolute the inhomogeneous broadening from the absorption band.

3.3.1 Homogeneous Linewidths of Metalloporphyrins and Chlorins.

There is a great deal of interest in determining the homogeneous linewidths of porphyrins and chlorophylls⁸⁰ because of the strong interdependence of the optical lineshapes and absolute rR cross-sections.¹³⁵ The homogeneous linewidths of some (metallo)porphyrins have been investigated in order to determine possible spectral broadening mechanisms of some important biomolecules.

There are several methods which may be used to "estimate" or observe the homogeneous linewidth of an electronic state. Even and Jortner¹³⁹ reported a homogeneous linewidth of 11 cm^{-1} for an Ar-porphine van der Waals complex which was supersonically expanded to yield vibrationless fluorescence spectra. In a similar study of Zn tetraphenyl porphyrin¹⁴⁰ the $S_0 \rightarrow S_1$ transition was found to be 1.2 cm^{-1} in spectral bandwidth, which was assigned as rotationally broadened. The same molecule in a benzene solution at 300 K exhibited a spectral bandwidth of about 400 cm^{-1} . This was assigned to unresolved vibrational sequence congestion and inhomogeneous broadening¹⁴¹ (equivalent to a gas at $> 421 \text{ cm}^{-1}$). For Zn tetrabenzoporphyrin¹⁴² the $S_0 \rightarrow S_1$ width was 1.5 cm^{-1} under supersonic expansion of He, while a pyridine solution of the same complex at 300 K exhibited a 300 cm^{-1} bandwidth (and 890 cm^{-1} for the vapour at 800 K). The $S_0 \rightarrow S_2$ transition into the Soret band was approximately twice as wide.

Another method of obtaining high-resolution fluorescence and absorption spectra is to incorporate the porphyrin into solid solutions at low temperatures (Shpol'skii matrices).^{143,144,145} This method¹⁴³ yields linewidths of $5\text{--}10\text{ cm}^{-1}$ for chlorophylls in ether at 5 K. The authors interpreted this as being due to mainly inhomogeneous broadening. This was removed by a photophysical holeburning technique which resulted¹⁴⁴ in a linewidth of $1\text{--}3\text{ cm}^{-1}$.

In general we see that, under similar conditions where possible, all the above-mentioned metalloporphyrins exhibit similar linewidths. In the recent rR study of myoglobin,¹³⁵ Champion and co-workers concluded that for that specific metalloporphyrin and most likely for others, there are low-frequency modes coupling¹³⁷ to the Soret electronic transition, which results in large homogeneous linewidths significantly greater than 50 cm^{-1} . In this work, the observed linewidth of Ni-pheo *a* in a dilute benzene solution at 25°C was about 300 cm^{-1} (HWHM) in the Q_y state. It will be shown that this absorption linewidth is satisfactory in reproducing the experimental REPs of this molecule, indicating that substantial inhomogeneous broadening is not present. The same is most likely true for Chl *a* in similar solutions except in the case where dimerisation occurs.⁵²

3.4 DIFFUSE REFLECTANCE SPECTROSCOPY.

In some instances, absorption spectra of opaque, heterogeneous, and highly scattering samples is required. This is the case in this work, where the absorption spectrum of Ni-pheo *a* deposited on a Na_2SO_4 substrate is required for resonance Raman transform analysis. It is clear that conventional transmission absorption

spectroscopy is not applicable for this sample because of its high optical density and highly scattering substrate; no detectable light is transmitted. For such samples, diffuse reflectance techniques^{146,147} are needed.

There are two types of reflection which may occur at a sample's surface:

- i) specular, regular surface, or mirror reflection;
- ii) diffuse reflection.

In specular reflection, the incident light is almost entirely reflected by the surface of the sample at the same angle to the normal. In diffuse reflectance, the incident light penetrates the surface of the sample into the interior of the particles, returning to the surface as a result of multiple scattering at the individual particle interfaces and being attenuated by absorption within the particle. The diffuse reflection occurs randomly and is not directional. It is important to note that specular and diffuse reflectance behave oppositely in the region of an absorption band, where an increase in the absorption coefficient leads to more specular and less diffuse reflection.

One approach to describe diffuse reflected light from a medium containing randomly distributed, and uniformly absorbing and scattering particles is to use Kubelka–Munk theory.^{148,149} The two differential equations which describe the light flux in a thin slice of thickness dx at a depth x beneath the irradiated surface are:

$$dI(x) = - (K+S) I(x) dx + S J(x) dx \quad \text{attenuation} \quad (3.24)$$

$$dJ(x) = (K+S) J(x) dx - S I(x) dx \quad \text{generation} \quad (3.25)$$

where K = absorption coefficient (distance⁻¹)
 S = scattering coefficient (distance⁻¹)
 $I(x)$ = light flux away from surface ($-x$ direction)
 $J(x)$ = light flux towards the surface.

The two light fluxes moving in opposite directions are considered perpendicular to the irradiated surface at $x = 0$.

Most chemists are familiar with Lambert's law for transparent media, which is obtained from Eq. (3.24) for $S = 0$ (i.e. no scattering)

$$dI(x) = -I(x) K dx \quad (3.26)$$

in which case $K = \alpha = \epsilon C$, where ϵ is the extinction coefficient and C denotes concentration.

When the sample is so thick that the layer containing the light flux is backed by material into which no light penetrates (i.e. increasing thickness x further does not affect reflectance, R), equations (3.24) and (3.25) may be solved¹⁴⁷ to give

$$\frac{(1 - R)^2}{2R} = \frac{K}{S} \quad (3.27)$$

where R is the observed reflectance ($R = J_0 / I_0$). A plot of $\log(K/S)$ versus wavelength will give the diffuse reflectance absorption spectrum.

4. EXPERIMENTAL

4.1 METHODOLOGY.

The main technical obstacle to overcome in this Raman study was the strong fluorescence of the chlorophyll Q_y state. An effective method of eliminating this undesirable fluorescence is to replace the Mg(II) ion in the centre of the Chl macrocycle with a heavier atom. This, via spin-orbit coupling, results in more effective intersystem crossing of the singlet excited state to the triplet manifold, thus quenching the strong fluorescence.

Surveying all the possible metals which could be inserted into a porphyrin ring (see Refs. 55, 149a, and 149b for a list of all metalloporphyrins synthesised in recent times), the Ni(II) metal ion was chosen for the following reasons:

1. Ni(II) porphyrins are totally non-luminescent^{55,58,149c,149d} as was explained in Chapter 2
2. Ni(II) porphyrins are stable, much more so than Mg(II) porphyrins¹⁵⁰, and less susceptible to photodamage and degradation¹⁵¹.
3. In terms of vibrational analysis, the vibrational spectra and normal coordinate analysis of Ni(II) octaethylporphyrin are the benchmarks for the interpretation of metalloporphyrin spectra.^{100,152,153}

With the choice of central metal ion completed, the next step is to insert the Ni(II) into the Chl *a*. This is done by established methods whereby the Chl *a* is pheophytinised (ie "de-metallated") and then nickel is inserted. The next step requires the isolation of the pure Ni pheophytin *a*, Ni pheo *a*, by chromatographic methods.

Because of trace amounts of highly-fluorescent impurities which remain after the separation procedure, a prepared sample of $\sim 10^{-4}$ M Ni pheo *a* in hexane or benzene will still exhibit enough fluorescence to significantly mask the Raman spectrum. It was found that depositing a film of Ni pheo *a* onto an inert substrate such as anhydrous Na_2SO_4 quenches the fluorescences of the impurities. As well, because of the strong SO_4^{2-} Raman band, it serves as an internal standard to measure the amount of resonance enhancement of the Ni pheo *a* Raman bands.

Still another problem is created when one deposits a film of highly absorbing material on a highly scattering substrate; under incident laser radiation the sample is heated, which results in dramatically lowered Raman signals and sample degradation. The heating problem was alleviated by rapidly spinning the sample with a special rotating disc assembly¹⁵⁴. To extend the life of the sample and prevent photo-oxidation, the entire sample-optical assembly was kept under a N_2 atmosphere.

Another technical constraint to this study is the limited choice for laser beam-sample geometry. Grazing geometry excitation has been used successfully in obtaining good quality Raman spectra of chlorophylls with a minimum amount of laser power^{8,48}. Although this geometry has the desirable effect of reducing sample re-absorption of both incident and scattered photons, it is undesirable when accurate

resonance Raman excitation profiles are required (by accurate we mean with respect to cross-section values). Wavelength-dependent reflectivity changes¹⁵⁵ caused by the dispersion of the absorption coefficients (or refractive indices) of the sample film and of the substrate (or the solution and quartz cell interface) cause photon losses¹⁵⁶ for both the incident and scattered photons as a complicated function of grazing incident angle and wavelength. This is very difficult to correct and take into account. A better geometry, which is also better for very highly absorbing samples, is the 180° backscattering geometry; it is the one used in this work. It has been used successfully by Cotton and van Duyne¹⁵⁷ in their resonance Raman studies of chlorophylls.

With the above rationale and strategy in mind, this chapter outlines the steps taken to measure the REPs of Ni pheo *a* excited in the Q_y state.

4.2 PURIFICATION OF CHLOROPHYLL FROM SPINACH.

In order to synthesise Ni pheo *a*, it was necessary to obtain the purified starting material, Chl *a*.

Chl *a* was isolated from fresh spinach according to the method of Omata and Murata^{158,159}. All procedures were performed in dim lighting to prevent photo-oxidation^{160,161,162,163} (see Ref. 164 for possible degradation products). This method was chosen because it is rapid and can be easily used for small preparations. The following procedure was used for the extraction of 1 kg of spinach leaves.

Fresh spinach leaves were shredded, washed with cold water, and then scalded

with boiling water (distilled) until the leaves softened (1–2 minutes). The leaves were strained, dried with paper towelling, and allowed to cool. The leaves were then immersed in a minimum amount of ice–cold acetone to which $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$ had been added (3.5 g per 20 ml acetone). This mixture was allowed to stand for several hours in an ice bath, in the dark until the acetone appeared a very dark green colour; the leaves were then removed.

Next, the extracted pigments were precipitated from the acetone with the addition of 1,4–dioxane (2.5 ml per 20 ml of acetone) followed by the addition of distilled water (~4 ml per 20 ml acetone) until a green precipitate was observed. This colloidal dioxane/water precipitate was allowed to settle for about 6 hours, in the dark. The precipitate was quickly filtered, under vacuum, using a fritted glass filter (coarse). The solid was then recovered by washing the glass filter with ethanol. The ethanol wash (containing the pigments) was quickly evaporated to dryness under vacuum, leaving a black, waxy solid. This residue was washed with 20 ml aliquots of hexane to remove soluble chlorophylls and leave behind less soluble undesired pigments (such as water–soluble carotenoids). The hexane was then evaporated under vacuum.

The next step in the process is the isolation and purification of Chl *a* using column chromatography to: i) remove carotenoids from the crude pigment extract, followed by ii) its purification from other chlorophyll pigments. Each step required different column materials.

Column material (Pharmacia Fine Chemicals) was prepared from aqueous suspensions of DEAE–sepharose CL–6B (the diethylamine derivative of the cross–linked agarose) and Sepharose CL–6B (a cross–linked agarose). The

DEAE-Sephrose was prepared by washing a 50 ml suspension, successively, with distilled water, 1 M sodium acetate buffer (pH 7.0), distilled water, and acetone. These washings were done by filtration under vacuum and immediate re-suspension. The Sepharose CL-6B material was prepared by successively washing 100 ml of the suspension with distilled water, acetone, 2/1 acetone/hexane mixture, 1/2 acetone/hexane mixture, 10/1 hexane/2-propanol mixture, and 20/1 hexane/2-propanol mixture. The materials were packed in water-cooled columns (1–2 cm in diameter) to a height of about 4 cm, stirred with a glass rod, and allowed to settle under solvent flow. Uniform packing was essential.

After use, the columns were regenerated to remove adsorbed pigments. The Sepharose CL-6B column was regenerated by washing successively with acetone, methanol, and 20/1 hexane/2-propanol mixture. The DEAE-Sephrose column was regenerated by successively washing with 80% aqueous acetone containing 1% ammonium acetate, and finally, acetone. Each column was carefully re-packed under solvent flow with the last solvent used. In general, each column was used five times before discarding. For the final passes, new column material was used.

The purification of Chl *a* from the isolated chlorophylls proceeded as follows.

Small amounts of the solid, crude pigment extract were dissolved in a minimum of acetone (usually 1 ml) and applied to the first column, DEAE-Sephrose. The column was developed with acetone at the constant flow rate of about 2 ml/minute until all of the pheophytins (grey band¹⁶⁴) and carotenes (yellow-orange bands¹⁶⁴) were eluted. At this point the chlorophylls are left adsorbed on the column. These were eluted by developing the column in a 10/3 acetone/methanol mixture (green, unresolved

band). Much of the fatty materials¹⁵⁸ were retained on the column and were only removed during the regeneration of the column.

The eluent containing the chlorophylls was immediately evaporated under vacuum and stored in the dark while the column was regenerated and further separations made. The above procedure was repeated, as necessary, (up to three times usually) until carotenoids could not be detected on a TLC plate (silica gel C, Merck). These plates were developed using a mixture of hexane/acetone/*t*-butanol (90/5/5).

