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**Laser-Induced Anisotropy
and Raman Intensities in
meso-tetrakis(N-methyl-4-pyridyl)porphine,
H₂TMpyP(4) monomer**

by

Don Dawson, B.Sc.

A thesis submitted to
the Faculty of Graduate Studies and Research
in partial fulfilment of the requirements of
Masters of Science

Department of Chemistry

Carleton University

Ottawa, Ontario

June, 1994

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meso-tetrakis(N-methyl-4-pyridyl)porphine, H₂TMpyP(4)
monomer"**

submitted by
Don Dawson, B.Sc.

in partial fulfilment of the requirements
for the degree of Masters of Science

Chairman, Department of Chemistry

Thesis Advisor

Carleton University

June, 1994

Abstract

Monobeam transmission and resonance Raman (RR) experiments were used to study the excited state $S_m \leftarrow S_3(B)$ spectra and the total depolarization ratios for naturally polarized light, ρ_n , of the $\nu_2(C_\beta-C_\beta)$, A_{1g} and $\delta(\text{pyr})$, A_{1g} RR peaks of the meso-tetrakis(N-methyl-4-pyridyl)porphine $H_2\text{TMpyP}(4)$ monomer, excited at 422 nm, observed at $\Delta\bar{\nu} = 1558 \text{ cm}^{-1}$ and $\Delta\bar{\nu} = 1643 \text{ cm}^{-1}$, in a pH=7 phosphate buffer, as a function of pump intensity, $I_1(\text{mW})$, over the range of 0-5 mW. A monobeam transmission effective population saturation, EPS, study of the $H_2\text{TMpyP}(4)$ monomer yielded an effective transmission saturation value of $T_{\text{eff}} = 0.192$. This resulted in the calculated maximum lifetime of the $S_3(B)$ state, $\tau(S_3(B))$ of (17-74) ps for the $H_2\text{TMpyP}(4)$ monomer. Within the level of the approximations present in the calculations, it can be stated that the lifetime is in the picosecond (ps) regime.

Three factors, (i) ground state depletion, (ii) induced anisotropy in the density distribution function of the concentration of ground state molecules, n_1^0 , and (iii) the anisotropic polarized excited state absorption spectrum effect, are discussed to interpret the behaviour of ρ_n versus I_1 for the $\nu_2(C_\beta-C_\beta)$ and $\delta(\text{pyr})$ RR peaks of the $H_2\text{TMpyP}(4)$ monomer. The results indicate that the polarization of the excited state $S_m \leftarrow S_3(B)$ spectrum of the monomer is in the YZ plane (i.e. the $T_{ZZ}^{\text{ans}}(f)$ and $T_{YZ}^{\text{ans}}(f)$ terms are active). Using the complete three-dimensional

Euler rotational matrix, $\mathbf{R}(\phi, \theta, \Psi)$, in the standard coordinate system of the right-handed screw in the X-convention, the expressions for the first-order anisotropic correction factors in the perpendicular excitation (Y-polarization) configuration, I_{YZ}^1 and I_{YX}^1 are derived in this thesis (added proof is presented in the Appendix). In addition, scattering tensors for the $\nu_2(\text{C}_\beta\text{-C}_\beta)$ and $\delta(\text{pyr})$ RR peaks of the $\text{H}_2\text{TMpyP}(4)$ monomer at excitations of 422, 476.5, and 514.5 nm are presented. The results imply that $a > b$, and could be interpreted as the presence of x- and y- splitting of the Soret B band.

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Chapter 1: Introduction

1.1 Introduction

The study of porphyrins over the last century has been spurred on by the fact that they play some of the most essential roles in the life processes of all the prokaryotic and eukaryotic organisms on this planet (eg., as chlorophyll, heme, and vitamin B₁₂). Their omnipresence in nature is no accident, and is due to the multifaceted and versatile functions (energy transfer, electron transfer, substrate ligand ($O_2(^3\Sigma_g^-)$) transfer, catalysis, etc.) that can be performed by variation of the tetrapyrrole porphyrin structure [1, 2]. Over this past decade, the wide spread use of resonance Raman spectroscopy (RR) [3-12] has yielded important structural information concerning porphyrin and porphyrin-biomacromolecule interactions, via the strong sensitivity to local environments of the resonance Raman shifts, $\Delta\bar{\nu}(\text{cm}^{-1})$, for ground state, S_0 , vibrations.

Arguably, this sensitivity and enhancement selectivity of specific chromophore moieties, makes resonance Raman spectroscopy the most versatile technique to study biomacromolecular structure [10]. Research in porphyrin-biomacromolecule interactions typically falls into one of these categories,

- (i) carotenoid-porphyrin systems (artificial photosynthesis research) [13, 14],
- (ii) porphyrin-protein systems (hemoglobin-ligation research) [6,15-17],
- (iii) hematoporphyrin and hematoporphyrin derivative sensitizer systems (producing tumour destroying singlet oxygen, $O_2(^1\Delta_g)$, in photodynamic therapy

(PDT)) [18-22], and (iv) porphyrin-DNA systems, dealing with the intercalation and groove-binding mechanisms of the drug (i.e. porphyrin) to DNA (chemotherapy of cancer cells) [1, 23-28].