The next step was to separate Chl *a* from Chl *b* (spinach is very rich in Chl *a* and contains very little of the other chlorophylls). The crude Chl *a* was dissolved in 0.5 ml aliquots of a 2/1 hexane/2-propanol mixture and applied to the Sepharose CL-6B column. This column was eluted with the 20/1 hexane/2-propanol mixture at a flow rate of about 1 ml/minute. The first band to be eluted was the grey band indicating pheophytins. The next band to be eluted was that of Chl *a* along with an unresolved, smaller band slightly ahead¹⁶⁴ (Cl. *a'*). As much as possible, Chl *a'* was not collected. The last band to remain on the column was Chl *b* (and Chl *b'*); this had to be eluted with a 10/1 hexane/2-propanol mixture because of its longer retention time. At this point only a dark green band (narrow) at the very top of the column remained (probably oxidised Chl material¹⁶⁴) which was completely removed only at the re-generation step. The crude Chl *a* eluent, once collected, was immediately evaporated under vacuum and stored in the dark while the column was regenerated and the remainder of the crude Chl *a* was processed. This procedure was repeated about five times by which time no Chl *b* was observable. The final pass was done on a column containing unused material.

The purity of the Chl *a* was checked by TLC (hexane/acetone/*t*-butanol 15/2/2) and HPLC. The HPLC analysis was performed on a Waters Associates M-6000 Series using a silica column (46x220 mm, Brownlee). A one-step solvent 20 minute program was used to separate the chlorophylls. The absorbance was measured at 430 nm using a SF-770 Spectroflow UV detector (Schoeffel Instruments). The only observable peaks were those of Chl *a* and Chl *a'* (this means that all other possible contaminants absorbing at ~ 430 nm are present at < 0.1% of the Chl *a* content¹⁶⁵), which were present at a ratio of Chl *a* : Chl *a'* = 10 : 3 (it is possible that the HPLC column material converted^{164,166} some of the Chl *a* to Chl *a'* and, in effect, actual samples contained ~15%¹⁶⁷ Chl *a'*). For this reason, it is difficult to estimate the amount of Chl *a'* present in the samples. It was assumed that the presence of Chl *a'* did not affect the results of this work, since the visible absorption spectra of these two compounds are essentially, identical^{71,72,166,168,169,170}. Chl *a'* is the C-10 epimer¹⁷¹ of Chl *a* and is always in equilibrium with Chl *a*.¹⁷² The above assumption also holds true for the Ni pheo *a* and its C-10 epimer.

Once verified, the purified Chl *a* was dried by co-distillation with 3 successive aliquots of dried carbon tetrachloride, followed by 3 successive aliquots of dry hexane.⁷² At this point, the solvents used were of spectral grade. They were deoxygenated by sparging with dry N₂ gas passed through a dessicant column (25 cm of Silica gel) for 20 minutes. The solvents were dried and kept dry by storing them over 3 Å molecular sieves¹⁷² (Aldrich, 8-12 mesh). Chl *a* stock solutions were prepared with dry hexane and kept in a dark, dry glove box with a N₂ overpressure. Purified Chl *a* films were similarly stored.

The purity of the Chl *a* was also verified by absorption spectroscopy

(Perkin–Elmer Lambda 4B uv/vis Spectrophotometer) by measuring the intensity ratio of the red and blue absorption bands (blue / red = $(1.30 \pm 0.5)^{164}$).

It was found that Chl *a* samples prepared and stored in the above manner could be used up to a year after isolation. The HPLC chromatogram of a 12-month old Chl *a* sample in dry hexane showed that the sample had maintained its integrity (although Chl *a'* content was higher).⁵²

4.3 PREPARATION AND PURIFICATION OF Ni pheo *a*.

The starting material for the preparation of Ni(II) pheophytin *a* (Ni pheo *a*) is purified Chl *a*. The following procedure is a compilation of several methods.^{46,53,173} First, the Chl *a* was de-metallated by contacting it with HCl.^{174,175} About 25 mg of the purified Chl *a* was dissolved in ether and transferred to a separatory funnel. Approximately 0.5 ml of concentrated HCl was added to the ether while quickly swirling for about 2 minutes, until the bright blue–green solution turned to a duller green colour, indicating pheophytin had been formed (i.e. de-metallated Chl *a*). The ether phase was then washed five times with de-ionised, N₂–sparged water (50 ml aliquots) to neutralise the phase. The ether phase was then quantitatively transferred to a 250 ml round-bottom flask, where the ether was quickly evaporated under vacuum. The pheophytin film was then re-dissolved in ether and evaporated two more times, followed by three treatments with dry hexane.

The flask containing the pheophytin film was then fitted with a water-cooled

condenser tube under argon atmosphere. The film was dissolved in 50 ml of chloroform and 50 ml of glacial acetic acid, some of which was used to dissolve 0.06 g of nickel acetate $\cdot 4\text{H}_2\text{O}$ (4-fold excess of Ni(II) over pheophytin). The mixture was refluxed for one hour under an argon atmosphere after which it was cooled. A silica gel TLC plate indicated only one spot for the cooled reaction mixture.

The mixture was cooled (30 minutes) and then washed five times with de-ionised, deoxygenated water. The chloroform was evaporated under vacuum and the resulting film dissolved (and evaporated) two times in dry hexane and two times in benzene. These co-distillations removed any traces of water which could hydrogen-bond to the Ni pheo *a*.

The crude Ni pheo *a* was then purified on the Sepharose CL-6B columns as described in the previous section for Chl *a*. During this step only one band was observed.

The HPLC analysis (as previously described) (isopropanol/ethyl acetate/hexane 0.5/14.5/85) indicated the presence of two bands only; Ni pheo *a* and Ni pheo *a'* at a ratio of 100:31. Again, it would seem that any other impurities absorbing at ~ 430 nm would be present at less than 0.1%. Once purity was verified, the Ni pheo *a* was co-distilled two times in dry, deoxygenated carbon tetrachloride and four times in dry, deoxygenated hexane.

The absorption spectrum of the purified material dissolved in chloroform agreed very well with the spectrum of Boucher and Katz⁵³ (see Table 4.1).

TABLE 4.1: Absorption Maxima of Ni pheo *a* in Chloroform

$\lambda^{\text{a)}}$ (nm)	Ratio ^{a)} Blue/Red	$\lambda^{\text{b)}}$ (nm)	Ratio ^{b)} Blue/Red	in hexane (nm)
652.6	0.95	652	0.95	647.5
421.8		423		419.2

a) This work.

b) From Ref. 53.

The second derivative of the absorption spectrum of Ni pheo *a* in dry hexane ($\sim 10^{-4}$ M) indicates that no dimerisation occurred, as is evident by the presence of only one minimum in the Q_y region. Under similar solution conditions, Chl *a* exhibits dimer formation.^{42,46} This also agrees with the observations of Boucher and Katz⁵³ and is further proof of the exchange of central metal ion in the chlorophyll molecule. Figure 4.1 shows the absorption spectrum, along with the corresponding second derivative curve, of Ni pheo *a* in dry hexane.

Stock solutions of the purified Ni pheo *a* were prepared with dry hexane and kept in stoppered flasks in the dark, in the glovebox with a N_2 overpressure. These solutions were stable for at least 1 month.

Fig. 4.1.

Absorption spectrum (and its 2nd derivative) of Ni pheo *a* in dry hexane ($\sim 10^{-5}$ M).

A Fly in the Ointment?

Despite the spectral and HPLC data, the Ni pheo *a* contained traces of fluorescent impurities which were enough to seriously affect any Raman spectrum. A fluorescence spectrum of the Ni pheo *a* sample excited with 432 nm laser radiation, exhibited a single band at $14,800\text{ cm}^{-1}$ with a lifetime of about 6 ns. Based on previous work in this laboratory^{175,176} this fluorescence spectrum and lifetime are consistent with those of pheophytin *a*, the free-base form of Chl *a*. A fluorescence excitation profile of the $14,800\text{ cm}^{-1}$ band measured with a Perkin-Elmer 204-S Fluorescence Spectrophotometer equipped with a Perkin-Elmer 150 Xenon Lamp (10 μm slits) showed a peak at $\sim 670\text{ nm}$ (poor resolution 20 μm slits). Pheophytin *a* in dry hexane absorbs at 670 nm, which agrees with the fluorescence excitation profile data. Also, the fluorescence spectrum did not change in shape or lifetime when excited at 432 nm and 442 nm, indicating that only one fluorescent impurity was present.

From the above information it was concluded that trace amounts of highly fluorescent unreacted (i.e. "un-metallated") pheophytin remained in the "purified" Ni pheo *a* sample.

Several attempts were made to remove the fluorescent impurity with column chromatography using:

- i) Sepharose CL-6B according to the method of Omata and Murata¹⁵⁸ using a slightly narrower, but longer column.

- ii) Sepharose CL-6B but eluting with benzene and 2-propanol mixture (100:1) according to the method of Fujiwara and Tasumi.¹⁷³
- iii) using grade III Alumina according to Boldt *et al.*⁴⁶ eluted with the same mixture as in ii). (This method formed more pheophytin on the column than it separated.) As well, an eluting mixture of 20:1 benzene:2-propanol did not work.
- iv) Extraction of the impurity in 6N HCl.^{177,178,179,180}
- v) Reverse-Phase HPLC using a C₁₈ column (RP-18 SPHERI-10, Brownlee) 4.6 x 220 mm with a programmed solvent gradient which linearly changed from 90% acetonitrile/10% ethyl acetate to 100% ethyl acetate over a 20 minute period.¹⁸¹

All of the above proved to be ineffective.

Purchased Ni pheo *a*.

Ni pheo *a* was also purchased from Porphyrin Products (Logan, Utah) and was used without further purification. The absorption spectrum of this material in dry hexane is shown in Fig. 4.2 and is similar to that of the prepared material in this work. Differences in the spectra arise from the fact that the spectrum in Fig. 4.2 is from a solution five times more concentrated than that of Fig. 4.1. The slight spectral broadening is indicating an increase in inhomogeneous broadening, but the second derivative of the absorption curve still indicates that there is no observable

2

of/de

2

MicroD

Fig. 4.2.

Absorption spectrum (and its 2nd derivative) of Ni pheo *a* purchased from Porphyrin Products in dry hexane ($\sim 10^{-4}$ M). This sample is approximately 5 X the concentration of the sample in Fig. 4.1.

dimerisation. Based on the spectral data, the purchased material was considered to be the same as the prepared material. The purchased Ni pheo *a* also contained traces of highly fluorescent impurities.

4.4 SAMPLE PREPARATION.

At this point it was not feasible to physically remove the trace amounts of fluorescent impurities, therefore the fluorescence had to be suppressed. This was done by depositing a film of Ni pheo *a* onto an inert substrate, such as anhydrous Na_2SO_4 . By means of the heavy atom effect and/or energy transfer, the fluorescence of the impurities is effectively quenched. In this work it was found that depositing a concentrated film of the Ni pheo *a* material onto a pressed pellet of anhydrous Na_2SO_4 completely quenched the fluorescence and produced good quality Raman spectra at minimum incident laser powers.

There were two types of samples used in this work, a liquid phase and a solid phase sample. The liquid phase sample was composed of the purified Ni pheo *a* dissolved in benzene and was contained in a 1 mm optical quartz cell (Helma 220/221 QS Suprasil) sealed with Teflon stopcocks (Rotaflo). This sample was fluorescent and thus was used in resonance Raman work excited in the Soret region, where the red emission would not obscure the Raman spectrum. The solid phase sample was comprised of a film of Ni pheo *a* deposited on a pressed pellet of anhydrous Na_2SO_4 . They are described below.

A. Liquid Phase Sample.

These samples were prepared from the stock solution in dry hexane kept in the dark glove box. All glassware was thoroughly cleaned and dried in an oven at 120°C for 24 hours before use. A measured amount of the stock solution was transferred to a 1 mm quartz optical cell (as described above) and the hexane was immediately evaporated under a flow of clean, dry N₂. The sample was then re-dissolved in dry de-gassed spectral grade benzene to yield a sample with an optical density of 1 as measured at the peak of the Soret band. The cell was then stoppered. All manipulations were performed in the glove box.

B. Solid Phase Sample (Raman Spectroscopy).

Because a solid phase sample of a highly absorbing material which was irradiated with laser light was used, special precautions were taken to prevent local heating that would result in weakened Raman signals and sample degradation. To overcome this problem we have used a rotating sample system which contains a pressed disc-shaped pellet of Na₂SO₄ upon which a film of Ni phthalocyanine was deposited. The rotating laser Raman sample device¹⁵⁴ is shown in Fig. 4.3. The sample holder consists of a round piece of stainless steel that contains a circular channel groove (6 mm wide, 1.5 mm deep, 57 mm middle diameter) in which the powdered Na₂SO₄ (that had been stored in an oven at 120°C) was placed. A press tool led by a central rod (19 mm diameter), which is part of the sample holder, was placed over the sample holder

c)

b)

Fig. 4.3.

Rotating laser Raman sample device. a) Apparatus for preparing the ring-shaped powder pellets. b) optical arrangement of the sample holder. (Adapted from Ref. 154.)

containing the Na_2SO_4 powder, and the unit was placed between the plates of a hydraulic press. The unit was pressed with 20 tons of pressure for about 20 minutes to obtain a hard pellet surface which would not loosen and crumble due to mechanical vibrations during high velocity rotation.

After the Na_2SO_4 circular disc pellet was pressed, the holder was placed in the glove box, where a film of Ni pheo *a* was deposited onto the pressed pellet portion of the holder. This was done by coating the pellet with a few millilitres of the stock solution and then evaporating the hexane solvent under a N_2 flow. This step was repeated several times until the sample appeared dark green in colour. It was found that about 15 mg of Ni pheo *a* produced the best sample. It was difficult to estimate how much material was actually deposited onto the Na_2SO_4 substrate itself due to losses on the sidewalls of the channel groove and on the holder plate itself.

After the desired film material was deposited, the press tool was used to cover the sample and the unit was placed in a sealed container under a N_2 atmosphere so that it could be removed from the glovebox.

Just prior to recording a Raman spectrum, the sample was removed from the container and placed under an Atmos-Bag (Aldrich) arranged over the optical set-up in front of the Raman spectrometer (described in

Section 4.5). The sample was now under a N_2 atmosphere. At this time the sample holder was mounted on a central rod which had a screw and flat plate on one end. The rod itself fit into a chuck which was connected to a motor drive that provided a variable rotation rate from 0 to 3000 rpm. The sample holder and motor were then mounted in front of the Raman spectrometer. The optical arrangement and Raman spectrometer will be described in Section 4.5.

C. Solid Phase Sample (Absorption Spectroscopy).

A slightly different solid phase sample was required for the diffuse reflectance absorption spectroscopy work. In this case, about 15 ml of the Ni pheo *a* stock solution (~ 1 mg/ml) in dry hexane was contacted with 1 gram of oven-dried Na_2SO_4 powder contained in a pear-shaped flask. The contents of the flask were swirled vigorously and the hexane solvent was quickly evaporated under vacuum. This technique has been used in the past¹⁸² to ensure an even coating of material over powdered substrates. All the above was done in the glovebox.

The coated powder was then transferred to a stainless steel container along with a stainless steel ball bearing (~ 3 mm in diameter) and more powdered Na_2SO_4 . The container was capped and vigorously shaken with a mechanical shaker for 30 seconds. This produced a more "dilute" (~ 10 -fold), homogeneous powdered sample which was then placed into a 1 cm quartz optical cell (square) and positioned in front of the diffuse reflectance spectrophotometer which will be described in the following

section.

4.5 ABSORPTION SPECTRAL MEASUREMENTS.

A. Liquid Phase Sample.

For the liquid phase samples, absorption spectra were recorded using a Perkin-Elmer Lambda 4B uv/vis Spectrophotometer. Slitwidth was better than 1 nm at 500 nm of the spectral region. The software package provided with the spectrophotometer allowed for the direct calculation of second order derivative curves for the absorption spectra.

B. Solid Phase Sample.

The diffuse reflectance absorption spectra of Ni pheo *a* on the powdered Na_2SO_4 was obtained using the apparatus of J.C. Scaiano.¹⁸³

The basic system consists of a pulsed (0.5 ms) 250 W Xenon arc which irradiates the powdered sample at glancing incidence. This angle was important in that it minimised the amount of specularly reflected light from the quartz cell passing through the entrance slits of the monochromator. The reflected light from the surface of the powder sample itself was focussed with a collecting lens through the entrance slit of the analysing monochromator equipped with an optical multichannel analyser (OMA) (512 channels). A computer controlled the pulsing of the Xenon lamp, the shutter control, the triggering of the gated OMA, and the transfer of digitised data. For

these experiments, 100 pulses were accumulated for the diffuse reflectance absorption spectrum, with entrance slits on the analysing monochromator set at 2 nm resolution (at 500 nm) and an OMA gate width of 500 μ s. For further information the reader is referred to Reference 146.

4.6 INSTRUMENTATION.

In this work, two separate optical systems were used to obtain resonance Raman spectra. During the course of this work, the availability of one of the systems was not possible and so another was used.

4.6.1 System 1.

This system was used in the resonance Raman work of the liquid phase sample. This system is described in detail in the thesis of Thibodeau⁵². The components consist of a nanosecond, pulsed N_2 laser, its associated dye laser, a monochromator, and a detection system. A schematic diagram is shown in Fig. 4.4.

Lasers.

A nitrogen laser (Lambda Physik M2000) (337 nm) was used to pump a dye laser (Lambda Physik F12000) at a repetition rate of 30 Hz. The nitrogen laser produced 3 ns pulses. The tunable two-stage dye laser is capable of providing laser radiation from 380 nm to 700 nm. In this work, the dye used was a 3×10^{-3} M ethanol solution of LD 423 (Exciton) which lased from 418 nm to 428 nm with a peak power of over 2 mW. In practice, only 0.6 mW at the sample was needed to obtain reasonable Raman spectra from the

Fig. 4.4.

Schematic diagram of the nanosecond laser Raman System 1.

M1 to M6: mirrors

CL: cylindrical

COLL: collecting lens

FP: fast photodiode

MD: monochromator drive mechanism

Ni phéo *a* samples (O.D. = 1 in a 1 mm cell), and this lower power prolonged the life of the sample (i.e. minimised photodecomposition). Light from the dye laser was passed through a Pellin–Broca prism to disperse the superradiance and then through an iris.

Optical Alignment.

A problem with resonance Raman spectroscopy is that of re-absorption of the emitted Raman photons. Also, because the laser is deliberately tuned to the wavelength region of an absorption band, heating and decomposition may present serious difficulties. In liquid samples, this may be overcome by using a flowing cell technique (i.e. essentially, rapidly moving the position of the laser focus on the sample¹⁸⁴), or by using low incident laser intensities either with an unfocussed beam, or one brought to a slit focus with a cylindrical lens.¹⁵⁶

In this work, a cylindrical lens (20 cm focal length) was used. It was found that a slightly defocussed slit just within the cell face (in a 180° geometric alignment) yielded the same signal intensity as a tightly focussed slit. Therefore, the defocussed slit was used.

The 180° backscattering geometry was used (see Fig. 4.5) because of the highly absorbing material studied. Using a 1 mm pathlength cell with a sample O.D. of 1, average powers of 0.85 mW – 2.0 mW were found to give reasonable spectra and minimised re-absorption interference.⁷⁶ Also, re-absorption effects were minimised by choosing a reference band which is

close in frequency to the Raman bands studied; this was the 992 cm^{-1} band of

Fig. 4.5.

Position of the optical components used in the 180° backscattering geometry used in System 1.

the solvent, benzene.

The above precautions were taken to minimise photodecomposition and re-absorption effects. We now proceed to describe the rest of the optical arrangement.

The two mirrors and the cylindrical lens shown in Fig. 4.5 were mounted on gimbal mounts for fine adjustments. The dispersed light from the dye laser was reflected by mirror, M2, and then focussed with a cylindrical lens onto a small mirror, M3, which reflected a slightly defocussed slit of light, horizontally, onto the sample. The Raman scattered light was collected and directed into the Raman monochromator using two coupled spherical lenses in front of the monochromator.

Raman Monochromator Spectrometer.

The monochromator used in System 1 is the Jobin–Yvon Ramanor HG2. It is a double monochromator (operating in additive mode) with concave holographic gratings ($(100)^2 \text{ mm}^2$), 2000 grooves/mm, a 1 m focal length and F/8 aperture. There are 4 slits which are independently controlled and can have a width of up to 2 mm with $1 \mu\text{m}$ definition. The entrance and exit slits are horizontal.

The mechanical range of the Jobin–Yvon is from $12,800 \text{ cm}^{-1}$ to $25,000 \text{ cm}^{-1}$ and the resolution is better than 0.5 cm^{-1} at 514.4 nm , depending, of course, on the slit widths and scanning speed. The stray light rejection is estimated at 10^{-14} at 20 cm^{-1}

away from the Rayleigh line. The dispersed light exiting the monochromator then falls onto a photomultiplier tube, PMT, which comprises part of the detection system. Error is stated at $\pm 1 \text{ cm}^{-1}$.

Detection System.

The detection of light (e.g. the Raman signal) is accomplished by transforming photons into electric current using a photomultiplier tube (PMT). This signal is then amplified and filtered using a gated boxcar averager/integrator.

A. Photomultiplier Tube.

The PMT used in this work was a cooled RCA C31034A operating at 1800 V and having a rise time of 2.5 ns^{185} . This PMT possesses a relatively high quantum efficiency over a broad spectral range, and thus makes it very useful in visible spectroscopic studies. Under these conditions, the Raman signals had a signal-to-noise ratio of better than 20/1 in the best situations. Of course, weaker Raman signals resulted in lower signal-to-noise ratios.

B. Boxcar Averager/Gated Integrator.

In pulsed laser-excited Raman spectroscopy, a fast, analog signal from a noisy background must be recovered. A boxcar averager offers two main advantages in this respect:

- i) it improves signal-to-noise ratio by averaging 1 to 10,000 samples (i.e. laser shots)
- ii) the gated integrator integrates the signal during the duration of the gate which can be delayed in time (variably) to coincide with the maximum of the signal in time. The output from the integrator is then normalised to the gate width to provide a voltage which is proportional to the average of the input signal during the sampling gate.

In this work, the Stanford Research Systems SR250 Gated Integrator and Boxcar Averager was used. It consisted of a gate generator, a fast gated integrator, and exponential averaging circuitry.

In practice, the sampling gate is set to a width which corresponds to the time duration of the Raman signal (which exists only when the exciting laser pulse is at the sample) and then delayed so that the averager collects a signal when the Raman signal is present. The result is a maximum Raman signal with a minimum level of background noise and interfering fluorescence.

Instead of triggering the boxcar with the output pulse provided by the nitrogen laser, it was found that less jitter was obtained if the trigger signal originated from a fast photodiode irradiated by the dye laser light pulse. The intrinsic trigger-to-sample delay time of the boxcar is typically 40 – 70 ns, which was slightly greater than the time taken for the laser pulse to travel from the position of the photodiode to the detecting PMT. Therefore, the signal from the PMT was passed through an electronic variable delay box (EG&G DB263) before it was fed into the boxcar signal input.

Data Acquisition.

The Raman spectral data were acquired using a laboratory computer which was interfaced to the boxcar detection system using the Stanford SR245 interface module. This converted the analog signal to a digital record which was then stored, managed, and processed in the computer. The computer was also used to control the stepper motor of the Ramanor HG2 (equipped with a 4-phase stepping motor drive). One hundred (100) samples were averaged and recorded at every spectral resolution step (0.1 cm^{-1}) before the monochromator gratings were moved. Typically, two complete Raman spectra were recorded on the computer and averaged.

Complete details of the data acquisition system are found in the thesis of Thibodeau.⁵²

Powermeter.

Laser power was measured with a Photodyne 66XLA power meter fitted with a silicon detector head (#350).

4.6.2 System 2.

This system was used in the resonance Raman studies of the solid phase Ni phco *a* samples (on Na_2SO_4). The SO_4^{2-} 992 cm^{-1} stretch served as the internal Raman standard by which resonance enhancement could be measured and quantified. The major differences between System 1 and System 2 are:

- i) CW lasers are used in System 2;
- ii) optical multichannel detection for System 2.

The schematic of System 2 is shown in Fig. 4.6.

Lasers.

The Raman experiments were performed using a Coherent 590 dye laser pumped with a Spectra Physics Ar^+ ion laser. In the all-lines mode, the Ar^+ ion laser provided 1.7 W of laser power. The resulting dye laser power pumped with the Ar^+ laser in the all-lines mode was a maximum of 150 mW at the source. This was measured for the maximum of the 1.5×10^{-3} M DCM dye (Exciton) dissolved in a 2/3 mixture of benzyl alcohol and ethylene glycol (0.456 g of DCM in 1 L of solvent). To cover the spectral range of interest, two dyes were used:

- i) 1×10^{-3} M DCM in 2/3 benzyl alcohol/ethylene glycol
- ii) 1×10^{-3} M Rhodamine 6G in ethylene glycol.

The superradiance from the dye laser light was filtered by passing the beam through a Pellin-Broca prism. The laser power at the sample was less than 10 mW (losses due to prisms and mirrors).

Optical Alignment.

A 180° backscattering geometry was again used. The Ar^+ laser and coupled dye laser were situated below the optical table. The light from the dye laser

Fig. 4.6.

Schematic diagram of laser Raman System 2.

M1 to M2: mirrors

P1: Pellin-Broca prism

L1 to L2: lenses

SPECT: Triplemate spectrograph

Fig. 4.7.

Expanded view of Fig. 4.6 showing the 180° backscattering geometry.

was dispersed through the Pellin–Broca prism and reflected from mirror M1 upwards through a hole in the optical table. A lens, L1, focussed this light onto a small mirror, M2, which was attached to a steel arm mounted on a precision micro–positioner (with XYZ translation capabilities). This backscattering mirror brought the laser beam co–linear with the axis of the monochromator (i.e. parallel to the optical table) which then irradiated the sample on the rotating sample holder. The position of lens L1 (20 cm focal length, long focal cylinder) was adjusted to give the appropriate focus of the laser beam on the sample surface (slightly defocussed). This lens was a spherical lens and the laser spot was about 50 μm in diameter. Because of the high speed rotation of the sample (~ 2000 rpm), this somewhat tight laser spot did not degrade sample integrity or Raman signal due to local heating. The light scattered from the sample was collected with a spherical lens, L2, (20 cm focal length) situated in front of the monochromator entrance slits.