This thesis deals with the properties and characteristics of the porphyrin monomer, meso-tetrakis(N-methyl-4-pyridyl)porphine, $H_2TMpyP(4)$, and can be used in future studies to yield information in a further category of porphyrin-biomacromolecule interaction research, (iv) porphyrin-DNA systems. The dependence of the depolarization ratios, $\rho = I_{\perp}/I_{\parallel}$, (where ρ , $I_{\perp}$, $I_{\parallel}$ denote the depolarization ratio, observed Raman scattered intensity in the perpendicular, and parallel configurations (see section 2.2), respectively) of several RR peaks of monomeric $H_2TMpyP(4)$ on exciting laser power $I_e(mW)$, is rationalized by three factors, (i) ground state depletion, (ii) induced anisotropy in the density distribution function of the concentration of ground state molecules, n_1^0 , and (iii) the anisotropic polarized excited state absorption spectrum effect. The possibility of the presence of excited state $H_2TMpyP(4)^*$ species and their effects on the intensities of an observed resonance Raman emission in regions of low transmission in their excited state absorption spectra, is of paramount importance not only in the interpretation of experimental resonance Raman spectra, but also in the information that is implicitly contained in the depolarization ratios of their ground state, S_0 RR peaks. Factors, (ii), induced anisotropy in the density distribution function of the concentration of ground state molecules, n_1^0 and (iii), anisotropic polarized excited state absorption spectrum effect have their basis in

anisotropic absorption, and fluorescence polarization studies and Laser-Induced Dichroism Effect (LIDE) spectroscopy studies respectively [11, 12, 29-31]. Important parameters, such as rotation relaxation times, τ_{rot} , for drugs and drug-biomacromolecule complexes, structural interactions, and time-resolved fluorescence lifetimes, have been obtained from these fields.

This thesis will be presented in five chapters, Chapter 1: Introduction, Chapter 2: Theoretical, Chapter 3: Experimental, Chapter 4: Results and Discussion, Chapter 5: Conclusion. Chapter 1 will deal with the structure, electronic and resonance Raman Spectra of porphyrins. Chapter 2 will deal briefly with the theory of vibrational infrared (IR) and resonance Raman (RR) spectroscopy and concentrates on the equations that govern the behaviour of the laser-induced anisotropic effects on the depolarization ratio, ρ , as a function of laser excitation power, $I_l(\text{mW})$. Chapter 3 will deal with the experimental arrangements of the lasers and optics [32] involved in the monobeam transmission excited state absorption [33], and resonance Raman spectroscopic studies of the $\text{H}_2\text{TMpyP}(4)$ monomer [11, 12]. Chapter 4 presents the experimental results of the monobeam transmission studies of $\text{H}_2\text{TMpyP}(4)$; the excited state $S_m \leftarrow S_3(B)$, and, $S_k \leftarrow S_1(Q_x)$, absorption spectra in the Soret B, and Q band regions, respectively; the resonance Raman spectra of $\text{H}_2\text{TMpyP}(4)$ under pulsed laser (XeCl excimer) $\lambda_l = 422$ and 460 nm, and continuous wave (CW Ar^+ laser) $\lambda_l = 476.5$ and 514.5 nm, excitation; and the depolarization ratio, ρ_n , measurements as a function of excitation laser power $I_l(\text{mW})$. Chapter 4 deals

with the rationalization of the results of Chapter 3 in terms of the framework presented of Chapter 2 dealing with how factors (i) (ii) and (iii) effect the $H_2TMpyP(4)$ monomer. In conclusion, Chapter 5 deals with the importance and limitations of this work, and suggestions for future work.

1.2 Structure, Electronic and Raman Spectra of Porphyrins

1.2.1 Structure, Energetics, and Absorption Spectra of Porphyrins

Volumes of research have been written on porphyrins, especially over the past 40 years. A commonality that all these works share is that the properties, such as electronic absorption, fluorescence, phosphorescence, Raman, IR spectra, etc., of porphyrins are rationalized on the basis of what is known as Gouterman's Four Orbital model [34-52]. Porphyrins and Metalloporphyrins are highly coloured compounds with strong absorption in the near UV-visible light region due to its cyclic conjugated tetrapyrrole chromophore structure. Several types of this structure are shown in Fig. 1 [1,28]. The exact position of several different peripheral substituents are labelled as the 1, 2, 3, 4, 5, 6, 7, 8 positions and the symbols a, b, c, d are used for pyrrole rings A, B, C, D, and meso-substituents. Alternately, when one is concerned with the vibrational modes of the porphyrin core, the C_α , C_β , C_m labelling systems are used where the substituents X and Y are at the β -pyrrole and α -meso carbon positions respectively. The porphyrin ring can be thought of as a delocalized planar resonance structure, with an inner 16-membered ring composed of the N, C_α and C_m atoms, contributing 18π