Spectrograph.

The collected light was dispersed with a SPEX 1877 B Spectrometer/Spectrograph Triplemate. This spectrograph provides low stray light (10^{-14} at 10 bandpass units from Rayleigh line) and a flat, undistorted focal plane, which is crucial for sensitive work with optical multichannel detectors.

There are two main sections to the Triplemate.

- i) The first section is a 0.22 meter double–monochromator with gratings locked in

a subtractive–dispersive mode. This stage acts as a variable–wavelength bandpass filter that feeds a non–dispersed segment of visible radiation from the scattering sample into the entrance slit of the second stage (the spectrograph stage).

- ii) This second stage is a 0.6 meter single–monochromator spectrograph stage which now disperses the radiation over the detector. The Triplemate may provide 3 gratings of different groove densities. In this work, a 1800 grooves/mm grating was used. At 500 nm, this would yield a $\sim 800 \text{ cm}^{-1}$ spectral window.

The optical path of the Triplemate is shown in Fig. 4.8. Slit S1 regulates the total amount of light entering the spectrograph. Slit S2 determines the bandpass of the filter stage. It is usually left wide open to maximise the spectral range. The slit S3 controls the ultimate resolution at the detector focal plane. In this work it was 200 μm wide or 4 cm^{-1} resolution.

The Triplemate was equipped with a computer–controlled, automatic drive (SPEX 1673C Mini Drive 2).

Detection System.

The light was detected using a Tracor–Northern intensified diode array detector (model TN 6112) with a multichannel analyser (TN 1710) operating with 1024 channels. This allowed the simultaneous acquisition of data over a $\sim 800\text{ cm}^{-1}$ range of the Raman spectrum. Typically, good quality spectra were obtained by acquiring 100 scans with an integration time of 2 seconds per scan. In this case, the total time to record a spectrum was less than 4 minutes (compared to 1.5 hours for System 1). All

Fig. 4.8.

Schematic diagram of the SPEX Triplemate.

M1 to M7: mirrors

G1 to G5: gratings

S1 to S3: slits (adjustable)

X: position of legs

spectra were stored on floppy disks and later plotted using a Hewlett–Packard plotter.

Powermeter.

CW laser power was measured using a Scientech 361 Powermeter.

4.7 CORRECTIONS.

Raman intensities are usually subject to wavelength–dependent changes which must be corrected in order to obtain undistorted resonance Raman excitation profiles (REPs). Such factors include sample re–absorption, detector efficiency, and monochromator efficiency. An effective, yet simple method to correct for these wavelength–dependent factors is to have an internal standard present in the sample, whose Raman intensity does not have any resonance or pre–resonance activity in the spectral region of interest. In order for this internal Raman standard to be most effective, its Raman band should be as near as possible to the band(s) being monitored. In this work, the 992 cm^{-1} stretch of the benzene solvent in the liquid phase Ni pheo *a* sample was used as the internal standard. In the solid phase sample, the SO_4^{2-} stretch of the Na_2SO_4 substrate was used as the internal standard; coincidentally, this Raman band was also at 992 cm^{-1} . The REPs were constructed by plotting the Raman intensity of particular modes divided by the Raman intensity of the internal standard, all as a function of laser excitation energy. As a matter of further precaution, the efficiency of the detection system was measured. As well, the intensity of the internal standard Raman band was measured as a function of wavelength. When the REPs were

corrected using this information, it was found that the shape of the REPs did not significantly change, within experimental error. In order to compensate for the variation in sensitivity with pixel # of the multichannel detection system, care was taken to ensure that all Raman bands to be measured appeared in the same pixel # when a new spectrum was recorded. When a Raman band could not be directly compared with the internal standard band (because these two bands could not simultaneously appear in the same spectral range), the Raman band was compared with one which had been previously compared with the internal standard.

For the solid phase samples, another type of correction had to be made which did change the shape of the REP. Along with dividing the Raman intensities by the SO_4^{2-} Raman intensity, an incident laser skin-depth penetration correction was made. This correction arises from the heterogeneous nature of the solid phase sample. When the laser light is tuned to a region in the absorption spectrum of the Ni pheo *a* film on Na_2SO_4 where the absorption coefficient is lower, the light penetrates the sample to deeper regions where more scattering from the underlying Na_2SO_4 solid can occur. The result is a higher SO_4^{2-} Raman intensity, and therefore a lowering of the measured Raman intensity for the particular mode. This was corrected using the absorption spectrum of the solid phase sample (note that the absorption coefficient possesses an inverse distance dependence). In the liquid phase sample, this problem does not occur because of its homogeneous nature. Also, the optical density was lower and the pathlength was fairly constant (thin cell) for the liquid phase sample.

In summary, we feel the REPs reported here are undistorted within experimental error. A total of two spectra for each point on the REP and four different samples were used. The integrity of the sample was monitored by absorption spectroscopy.

5. RESULTS AND DISCUSSION

5.1 RESONANCE RAMAN SPECTRA.

The resonance Raman spectra of Ni pheo *a* excited in the Soret, Q_x , $Q_y(0-0)$ and $Q_y(0-1)$ spectral regions are shown in Figures 5.1, 5.2, 5.3, and 5.4, respectively. Exact wavelengths (and wavenumber energies) of excitation are given in the figure captions. The spectra reported in this work are quite similar to those reported by Boldt *et al.*⁴⁶ for Ni(II)-methylpyropheophorbide *a* (Ni(II)-PPh). This molecule is similar to Ni pheo *a* except that Ni pheo *a* possesses a carbomethoxy group at the C_{10} position of ring V (PPh possesses a hydrogen atom there) and a phytol chain replaces the methyl group of the ester at C_7 (see Fig. 4.1). The B-state excited resonance Raman spectrum of solid samples of Ni(II)-pheophorbide *a* has been reported by Fujiwara and Tasumi.¹⁸⁶ This molecule does not possess the phytol chain but is otherwise the same as Ni pheo *a*. The Raman frequencies observed for these three complexes are similar. This is a good indication that the group substitutions mentioned above do not drastically change the normal modes of those vibrations which are Raman active upon B-state excitation (when the exciting radiation is in resonance with the conjugated ring chromophore).

From the work of Boldt *et al.*⁴⁶, it is apparent that the rR spectrum of Ni(II)-octaethylchlorin (NiOEC) exhibits anomalously polarised (ap) bands in addition to the polarised (p) and depolarised (dp) bands expected for a molecule described by a C_2 symmetry. The depolarisation ratios for the ap modes are in the range of $\rho \sim 1.0 - 1.2$, while those of the p modes are $\rho \sim 0.33$. Polarised bands dominate the

Fig. 5.1.

Resonance Raman spectrum of Ni pheo *a* (liquid phase sample) in benzene excited in the Soret band ($\lambda_{\text{ex}} = 422.1 \text{ nm} = 23691 \text{ cm}^{-1}$).

Fig. 5.2.

Resonance Raman spectrum of Ni pheo *a* (solid phase sample) on Na₂SO₄ excited in the Q_x band ($\lambda_{ex} = 514.5 \text{ nm} = 19436 \text{ cm}^{-1}$).

Fig. 5.3.

Resonance Raman spectrum of Ni pheo *a* (solid phase sample) on Na_2SO_4 excited in the $Q_y(0-0)$ band ($\lambda_{\text{ex}} = 638.7 \text{ nm} = 15657 \text{ cm}^{-1}$).

Fig. 5.4.

Resonance Raman spectrum of Ni phco *a* (solid phase sample) on Na_2SO_4 excited in the $Q_y(0-1)$ band ($\lambda_{\text{ex}} = 602.0 \text{ nm} = 16611 \text{ cm}^{-1}$).

NiOEC spectra obtained with B and Q_y excitation, as has been observed for other metallochlorins^{62,64,101,102}, while ap and dp modes dominate the Raman spectrum obtained with Q_x excitation. However, comparison of the intensities of certain ap (1590 cm^{-1} and 1308 cm^{-1}) and dp (1402 cm^{-1}) modes to the intensity of the internal standard reveals that the absolute intensity enhancements of these modes are approximately the same with Q_x and Q_y excitations. It is also important to note that these ap and dp modes are clearly observed with B-state excitation. This observation indicates that the resonance enhancement mechanisms for the non-totally symmetric modes of metallochlorins are distinctly different from those of metalloporphyrins. In the case of NiPPh (as well as its analogues) all observed rR bands appeared to be polarised with $\rho \sim 0.3 - 0.6$, depending on excitation wavelength.⁴⁶ A similar observation was reported by Keller *et al.*⁴⁵ In their study of Q_y -excited Cu(II)-pheophytin *a*, they observed all rR bands to be polarised (with the possible exception of a weak band at 320 cm^{-1} which appeared anomalously polarised). Similar observations were also reported by Lutz¹³ for chlorophyll *a* (Chl *a*) which may be regarded as Mg(II)-pheo *a*. In this case, again, nearly all the rR bands observed were polarised.

Depolarisation ratios for Ni pheo *a* were not available in this work but in light of the similar trends in depolarisation ratios for Chl *a* and Cu pheo *a*, we will assume that such similarities also apply to Ni pheo *a*.

Excitation in the porphyrin Q_y absorption band usually promotes resonance enhancement of Herzberg–Teller (or B–term) vibronic modes which are dp or ap in D_{4h} symmetry.¹⁸⁷ The observed prevalence of polarised modes in the metallopeophytins reflects the lowered symmetry of these molecules (formally C_1). Even large distortions of the D_{4h} symmetry in heme a results in the observation of an increased number of polarised rR bands with α excitation.¹⁸⁸

The Raman frequencies observed in this work under different excitations are listed in Table 5.1. Included in this table are the observed bands of Ni PPh, Chl a , and the corresponding modes (and their respective symmetry) for the parent NiOEC complex. The reported polarisations are also listed.

TABLE 5.1: Observed Lines (in cm^{-1}) for Ni pheo *a*

Region of Excitation ^{a)}				NiPPh ^{b)}	Chl <i>a</i> ^{c)}	NiOEC ^{d)}
Q_y	$Q_{y,vib}$	Q_x	Soret			
				330		342 A _p ^{e)}
				380		
				526		
				660	723	681 A _p
755	755	755		752	745	778 B
		804			802	
891						
924	924	924			907	A
						B
						1026 B _{ap}
						A
						B
						B
		1095		1100		B
		1120		1122	1115	1124 B _{ap} , B
						1152 B _{dp}
1134	1134	1134 [#]	1134	1134	1149	A
		1162		1154	1160	1152 B _{dp} , 1124 B _{ap}
1188	1188	1188	1188	1188	1189	1204 A _p , 1152 B _{dp}
		1201			1214(sh)	B
1220	1220			1218		1204 A _p , 1124 B _{ap}

			A		
			B		
1235	1235		1234	1230	1232 A _p
1269	1269	1269	1268	1270	1274 A _p
1282	1282		1282	1292	1308 B _{ap}
	1295	1295	1312	1312	1392 B _{ap} , 1349 A
1337	1337		1338		
1359			1356		1349 A, 1274 A _p
1365			1362	1333	1367 A _p , 1318 B
	1380 [#]		1380	1351	1382 A _p
			1392	1381	1392 B _{ap}
			1398	1392	1492 A, A
			1454	1440	A
					B
			1484		1512 A _p , 1402 B _{dp}
	1490 [#]		1496		1492 A
1522	1522	1522	1574	1530	1608 A _p , 1572 B, A
			1584		1590 B
1568	1568		1604	1560	1608 A _p , 1572 B, A
		1578	1580 [#]	1560	1608 A _p , 1572 B, A
1598	1598	1598	1604	1560	1608A _p , 1572 B, A
	1618		1624	1615	1614 A _p
					1608 A _p , 1572 B
			1652		1648 A _p , 1644 B _{ap}

1659	1664	1658	1630	1648 A _p	1644 B _{ap}
		1700	1690	1648 A _p	

- a) This work.
- b) From Ref. 46.
- c) From Ref. 13.
- d) From Ref. 46 and 100.
- e) SYMMETRY_{polarisation}
- # Broad, unresolved bands.

It is interesting to correlate the frequencies of the Ni pheo *a* Raman modes with those of the parent chlorin NiOEC. Most of the Raman active modes of Chl *a* and NiPPh have been assigned by Lutz¹³ and Boldt *et al.*⁴⁶, respectively.

C_aC_m and C_bC_b Modes.

These modes lie in the 1500–1700 cm⁻¹ range and are expected to change the most upon adding the ring V isocyclic group to the basic chlorin structure. It is this ring V group which is present in Chl *a* and Ni pheo *a*. In general, these modes (1658, 1652, 1626, and 1584 cm⁻¹) in NiPPh are up-shifted in frequency compared with those corresponding modes (1648, 1644, 1614, and 1590 cm⁻¹) in NiOEC, but not so much so for Ni pheo *a* and Chl *a*. The absence of the carbomethoxy group at C₁₀ for NiPPh may explain these observations.

Other Skeletal Modes.

Below 1500 cm⁻¹, the rR spectra of ring V – containing molecules are, in

general, quite similar to those of NiOEC.

The C_9 carbonyl stretching vibration is observed near 1700 cm^{-1} for NiPPh.⁴⁶ This mode has been previously identified in the rR spectra of various chlorophylls.^{8,13} Boldt *et al.*⁴⁶ have attributed the $1180\text{--}1190\text{ cm}^{-1}$ region as belonging to the C_mC_{10} stretching vibration. The 1450 cm^{-1} mode of NiPPh is thought to be a mode mixed with a large amount of in-plane skeletal motion although it is nominally an out-of-plane vibration. This mixing could be responsible for the rR intensity of the mode.

Vinyl Modes.

The band at 1338 cm^{-1} in the NiPPh rR spectrum has no apparent analogue in a similar molecule which does not contain the vinyl group.⁴⁶ This band was assigned to the vinyl CH_2 scissoring motion based on analogy to the results obtained by Choi *et al.*¹⁸⁹

The important difference between Chl *a* and Ni pheo *a* is the metal present in the centre of the macrocycle. The phytol chain is expected to have only small effects on the rR spectrum of Ni pheo *a* as compared with NiPPh, because of its lack of participation in the conjugated ring system which is the actual chromophore. The change in metal ion from Ni(II) to Mg(II) has a substantial effect on the core size, and, hence, certain vibrational frequencies. This has been extensively investigated by several groups.^{98,94,103}

The presence of the carbomethoxy group at the C₁₀ position of Chl *a* and its Ni analogue is not observed to be a large perturbation of the rR spectrum.

As a result of the larger Ni(II) metal ion, the C_aC_m, C_bC_b, and C_aN stretching modes are shifted to lower frequencies by approximately 20 – 30 cm⁻¹. However, the rR intensity pattern of Chl *a* is comparable to that of NiPPh, which suggests that the normal coordinate descriptions for Chl *a* are similar to those of its Ni analogues. In addition, except for the shifts in the metal-sensitive bands, Ni pheo *a* rR spectra are very similar to those of Chl *a*.

It has been observed⁴⁶ that rR spectra of NiOEC obtained with Q_y excitation exhibit symmetric modes which are predominantly derived from the B_{1g} modes of metalloporphyrins (D_{4h}), whereas the spectra obtained with B-state excitation exhibit symmetric modes which are predominantly derived from the A_{1g} modes. This observation indicates that the Franck–Condon factors for the symmetric vibrations are quite different between the Q_y and B (or Soret) electronic states. The enhancement of the B_{1g}-derived modes of the chlorins with Q_y excitation suggests that the origin shifts along these normal coordinates might be qualitatively viewed as originating from distortions similar to the Jahn–Teller displacements experienced by higher symmetry porphyrins.⁹⁸ Furthermore, the enhancement behaviour of the A_{1g}-derived modes of chlorins suggests that the nature of the origin shifts of these modes could be similar to those of the parent porphyrin. The observation that the asymmetric modes of NiOEC exhibit rR intensity with excitation in both the Q_x and Q_y states indicates that a Herzberg–Teller scattering mechanism is operative for those states. The fact that the rR enhancements of the asymmetric modes are approximately equal for Q_x and Q_y excitation suggests that the Q_x state is vibronically coupled to the Q_y state in addition

to the B state. If this were not the case, the Q_x scattering intensity would be substantially less than the Q_y intensity owing to the lower oscillator strength of the Q_x state. It should be noted that the proximity of the Q_x state to the B state is not sufficient to account for the equal rR intensities of the asymmetric modes with Q_x and Q_y excitation (presuming $\partial H/\partial Q$ is the same for a given mode in the two states).

Striking features of the resonance Raman spectra of Ni pheo *a* are the differences in relative intensities of certain modes when the Raman spectrum is excited in various regions of its absorption spectrum. When the Raman spectrum is excited in the Soret region, the vibrational modes at $\sim 1580 \text{ cm}^{-1}$ are the most intense. This also seems to be the case when the Raman spectrum is excited in the Q_x region. However, when the Raman spectrum is excited in the $Q_y(0-0)$ spectral region, the vibrational modes at $\sim 1130 - 1330 \text{ cm}^{-1}$ seem to dominate the Raman spectrum, thus indicating different vibrational modes coupling to this state as compared to the Soret state and the Q_x state. Furthermore, when the Raman spectrum is excited in the $Q_y(0-1)$ region, there was a strong enhancement of the 1568 cm^{-1} and 1598 cm^{-1} bands as well as the appearance of the 1618 cm^{-1} and 1659 cm^{-1} bands. This resulted in a Raman spectrum which appeared similar to those excited in the Soret and Q_x spectral regions, indicating the possible vibronic character of the $Q_y(0-1)$ band and its participation in the coupling of the Q_y state with the Soret state. This will be further discussed in the following section.

5.2 TRANSFORM ANALYSIS OF MEASURED REPs.

The theoretical REPs in this work were generated with the previously described transform method, using the experimental absorption spectrum of Ni pheo *a* in benzene solution (10^{-4} M assuming a comparable molar absorption coefficient as Chl *a*) (see Fig. 5.5). This presumes that the observed absorption spectrum exhibits the proper homogeneous linewidth of the resonant state, or that very little site-broadening or inhomogeneous broadening is present. This is a reasonable assumption, since the solution is quite dilute (i.e. 10^{-4} M). It is expected, however, that the observed linewidth is slightly greater than the true homogeneous linewidth and that temperature effects are present. Although not shown, the second derivative of the absorption spectrum (Fig. 5.5) of Ni pheo *a* in solution indicates that it is present mainly as a monomer and not as a dimer, which would be the case for a 10^{-4} M solution of Chl *a* in dry hexane. The presence of a single minimum in the vicinity of the Q_y absorption maximum indicates that there are no shoulders or convoluted bands which could represent

a

Fig. 5.5. Absorption spectrum of Ni pheo *a* in benzene (liquid phase sample).

Fig. 5.6.

Absorption spectrum of Ni pheo *a* on Na_2SO_4 (solid phase sample).

dimeric form being appreciably present. The absence of this dimeric form of Ni pheo *a* at these concentrations in benzene and hexane⁵² suggests a very weak carbonyl–metal(Ni) interaction due to bonding saturation of the Ni metal atom. Boucher and Katz⁵³ observed and concluded the same. Since the REPs of the various Raman modes excited in the Soret region were measured using the solution or liquid sample, it is logical to use the corresponding absorption spectrum in the calculation of the theoretical REPs.

The REPs of the Ni pheo *a* modes which were excited in the Q_y spectral region were measured from a sample deposited onto a Na_2SO_4 substrate in the form of a pressed pellet. The absorption spectrum of this sample, which was recorded using a diffusive reflectance apparatus, is shown in Fig. 5.6. We first note that the peak position is, within experimental accuracy, the same as that for the benzene solution, i.e. 654 nm. Secondly, we note that the bandwidth for the solid sample is very much larger than that of the benzene solution sample. Table 5.2 lists the spectral parameters of the different samples.

**TABLE 5.2: Absorption Spectrum Parameters
of Ni pheo *a* Samples**

SAMPLE	PEAK(nm)	BANDWIDTH(cm^{-1})	RESOLUTION
			(at 500 nm)
solution	654	595	1 nm
solid	654	2247	2 nm

The much broader linewidth of the solid sample results from inhomogeneous broadening, whereas the peak position of the absorption band reflects the amount of interaction experienced by the Ni pheo *a* from the solvent. For the solid sample, we may regard the Ni pheo *a* as being its own solvent because of the high local concentration at the surface of the Na_2SO_4 pellet. If we assume that the peak position reflects the degree of interaction between the solvent^{190,191} and the Ni pheo *a* (with no ligation effects), then we may imply that the Ni pheo *a* self-interaction on a Na_2SO_4 substrate is similar to the benzene solvent interactions the molecule experiences, since the peak positions of the two absorption bands are similar.

As we have seen in Chapter 3, the linewidth of the absorption band used in the transform method plays an important role in the appearance of the calculated REP. In this work, when REPs were calculated using the absorption spectrum of the solid sample, they appeared abrupt and unnatural. This is due to the excessively large inhomogeneously broadened linewidth exhibited by this spectrum, which greatly differed from the true homogeneous linewidth of the resonant state. The calculated REP of the 1134 cm^{-1} mode is shown in Fig. 5.7 using both experimental absorption

bands.

For the reasons mentioned above, all REPs were calculated using the absorption spectrum of the dilute benzene sample.

Fig. 5.7.

REP of 1134 cm^{-1} mode generated by the transform method using (above) the absorption band of the liquid phase sample (Fig. 5.5) and (below) using the absorption band of the solid phase sample (Fig. 5.6).

B-State Excited REPs.

For Raman spectra excited in the blue spectral region, the three most prominent modes were monitored to construct the experimental REPs. These were the 1134, 1188, and 1580 cm^{-1} (broad, unresolved) modes. In general, these modes showed very similar behaviour in their respective REPs. Despite the limited spectral range of measurement, the data are interpreted as showing a maximum in REPs for these three modes at 418.5 nm ($23,895\text{ cm}^{-1}$). This maximum is 240 cm^{-1} blue-shifted from the absorption maximum of 422.7 nm ($23,657\text{ cm}^{-1}$). This observation for B-state excitation is quite consistent with the observations of Lutz¹³, who has measured the REPs of various modes of Chl *a* and Chl *b* in solution. It was found that when excited in the Soret region, all the measured modes of Chl *b* showed similar behaviour in their REPs, which all peaked $\sim 392\text{ cm}^{-1}$ from the absorption maximum. Similar results were mentioned for Chl *a* in solution.¹³

In addition, there appears to be a relatively weak maximum in each of the three REPs at $\sim 23,100\text{ cm}^{-1}$, made especially evident by the 1188 cm^{-1} mode. Similar behaviour was again observed by Lutz.^{13,104} Resonance Raman bands with wavenumbers between 800 and 1655 cm^{-1} all exhibited maxima in a narrow 150 cm^{-1} range in their respective REPs. On this basis, it was proposed that these maxima corresponded to a purely electronic transition and that a "helping mode" effect was unlikely under those conditions.

Figures 5.8, 5.9, and 5.10 show the experimental REPs as well as the theoretical curves which best fit the data obtained for Soret-excited rR modes of Ni pheo *a*. As previously mentioned, we shall assume that despite the limited number of data, the

Fig. 5.8.

REP of the 1134 cm^{-1} mode in the Soret region. $C_r = -0.10$. Above is the absorption spectrum of Ni phéo a in the Soret region.

Fig. 5.9. REP of the 1188 cm^{-1} mode in the Soret region. $C_r = -0.1$.

Fig. 5.10. REP of the 1580 cm^{-1} mode in the Soret region. $C_T = -0.12$.

REPs do peak at $\sim 23,895 \text{ cm}^{-1}$ and are, therefore, blue-shifted from the absorption maximum. This is found to be consistent with Raman modes of Chl *a* also excited in the Soret region. Secondly, we shall assume that the observed linewidth of the absorption spectrum is slightly larger than the true homogeneous linewidth of the corresponding resonant electronic state. With these two presumptions in mind, then we find that appropriate fits of the transform-generated REPs to the experimental REPs occur when small non-Condon contributions (i.e. $\sim 10\%$) are included in the transform calculation. For all the three modes monitored in this series of experiments, the best fits occurred when $C_r \sim -0.1$.

Figure 5.11 shows the REPs for the 1580 cm^{-1} (broad, unresolved) mode calculated with three different values of C_r : 0.0, -0.12 , and -0.4 . We note that increasing the absolute value of C_r from 0.0 (Fig. 5.11a) to -0.12 (Fig. 5.11b) results in an increase in intensity in the 0–1 region of the REP, which has the effect of slightly blue-shifting the apparent 0–0 transition. This value of $C_r = -0.12$ places the apparent maximum of the REP in the 0–0 region in the same vicinity as the experimental REP. Increasing the value of C_r to -0.4 (Fig. 5.11c) produces a maximum of the calculated REP at the 0–1 region of the 1580 cm^{-1} mode, which we feel is unrealistic for this case.

The observation that small non-Condon effects with $C_r \sim -0.1$ are needed to generate a good fit between experimental REPs and transform REPs is in complete agreement with other reported REPs of metalloporphyrins excited in the B-state.^{81,82} This is particularly interesting because it may explain the blue-shift of the REP with respect to the absorption spectrum without proposing the presence of a hidden

a *b* *c*

Fig. 5.11.

Various C_r values used in the transform generated REPs of the 1580 cm^{-1} mode.

- a) $C_r = 0.0$
- b) $C_r = -0.12$
- c) $C_r = -0.40$

electronic origin $\sim 300 \text{ cm}^{-1}$ from the 0–0 of the Soret.

It should be noted, however, that there is another point-of-view which may explain the slight blue-shift. The excitation profiles in the B band region for the totally symmetric A_{1g} mode (1363 cm^{-1}) in cytochrome c^{2+} has been carefully investigated⁸⁶ and it exhibits a pronounced asymmetry toward the blue side of the B band in the absorption spectrum. Champion and Albrecht⁸⁶ have shown that this blue shift could result from inclusion of the complete sub-space of Franck–Condon active vibrations, rather than the particular Raman mode alone, in their scattering formalism. However, it was also shown^{88,89,90,91,92} that a superposition of non–Condon scattering, that is, scattering by vibronic coupling, on a one–mode Franck–Condon model could also produce the asymmetry of the REP in the B region, although the proper intensity ratio of B and Q scattering was not accurate.

The relative importance of the multimode and non–Condon effects continue to be debated along with the development of the transform theory that relates the REPs and the absorption band shapes. The transform theory implicitly includes both multimode and non–Condon effects. Careful analysis of the 1363 cm^{-1} mode in cytochrome c^{2+} has established that a $\sim 13\%$ contribution of non–Condon scattering provides a near perfect fit of the transform calculated REP to the experimental one.¹⁹²

With the data available, we conclude that the REPs of the modes studied in this work for Ni pheo *a* excited in the Soret band behave as similarly excited modes in those metalloporphyrins studied. This behaviour may be explained by a small contribution to the intensity of these modes of about 10% non–Condon scattering, an

amount found to be the same for the metalloporphyrins. Based on the striking similarity between the Raman spectra of Chl *a* and Ni pheo *a*, especially in the intensity ratio patterns of the respective modes excited in the Soret band, similar conclusions may be inferred for Chl *a* from the Ni analogue data.

Q_y -State-Excited REPs

The experimental REPs, along with their corresponding calculated profiles for Raman modes excited in the Q_y spectral region, are shown in Figures 5.12 to 5.19.

In general, these modes all showed similar behaviour except for the 1522 cm^{-1} mode, which will be discussed later.

The one common and striking feature which all the modes exhibited was the pronounced enhancement of the REPs in their respective 0-1 region. This behaviour is markedly different from those modes excited in the Soret band, which showed maxima near the 0-0 region, indicating small non-Condon effects. For these REPs in the Q_y region satisfactory agreement between theory and experiment could only be obtained if significant non-Condon effects ($\sim 40\%$) were included in the transform calculation. In general, C_r values of about -0.4 were required in fitting the experimental REPs. This is indicative of significant non-Condon scattering. Furthermore, the degree of non-Condon scattering seemed to increase for the higher frequency modes.

As suggested by Andersson *et al.*^{62,64}, symmetry considerations and the predominance of polarised bands in the resonance Raman spectra of C_{2v} metallochlorins should yield REPs consistent with dominant Franck–Condon scattering. This is also suggested by the relatively strong Q_y absorption band. Indeed, their poorly resolved REPs did show dominant 0–0 enhancement, but this is not consistent with simple Franck–Condon or A–type scattering.

Fig. 5.12.

REP of the 1134 cm^{-1} mode in the Q_y region. $C_r = -0.40$.

Fig. 5.13. REP of the 1188 cm^{-1} mode in the Q_y region. $C = -0.40$.

Fig. 5.14.

REP of the 1235 cm^{-1} mode in the Q_y region. $C_r = -0.35$.

Fig. 5.15. REP of the 1269 cm^{-1} mode in the Q_y region. $C_r = -0.40$.

Fig. 5.16.

REP of the 1282 cm^{-1} mode in the Q_y region. $C_r = -0.45$.

Fig. 5.17. REP of the 1337 cm^{-1} mode in the Q_y region. $C_r = -0.45$.

Fig. 5.18.

REP of the 1568 cm^{-1} mode in the Q_y region. $C_r = -0.60$.
Deviation from the transform-generated REP is probably due to
interferences from nearby or underlying bands.

Fig. 5.19.

REP of the 1598 cm^{-1} mode in the Q_y region. $C_r \approx -0.80$.

It is well documented^{77,94,98} that for metalloporphyrins the intensities of the Q and B states are greatly influenced by Herzberg–Teller, Jahn–Teller, *intrinsic* Jahn–Teller, and even configuration interaction coupling mechanisms. It is therefore quite evident that the Q and B electronic states are intrinsically coupled. Measured REPs of metalloporphyrins excited in the Q band support the above.

In the work of Andersson *et al.*^{62,64} REPs obtained in the Q states of metallochlorins show a predominance of polarised Raman bands, which was interpreted as arising from dominant Franck–Condon scattering. The REPs, however (which were poorly resolved), did not show nearly equal 0–0 and 0–1 enhancements. Keller *et al.*⁴⁵, as well as Boldt *et al.*⁴⁶, report the same predominance of polarised bands, but REPs were not measured.

With this work representing the first report of well-resolved (2 nm resolution) REPs of a chlorophyll in the Q_y state, it is quite clear that equal 0–0 and 0–1 enhancements were not observed. From this we must conclude that scattering of these supposedly totally symmetric modes is not purely A-type scattering. As was stated by Champion and Albrecht⁸⁶, totally symmetric modes, in general, can derive intensity from both A- and B-term sources. The B-term coupling of the x,y-polarised Soret states with similarly polarised states elsewhere (i.e. α -bands in metalloporphyrins) can occur, but such B-term contributions to totally symmetric scattering are thought to be weak when compared to strong A-term activity to be expected when in resonance with a strongly absorbing state.

Despite the high relative oscillator strength of the Ni pheo $a Q_y$ state and low

molecular symmetry, coupling between the Q_y and (an)other electronic state(s) seem(s) to be present. In a recent linear dichroism study of oriented Chl *a*⁷⁰, it was concluded that the traditional assignment of the absorption envelopes to two mutually perpendicular Q_x and Q_y transitions could not be sustained, owing to the resolution of several x- and y-polarised electronic origins. This implies a breakdown of the four-orbital model. With several electronic origins present, the chances for coupling become much greater. As well, interferences between these origins may also manifest themselves. And still further, closely lying origins will significantly increase nonadiabatic effects, which may account for the pronounced 0-1 enhancement in the REP without needing to invoke excessive vibronic coupling strength.

The 1522 cm^{-1} Mode.

The one seemingly anomalous REP was that of the 1522 cm^{-1} Raman mode (see Fig. 5.20). Inspection of the resonance Raman spectrum in this frequency region shows that this band appears free of any interfering nearby band, and yet the REP shows a maximum in a region not predicted by the transform method. Because the data is not complete for this REP we can only speculate as to the cause of this anomaly. First of all, the maximum of the REP could be indicating an electronic origin. From the work of Fragata *et al.*⁷⁰ on Chl *a* electronic origins, it is possible that Ni pheo *a* possesses an electronic origin in the spectral region where the REP of the 1522 cm^{-1} mode shows a maximum. As another possible explanation, the maximum in the REP could be showing the resonance of a "helping" mode at $\sim 860\text{ cm}^{-1}$ in

Fig. 5.20.

REP of the 1522 cm^{-1} mode in the Q_y region. $C_r = 0.0$.

frequency. As has been pointed out by Spiro and Stein⁷³, N-heterocyclic compounds such as tetrapyrroles possess a forbidden $n \rightarrow \pi^*$ transition which effectively mixes with a strongly allowed $\pi \rightarrow \pi^*$ transition via an out-of-plane C-H bending mode near 900 cm^{-1} , which could represent the assumed "helping" mode. At this time, further work is needed to confirm the above speculation.

6. SUMMARY AND CONCLUSIONS.

With the data presently available, the thesis may be concluded as follows.

First, we have demonstrated that it is possible to obtain good quality resonance Raman excitation profiles (REPs) of a non-fluorescent chlorophyll sample with traces of highly fluorescent impurities using low incident laser powers. To our knowledge, this work reports the first REPs obtained for isolated chlorophyll samples excited in the Q_y spectral region.

From these spectra, it was evident that there are different vibronic coupling mechanisms at work. Resonance Raman spectra excited in various regions of the Ni pheo a absorption spectrum showed differences in the relative intensities of certain observable Raman modes. In particular, the dominant Raman modes in the Soret-excited Raman spectrum are those centred at 1580 cm^{-1} while in the $Q_y(0-0)$ -excited region the dominant modes are those in the range of 1130 cm^{-1} to 1337 cm^{-1} . Resonance Raman spectra excited in the Q_x region (specifically, at 514.5 nm) showed relative intensities of the $\sim 1580\text{ cm}^{-1}$ bands and the $\sim 1130 - 1330\text{ cm}^{-1}$ bands to be similar to those in the Soret-excited Raman spectrum. This, and the fact that the Q_x -excited Raman spectra are much more intense than can be accounted for by simply the oscillator strength of the Q_x absorption band, leads to the conclusion that the Q_x state is vibronically coupled to an excited state in the Soret region.

Resonance Raman spectra excited on the high energy side of the $Q_y(0-1)$ vibronic band showed relative intensities of various bands to be different from those excited in the $Q_y(0-0)$ region, in particular, the strong enhancement of the 1568 cm^{-1} and 1598 cm^{-1} bands and the appearance of the 1618 cm^{-1} and 1659 cm^{-1} bands. For these observations we propose two explanations, each of which involves vibronic coupling to an excited electronic state in the Soret band. First, despite the strong intensity of the $Q_y(0-0)$ band, the Raman scattering mechanism is not dominated by Franck-Condon or A-term scattering, in which case non-Condon (Herzberg-Teller or B-term) scattering may be observable. This may be the case as is evident by the remaining presence of the vibronic band, as in the absorption spectra of metalloporphyrins that exhibit Herzberg-Teller scattering from that state. A second explanation is based on the results of Fragata *et al.*⁷⁰, who report the presence of several electronic origins in the Q_y region, of both x and y polarisation. One of these bands may be coupled to an excited state in the Soret region while the other, the one that has gained intensity via the various changes associated with transforming the metalloporphyrin to the metallophycocyanin, may or may not be vibronically coupled to a state in the Soret region. Probably, a combination of these two factors is operational. Because of the complicated nature of the absorption spectrum we can only conclude that the associated vibronic coupling schemes are also complex.

From the measured REPs of most of the observed Raman modes excited in the Q_y region, transform theory supports the first explanation. It was found that reasonable agreement between experiment and theory could only be achieved when significant non-Condon effects (~40%) were included. The result was a more intense maximum in the 0-1 region in the REP over that of the 0-0 region. This enhancement is then explained by interferences between A-term and B-term scattering mechanisms at the

amplitude level. Furthermore, for higher frequency modes the amount of non-Condon scattering is higher. This is consistent with the Herzberg-Teller mechanism, where it is predicted that higher frequency modes are more effective in vibronically coupling two or more excited states. Also, the ratio of the 0-1 maximum over that of the 0-0 maximum is too large to be due to non-adiabatic effects, which in the weak coupling limit is 2.

The second explanation, which involves the presence of another electronic origin, may be supported by the REP of the 1522 cm^{-1} mode, which cannot be adequately explained by the simple transform theory. Fragata *et al.*⁷⁰ reported an electronic origin for Chl *a* at $15,750\text{ cm}^{-1}$ (635 nm) in the absorption spectrum. When we extrapolate this value to correspond to the Ni pheo *a* absorption spectrum, we arrive at $16,114\text{ cm}^{-1}$ (620 nm). Although important data are not available in this region, it is clear from the REP of the 1522 cm^{-1} mode that a maximum is interpolated in the $16,100\text{ cm}^{-1}$ region. Similarly, an origin may be present which is 1522 cm^{-1} from the peak of the observed REP. More work is required to pursue this speculation.

Since the lineshape of the liquid phase sample absorption spectrum was found to possess a linewidth satisfactorily fitting the observed REPs, it is clear that significant inhomogeneous broadening is not exhibited in this absorption spectrum. The apparently broad lineshape is probably due to low frequency vibrational modes broadening the homogenous linewidth. The lineshape of the solid phase sample was shown to exhibit large inhomogeneous broadening, and thus was not suitable for use with the transform method.

In direct contrast to the REPs excited in the Q_y region, the REPs excited in the

Soret region seem to be almost entirely due to A-term scattering. The observed maximum in the REPs of three prominent modes, all of which peak $\sim 300\text{ cm}^{-1}$ blue-shifted from the absorption maximum, can be explained with the inclusion of only 10% non-Condon scattering in the transform theory, and thus one need not invoke the presence of an electronic origin at that position.

Finally, it is clear that the scattering mechanisms present for Ni pheo *a* are not simple, but some useful information may be extracted from a resonance Raman study of this molecule. In general, we conclude that there is more than one scattering mechanism at work, which indicates the presence of vibronic coupling of excited states with other electronic excited states. At present, it remains to be determined whether the complicated coupling schemes have any significant impact on the efficiency of certain primary photosynthetic processes. At the very least, it should raise questions as to the validity of certain electron transfer theories which have been assumed to have separable electronic and vibrational factors. As is evident with the present study, at least for Ni pheo *a*, this may not be a good assumption and further modelling should be undertaken.

Because of the obvious similarities between the Raman scattering of Ni pheo *a* and Chl *a*, results from this work may be extended to the chlorophylls, in general, and especially, those present in the photosynthetic reaction centres.

In order to complete this work, it would be fruitful to measure the depolarisation ratios of the various Raman bands and to observe how they behave as a function of exciting frequency within a resonant band. This will provide more information as to which states the bands are coupled. Also, it would be satisfying to

verify the results of this work by actually undertaking a resonance Raman study of Chl *a* itself. To overcome the fluorescence of the Q_y state, one could use a resonance Raman gain technique.¹⁹³ This has advantages over CARS in that significantly lower laser powers are required, and the strict beam angle requirements are relaxed.

REFERENCES

1. H. Michel, *J. Mol. Biol.* 158 (1982) 562.
2. J. Deisenhofer, O. Epp, K. Miki, R. Huber, and H. Michel, *J. Mol. Biol.* 180 (1984) 385.
3. D.E. Budil, P. Gast, C.H. Chang, M. Schiffer, and J.R. Norris, *Ann. Rev. Phys. Chem.* 38 (1987) 561.
4. M. Calvin, in *Photochemical Conversion and Storage of Solar Energy*, J. S. Connolly, ed., Academic Press, New York, 1981, p. 1.
5. *The Light Reactions*, J. Barber, ed., Elsevier, Amsterdam, 1987.
6. *The Photosynthetic Bacterial Reaction Center, Structure and Dynamics*, J. Breton and A. Vermeglio, eds., Plenum Press, New York, to be published.
7. G. Feher and M. Okamura, in *The Photosynthetic Bacteria*, R.K. Clayton and W.R. Sistrom, eds., Plenum Press, New York, 1978, p. 349.
8. M. Lutz and B. Robert, in *Biological Applications of Raman Spectroscopy*, Vol. 3, T.G. Spiro, ed., Wiley-Interscience, New York, 1987, Chpt. 8.
9. J. Breton and E. Navedryk, Reference 5, Chpt. 4.
10. N.K. Packham and J. Barber, in Reference 5, Chpt. 1.
11. G. Garab, T. Szito, and A. Faludi-Daniel, in Reference 5, Chpt 7.
12. B. Ke and V.A. Shuvalov, in Reference 5, Chpt. 2.
13. M. Lutz, in *Advances in Infrared and Raman Spectroscopy*, Vol. 11, R.J.H. Clark and R.E. Hester, eds., Wiley-Heyden, London, 1984, Chpt. 4.
14. J.R. Norris, R.A. Uphaus, H.L. Crespi, and J.J. Katz, *Proc. Natl. Acad. Sci. USA* 68 (1971) 625.
15. G. Feher, A.J. Hoff, R.A. Isaacson, and L.C. Ackerson, *Ann. N.Y. Acad. Sci.* 244 (1975) 239.
16. N. Lubitz, R.A. Isaacson, E.C. Abresch, and G.E. Feher, *Proc. Natl. Acad. Sci. USA* 81 (1984) 7792.
17. M. Lutz and J. Kleo, *Biochim. Biophys. Acta* 546 (1979) 365.

18. J.J. Katz, J.R. Norris and L.L. Shipman, *Brookhaven Symp. Biol.* 28 (1977) 16.
19. J. Breton, *Biochim. Biophys. Acta* 810 (1985) 235.
20. W. Zinth, W. Kaiser, and H. Michel, *Biochim. Biophys. Acta* 723 (1983) 128.
21. G.R. Fleming, J.L. Martin, and J. Breton, *Nature* 333 (1988) 190.
22. M. Plato, K. Mobius, M.E. Michel-Beyerle, M. Bixon, and J. Jortner, *J. Am. Chem. Soc.* 110 (1988) 7279.
23. M.E. Michel-Beyerle, M. Bixon, and J. Jortner, *Chem. Phys. Lett.* 151 (1988) 188.
24. D. De Vault, *Q. Rev. Biophys.* 13 (1980) 387.
25. J. Jortner, *Biochim. Biophys. Acta* 594 (1980) 193.
26. R.A. Marcus, and N. Sutin, *Biochim. Biophys. Acta* 811 (1985) 265.
27. R.A. Marcus, *J. Chem. Phys.* 24 (1956) 966, 979.
28. J. Jortner, *J. Am. Chem. Soc.* 102 (1980) 6676.
29. N.N. Parson and A. Warshel, *J. Am. Chem. Soc.* 109 (1987) 6152.
30. R. Friesner and R. Wertheimer, *Proc. Natl. Acad. Sci. USA* 79 (1982) 2138.
31. Y. Won and R.A. Friesner, *Proc. Natl. Acad. Sci. USA* 84 (1987) 5511.
32. M. Lutz, B. Robert, Q. Zhou, J.M. Neumann, W. Szponarski, and G. Berger, in Reference 6, p. 41.
33. D. Bocian, N.J. Boldt, B. Chadwick, and H.A. Frank, *FEBS Lett.* 214 (1987) 92.
34. B. Robert and M. Lutz, *Biochem.* 27 (1988) 5108.
35. A. Warshel, *Proc. Natl. Acad. Sci. USA* 77 (1980) 3105.
36. W. Siebrand and M. Zgierski, in *Excited States*, Vol. 4, E.C. Lim, ed., Academic Press, New York, 1979, p.1.
37. I. Ozkan and L. Goodman, *Chem. Rev.* 79 (1979) 275.
38. *The Chlorophylls*, L.P. Vernon and G.R. Seely, eds., Academic Press, New York, 1966.
39. *The Chemistry and Biochemistry of Plant Pigments*, 2nd ed., T.N. Goodwin, ed., Academic Press, New York, 1972.
40. C. Weiss, *J. Mol. Spectrosc.* 16 (1972) 415.

41. C. Weiss, in *The Porphyrins*, Vol. 3, D. Dolphin, ed., Academic Press, New York, 1978, p. 211.
42. J.J. Katz, L.L. Shipman, T.M. Cotton, and T.R. Janson, in *The Porphyrins*, Vol. 5, D. Dolphin, ed., Academic Press, New York, 1978, p. 401.
43. S.G. Boxer and G.L. Closs, *J. Am. Chem. Soc.* **98** (1976) 5406.
44. C. Houssier and K. Sauer, *J. Am. Chem. Soc.* **92** (1970) 779.
45. J.S. Keller, J.J. McMahon, R. Chang, and R.L. Carter, *Spectroscopy Lett.* **17** (1984) 145.
46. N.J. Boldt, R.J. Donohoe, R.R. Birge, and D.F. Bocian, *J. Am. Chem. Soc.* **109** (1987) 2284.
47. E. Hoxtermann, N. Werncke, I.N. Stadnichuk, A. Lau, and P. Hoffman, *Studia Biophysica* **92** (1982) 147.
48. A.C. de Wilton and J.A. Koningstein, *J. Raman Spectrosc.* **14** (1983) 288.
49. A.C. de Wilton, L.V. Haley, and J.A. Koningstein, *Chem. Phys. Lett.* **97** (1983)
50. N.E. Binnie, T.L. Collier, L.V. Haley, T.A. Mattioli, D.L. Thibodeau, W. Wang, and J.A. Koningstein, *J. Luminescence*, **37** (1987) 39.
51. N.E. Binnie, L.V. Haley, T.A. Mattioli, D.L. Thibodeau, J.A. Koningstein, and W. Wang, *Can. J. Chem.* **66** (1988) 1728.
52. D.L. Thibodeau, Ph.D. Thesis, Carleton University, Ottawa, Canada, 1988.
53. L.J. Boucher and J.J. Katz, *J. Am. Chem. Soc.* **89** (1967) 4703.
54. M. Gouterman, *J. Mol. Spectrosc.* **6** (1961) 138.
55. M. Gouterman, in *The Porphyrins*, Vol. 3, D. Dolphin, ed., Academic Press, New York, 1978, p.1.
56. G. Herzberg and E. Teller, *Z. Phys. Chem. (Leipzig)* **B21** (1933) 410.
57. T.G. Spiro and T.C. Streakas, *J. Am. Chem. Soc.* **96** (1974) 338.
58. B.M. Dzhagarov, V.S. Chirvonyi and G.P. Gurinovich, in *Laser Picosecond Spectroscopy and Photochemistry of Biomolecules*, V.S. Letokhov, ed., Adam Hilger, Bristol, 1987, p. 137.
59. R.L. Ake and M. Gouterman, *Theor. Chim. Acta* **15** (1969) 20; A. Antipas and M. Gouterman, *J. Am. Chem. Soc.* **105** (1983) 4896.

60. W. Hoppe, G. Will, J. Grassmann, and H. Weichselgartner, *Kristallog.* 128 (1969) 18.
61. S.M. Strauss, M.E. Silver, and J.A. Ibers, *J. Am. Chem. Soc.* 105 (1983) 4108.
62. L.A. Andersson, T.M. Loehr, C.K. Chang, and A.G. Mauk, *J. Am. Chem. Soc.* 107 (1985) 182.
63. H. Scheer and H. Inhoffen, in *The Porphyrins*, Vol. 2. Part B. D. Dolphin, ed., Academic Press, New York, 1978, p. 45.
64. L.A. Andersson, T.M. Loehr, C. Sotiriou, W. Nu, and C.K. Chang, *J. Am. Chem. Soc.* 108 (1986) 2908.
65. J.D. Petke, G.M. Maggiora, L.L. Shipman, and R.E. Christoffersen, *Photochem Photobiol.* 30 (1979) 203.
66. R.E. Christoffersen, *Int. J. Quantum Chem.* 16 (1979) 573.
67. J.D. Petke and G.M. Maggiora, *J. Chem. Phys.* 84 (1986) 1640.
68. J.D. Petke and G.M. Maggiora, *Chem. Phys. Lett.* 97 (1983) 231.
69. J.D. Petke and G.M. Maggiora, in *Porphyrins: Excited States and Dynamics*, M. Gouterman, P.M. Rentzepis, and K.D. Straub, eds., ACS Symposium Series, Vol. 321, 1986, p. 20.
70. M. Fragata, B. Norden, and T. Krucsev, *Photochem. Photobiol.* 47 (1988) 133.
71. L.L. Shipman, T.M. Cotton, J.R. Norris and J.J. Katz, *J. Am. Chem. Soc.* 98 (1976) 8222.
72. T.M. Cotton, P.A. Loach, J.J. Katz, and K. Ballschmitter, *Photochem. Photobiol.* 27 (1978) 735.
73. T.G. Spiro and P. Stein, *Ann. Rev. Phys. Chem.* 28 (1977) 501.
74. R.J.H. Clark and T. Dines, in *Advances in Infrared and Raman Spectroscopy*, Vol. 9, R.J.H. Clark and R.E. Hester, eds., Wiley and Heyden, London, 1982, Chpt. 5.
75. W. Siebrand and M.Z. Zgierski, *J. Raman Spectrosc.* 13 (1982) 78.
76. A.B. Myers and R.A. Mathies, in *Biological Applications of Raman Spectroscopy*, Vol. 2, T.G. Spiro, ed., Wiley-Interscience, New York, 1987, Chpt. 1.
77. T.G. Spiro and Z.-Y. Li, in *Biological Applications of Raman Spectroscopy*, Vol. 3, T.G. Spiro, ed., Wiley-Interscience, New York, 1988, Chpt. 1.
78. T.C. Strekas and T.G. Spiro, *Biochim. Biophys. Acta* 263 (1972) 830.

79. T.G. Spiro and T.C. Strekas, *Proc. Natl. Acad. Sci. USA* **69** (1972) 2622.
80. V. Srajer, K.T. Schomacker, and P.M. Champion, *Phys. Rev. Lett.* **57** (1986) 1267.
81. B. Cartling, in *Biological Applications of Raman Spectroscopy*, Vol. 3, T.G. Spiro, ed., Wiley-Interscience, New York, 1988, Chpt. 5.
82. P.M. Champion, in *Biological Applications of Raman Spectroscopy*, Vol. 3, T.G. Spiro, ed., Wiley-Interscience, New York, 1988, Chpt. 6.
83. T.C. Strekas, A. Packer, and T.G. Spiro, *J. Raman Spectrosc.* **1** (1973) 197.
84. T. Kitagawa, M. Abe, K. Kyogoku, H. Ogoshi, H. Sugimoto, and Z. Yoshida, *Chem. Phys. Lett.* **48** (1977) 55.
85. P.K. Dutta, R. Dallinger, and T.G. Spiro, *J. Chem. Phys.* **73** (1980) 3580.
86. P.M. Champion and A.C. Albrecht, *J. Chem. Phys.* **71** (1979) 1110.
87. J.A. Shelnutt, *J. Chem. Phys.* **72** (1980) 3948.
88. P.M. Champion and A.C. Albrecht, *J. Chem. Phys.* **72** (1980) 6498.
89. P.M. Champion and A.C. Albrecht, *J. Chem. Phys.* **75** (1981) 3211.
90. P.M. Champion and A.C. Albrecht, *Ann. Rev. Phys. Chem.* **33** (1982) 353.
91. N. Siebrand and M.Z. Zgierski, *Chem. Phys.* **77** (1983) 35.
92. B.R. Stallard, P.M. Champion, P.R. Callis, and A.C. Albrecht, *J. Chem. Phys.* **78** (1983) 712.
93. A. Warshel and P.J. Dauber, *J. Chem. Phys.* **66** (1977) 5477.
94. T.G. Spiro, in *Iron Porphyrins*, Part II, A.B.P. Lever and H.B. Gray, eds., Addison-Wesley, Reading, Mass., 1983, Chpt. 3.
95. J.A. Shelnutt, D.C. O'Shea, N.T. Yu, D. Cheung, and R.H. Felton, *J. Chem. Phys.* **64** (1976) 1156.
96. M. Mingardi and W. Siebrand, *J. Chem. Phys.* **62** (1975) 1074.
97. J.M. Friedman and R.M. Hochstrasser, *Chem. Phys.* **1** (1973) 457.
98. R.M. Felton and N.T. Yu, in *The Porphyrins*, Vol. 3, D. Dolphin, ed., Academic Press, New York, 1978, Chpt. 8.
99. G.A. Schick and D.F. Bocian, *J. Am. Chem. Soc.* **106** (1984) 1682.
100. T. Kitagawa and Y. Ozakin, *Structure and Bonding* **64** (1987) 71.

101. L.A. Andersson, T.M. Loehr, A.R. Lim, and A.G. Mauk, *J. Biol. Chem.* 259 (1984) 15340.
102. L.A. Andersson, C. Sotiriou, C.K. Chang, and T.M. Loehr, *J. Am. Chem. Soc.* 109 (1987) 258.
103. M. Tasumi and M. Fujiwara, in *Spectroscopy of Inorganic-Based Materials*, R.J.H. Clark and R.E. Hester, eds., Wiley and Sons, New York, 1987, Chpt. 6.
104. M. Lutz, *J. Raman Spectrosc.* 2 (1974) 497.
105. G. Placzek, in *Rayleigh and Raman Scattering*, UCRL Transl. No. 526L from *Handbuch der Radiologie*, E. Marx, ed., Vol. 2, Akad. Verlagsgesellschaft, Leipzig, 1934, p. 209.
106. J.A. Koningstein, *Introduction to the Theory of the Raman Effect*, Reidel, Dordrecht, Holland, 1972.
107. A.C. Albrecht, *J. Chem. Phys.* 34 (1961) 1476.
108. P.R. Carey, *Biochemical Applications of Raman and Resonance Raman Spectroscopies*, Academic Press, New York, 1982, p. 44.
109. S. Hassing and O. Sonnich Mortensen, *J. Chem. Phys.* 73 (1980) 1078.
110. J. Tang and A.C. Albrecht, in *Raman Spectroscopy*, H.A. Szymanski ed., Plenum, New York, 1978, p. 347.
111. S.-Y. Lee and E.J. Heller, *J. Chem. Phys.* 71 (1979) 4777.
112. D.J. Tannor and E.J. Heller, *J. Chem. Phys.* 77 (1982) 202.
113. E.J. Heller, R.L. Sundberg, and D. Tannor, *J. Chem. Phys.* 86 (1982) 1822.
114. A.B. Myers, R.A. Harris, and R.A. Mathies, *J. Chem. Phys.* 79 (1983) 603.
115. G.R. Loppnow and R.A. Mathies, *J. Biol. Chem.*, to be published.
116. R.A. Harris, R.A. Mathies and W.T. Pollard, *J. Chem. Phys.* 85 (1986) 3744.
117. D.C. Blazej and W.L. Peticolas, *J. Chem. Phys.* 72 (1980) 3134.
118. D.L. Tonks and J.B. Page, *Chem. Phys. Lett.* 66 (1979) 449.
119. J.B. Page and D.L. Tonks, *J. Chem. Phys.* 75 (1981) 5694.
120. J. Michl and E.W. Thulstrup, *Spectroscopy with Polarized Light*, VCH Publishers, New York, 1986, p. 39.
121. J.J. Sakurai, *Advanced Quantum Mechanics*, Addison-Wesley, Reading, Mass., 1967, p.57.

122. V. Hizhnyakov and I. Tehver, *Phys. Stat. Sol.* **21** (1967) 755.
123. P.M. Champion and A.C. Albrecht, *Chem. Phys. Lett.* **82** (1981) 410.
124. C.K. Chan and J.B. Page, *J. Chem. Phys.* **79** (1983) 1976.
125. R.N. Bracewell, *The Fourier Transform and Its Applications*, McGraw-Hill, New York, 1978. p. 268.
126. B.R. Stallard, P.R. Callis, P.M. Champion and A.C. Albrecht, *J. Chem. Phys.* **80** (1984)
127. K.T. Schomacker, O. Bangcharoenpaurpong, and P.M. Champion, *J. Chem. Phys.* **80** (1984) 4701.
128. B. Stallard, P.M. Champion, P.R. Callis, and A.C. Albrecht, *J. Chem. Phys.* **78** (1983) 712.
129. R.J. Sension and H.L. Strauss, *J. Chem. Phys.* **85** (1986) 3791.
130. A.H. Zewail, *Acc. Chem. Res.* **13** (1980) 360.
131. S. Mukamel, *J. Chem. Phys.* **82** (1985) 5398.
132. W. Heitler, *The Quantum Theory of Radiation*, 3rd ed., Clarendon Press, Oxford, 1954, p. 181.
133. R.M. Hochstrasser, and C.A. Ny, *J. Chem. Phys.* **70** (1979) 1112.
134. F.A. Novak, J.M. Friedman, and R.M. Hochstrasser, in *Laser and Coherence Spectroscopy*, J.L. Steinfield, ed., Plenum, New York, 1978, p. 451.
135. K.T. Schomacker and P.M. Champion, *J. Chem. Phys.* **84** (1986) 534.
136. P.M. Champion and R. Lange, *J. Chem. Phys.* **73** (1980) 5947.
137. K. Kodama and A.D. Bandrauk, *Chem. Phys. Lett.* **80** (1981) 248.
138. L.C. Hoskins, *Spectrochim. Acta* **42A** (1986) 169, and references therein.
139. U. Even and J. Jortner, *Can. J. Chem.* **63** (1985) 2073.
140. U. Even, J. Magen, J. Jortner, J. Friedman, and H.J. Levanon, *J. Chem. Phys.* **77** (1982) 4374.
141. G.D. Dorough, J.J. Miller and F.M. Heunneken, *J. Am. Chem. Soc.* **73** (1951) 4315.
142. U. Even, J. Magen, J. Jortner and J. Friedman, *J. Chem. Phys.* **77** (1982) 4384.
143. K.K. Rebane, and R.A. Avarmaa, *Chem. Phys.* **68** (1982) 191.

144. I. Renge, K. Mäuring, P. Sarv, and R. Avarmaa, *J. Chem. Phys.* **90** (1986) 6611.
145. J. Funfschilling and D.F. Williams, *Photochem. Photobiol.* **22** (1975) 151; **26** (1977) 109.
146. F. Wilkinson and C.J. Willsher, *Tetrahedron* **43** (1987) 1197.
147. F. Wilkinson and G. Kelly, Diffuse Reflectance Flash Photolysis, to be published.
148. P. Kubelka, *J. Opt. Soc. Am.* **38** (1948) 448.
149. H.G. Mecht, *Reflectance Spectroscopy*, Wiley, New York, 1966.
- 149a. J.-H. Fuhrhop and K. Smith, *Laboratory Methods in Porphyrin and Metalloporphyrin Research*, Elsevier, Amsterdam, 1975.
- 149b. J.W. Buchler, in *The Porphyrins*, Vol. 1, D. Dolphin ed., Academic Press, New York, 1978, Chpt. 10.
- 149c. A. Antipas and M. Gouterman, *J. Am. Chem. Soc.* **105** (1983) 4896, and references therein.
- 149d. D. Kim and D. Holten, *Chem. Phys. Lett.* **98** (1983) 584.
151. J.A. Shelnutt, K. Alston, E.W. Findsen, M.R. Ondrias, and J.A. Rifkind, in *Porphyrins: Excited States and Dynamics*, M. Gouterman, P.M. Rentzepis, and K.D. Straub, eds., ACS Symposium Series 321, 1985, p. 232.
152. T. Kitagawa, M. Abe, and H. Ogoshi, *J. Chem. Phys.* **69** (1978) 4516.
153. M. Abe, T. Kitagawa, and Y. Kyogaku, *J. Chem. Phys.* **69** (1978) 4526.
154. W. Kiefer and H.J. Bernstein, *Applied Spectroscopy* **25** (1971) 609.
155. N.J. Harrick, *International Reflection Spectroscopy*, Wiley and Sons, New York, 1967, p. 80.
156. D.N. Batchelder and D. Bloor, *Advances in Infrared and Raman Spectroscopy*, Vol. 11, R.J.H. Clark and R.E. Hester, eds., Wiley-Heyden, New York, 1984, Chpt. 4.
157. T.M. Cotton and R.P. Van Duyne, *Biochem. Biophys. Res. Comm.* **82** (1978) 424.
158. T. Omata and N. Murata, *Photochem. Photobiol.* **31** (1980) 183.
159. K. Ireiyama, M. Yoshiura, T. Ishii, and N. Shiraki, *J. Liq. Chromatog.* **4** (1981) 533.
160. M. Holden, in *Chemistry and Biochemistry of Plant Pigments*, 2nd ed., T.W. Goodwin, ed., Academic Press, New York, 1976, Chpt. 1.

161. T. Braumann and H.J. Grimme, *J. Chromatogr.* 170 (1979) 264.
162. D. Davies and E.S. Holdsworth, *J. Lq. Chromatogr.* 3 (1980) 123.
163. P.M. Shaber, J.E. Hunt, R. Fries, and J.J. Katz, *J. Chromatogr.* 316 (1984) 25.
164. W. Svec, in *The Porphyrins*, Vol. 5, D. Dolphin, ed., Academic Press, New York, 1978, Chpt. 8.
165. T.L. Collier, personal communications.
166. P. Hynninen, *Acta Chem. Scand.* 27 (1973) 1487.
167. S.G. Boxer, *Biochim. Biophys. Acta* 726 (1986) 265.
168. P.H. Hynninen, M.R. Wasielewski, and J.J. Katz, *Acta Chem. Scand.* B33 (1979) 637.
169. T. Watanabe, M. Nakazato, and K. Honda, *Chem Lett.* 1986, p. 253.
170. T. Watanabe, M. Nakazato, M. Konno, S. Saitoh, and K. Honda, *Chem. Lett.* 1984, p. 1411.
171. P.H. Hynninen and G. Sievers, *Z. Naturforsch.* 36B (1981) 1000.
172. D.R. Burfield, K.H. Lee, and R.M. Smithers, *J. Org. Chem.* 42 (1977) 3060.
173. M. Fujiwara and M. Tasumi, *J. Phys. Chem.* 90 (1980) 5646.
174. J.J. Katz, G.L. Closs, F.C. Pennington, M.R. Thomas, and H.H. Strain, *J. Am. Chem. Soc.* 85 (1963) 3801.
175. F.C. Pennington, H.H. Strain, W.A. Svec, and J.J. Katz, *J. Am. Chem. Soc.* 86 (1964) 1418.
176. N.E. Binnie, L.V. Haley, T.A. Mattioli, D.L. Thibodeau, W. Wang and J.A. Koningstein, *J. Phys. Chem.* 90 (1986) 4938.
177. D.W. Hughes and A.S. Holt, *Can. J. Chem.* 40 (1962) 171.
178. V. Vardi, F.R. Longo, and A.D. Adler, in *The Porphyrins*, Vol. 1, D. Dolphin, ed., Academic Press, New York, 1978, Chpt. 13.
179. Reference 150, p.73.
180. G.R. Seely, in *The Chlorophylls*, L.P. Vernon and G.R. Seely, eds., Academic Press, New York, 1966, p.75.
181. S.W. Wright and J.D. Shearer, *J. Chromatogr.* 294 (1984) 281.

182. T.A. Mattioli, L.V. Haley, and J.A. Koningstein, *J. Raman Spectrosc.* 18 (1987) 53.
183. Division of Chemistry, NRC, Ottawa.
184. R.J.H. Clark, in *Advances in Infrared and Raman Spectroscopy*, Vol. 1, R.J.H. Clark and R.E. Hester, eds., Heyden, London, 1975, p.143.
185. RCA Data Sheet C31034A.
186. M. Fujiwara and M. Tasumi, *J. Phys. Chem.* 90 (1986) 424.
187. H.J. Bernstein, *Phil. Trans. Roy. Soc. Lond.* A293 (1979) 287.
188. G.T. Babcock, M.R. Ondrias, D. Gobeli, J. Van Steelandt, and G.E. Leroi, *FEBS Lett.* 108 (1979) 147.
189. S. Choi, T.G. Spiro, K.C. Langry, and K.M. Smith, *J. Am. Chem. Soc.* 104 (1982) 4337.
190. D. Gazdaru, V. Gheorghe and A. Glodneau, *Biophysique* 32 (1987) 1091.
191. G.R. Seely and R.G. Jensen, *Spectrochim. Acta* 21 (1965) 1835.
192. B.R. Stallard, P.R. Callis, P.M. Champion, and A.C. Albrecht, *J. Chem. Phys.* 80 (1984) 70.
193. P. Esherick and A. Owyong, in *Advances in Infrared and Raman Spectroscopy*, Vol. 9, R.J.H. Clark and R.E. Hester, eds., Wiley-Heyden, London, 1982, p. 130.

APPENDIX

The following Table summarises the C_r values used in the transform-generated REPs to obtain the best fit with respect to the experimental REPs.

TABLE A1: C_r Values

REGION OF EXCITATION	MODE (cm^{-1})	C_r
SORET	1134	-0.10
	1188	-0.10
	1580	-0.12
Q_y	1134	-0.40
	1188	-0.40
	1235	-0.40
	1269	-0.40
	1282	-0.45
	1337	-0.45
	1568	-0.60
	1598	-0.80

END

1|6·0|2·90

FIN

these experiments, 100 pulses were accumulated for the diffuse reflectance absorption spectrum, with entrance slits on the analysing monochromator set at 2 nm resolution (at 500 nm) and an OMA gate width of 500 μ s. For further information the reader is referred to Reference 146.

4.6 INSTRUMENTATION.

In this work, two separate optical systems were used to obtain resonance Raman spectra. During the course of this work, the availability of one of the systems was not possible and so another was used.

4.6.1 System 1.

This system was used in the resonance Raman work of the liquid phase sample. This system is described in detail in the thesis of Thibodeau⁵². The components consist of a nanosecond, pulsed N_2 laser, its associated dye laser, a monochromator, and a detection system. A schematic diagram is shown in Fig. 4.4.