electrons. The 16-membered ring is bonded at four opposite corners to four C_β - C_β double bond systems, with 2π electrons each giving a total of 26π -electrons to the delocalized porphyrin core. In the cases of benzoporphyrin and phthalocyanine; the C_β - C_β double bond is replaced by a 6-membered benzene ring; and the C_m carbons are replaced by N, as well, the C_β - C_β system replaced by benzene, respectively. Thus, it is best to visualize the electron density of the porphyrin evenly delocalized all over the ring, with the centre 16-member N, C_α , C_m ring having bond orders about 1.5, the C_α - C_β , and the C_β - C_β bonds having orders slightly less and slightly more than 1.5, respectively [1, 40, 41]. On the other hand, since this is a resonance bond system, many other resonance structures can be proposed to contribute, such as the 18 member N, C_α , C_m , C_α , C_β (excluding opposite N atoms) ring system [41].

When a metal is inserted in the centre of the porphyrin it bonds to the four N atoms. The metalloporphyrin, then has D_{4h} symmetry [1], as opposed to its "free-base" demetallated form, with two H atoms "ring-wizing" between opposite N atoms, [1]. The porphyrin electronic orbitals which can be seen spectroscopically are the highest occupied molecular orbital (HOMO), $a_{1u}(\pi)$, $a_{2u}(\pi)$, and the lowest unoccupied molecular orbital (LUMO), the doubly degenerate $e_g(\pi^*)$, shown in Fig. 2 [36, 37, 44]. On substitution at the eight C_β carbons with ethyl groups, the $a_{1u}(\pi)$ orbital is destabilized and rises in energy to become the HOMO of octaethylporphyrin. On the other hand, substitution of the four C_m carbons with phenyl groups, the $a_{2u}(\pi)$, orbital is destabilized, and rises

Fig. 1. Structures of porphine (P) a metalloporphyrin (MP), a chlorin (PH_2), phlorin (PH_2), bacteriochlorin (PH_4), and isobacteriochlorin (PH_4); numbering scheme for peripherally substituted porphyrin derivatives structural diagram of a D_{4h} metalloporphyrin with generalized peripheral substituents X and Y. The atom labelling scheme ($C_{\alpha, \beta, m}$) is shown [1, 28].

in energy to become the HOMO of tetraphenylporphine.

In any case, the energy difference between the HOMO and LUMO in most porphyrins is about 2 eV and is responsible for their strong absorptions in the visible and near-UV region of the spectrum. Fig. 3. [6, 28] illustrates the absorption spectrum of Fe(II)cyclochrome c showing the classic features of metalloporphyrin spectra: a very intense band, known as the Soret B band, near 400 nm, and a pair of weaker bands called Q_0 and Q_v , or $Q(0,0)$ and $Q(1,0)$, between 500 and 600 nm [5]. These are assigned using Gouterman's four-orbital model [36, 37, 44] (see the inset of Fig. 3). From the doubly degenerate LUMO $e_g(\pi^*)$ and the HOMO $a_{1u}(\pi)$ and $a_{2u}(\pi)$ orbitals (these filled orbitals have nearly the same energy) there is a strong configuration interaction between the two E_u orbital excitations $e_g(\pi^*) \leftarrow a_{1u}(\pi)$ and $e_g(\pi^*) \leftarrow a_{2u}(\pi)$ [6, 28]. The transition dipoles add up to the intense Soret B band transition and nearly cancel out for the weaker Q band transition. As well, some intensity (about 10%) is borrowed via vibronic mixing with the Soret B band transition, leading to the Q_v side-band, which is an envelope of vibrational bands built on the Q_0 band. The four-orbital model also accounts satisfactorily for the principal features of metalloporphyrin RR spectra, as will be discussed later [6, 28].

Metalloporphyrins may be placed in the following groups, known as either regular or irregular [1], and as well on the basis of their absorption spectra, into three subgroups known as, normal, hypso, and hyper types. A regular porphyrin has absorption and emission spectra governed mainly by the π electrons of the

Fig. 2. (A and B) Four-orbital and state energy level diagrams for the D_{4h} porphyrin, porphine dianion, respectively. (C and D) Four-orbital and state energy diagram for a D_{2h} porphyrin, porphine free-base. Protons are placed along the vertical axis (defined to be the x-axis). The principal effect of octaethyl substitution is indicated by the dashed line and arrow. (E) Porphine HOMO and LUMO orbitals. The sizes of the circles are proportional to the size of the atomic coefficients. The orbital symmetry labels a_{1u}, a_{2u}, e_g2 and e_g1 are appropriate for D_{4h} porphyrins. Their corresponding descendants for porphyrins of lower symmetry are labelled b₂, b₁, c₂ and c₁ [36, 37, 44].

Fig. 3. Absorption spectrum of Fe(II)cytochrome c, with a schematic representation of the four-orbital excitations, 1 and 2, are subject to strong configuration interaction, with the transition dipoles adding in the higher energy B transition, and nearly cancelling in the lower energy Q₀ transition. The Q_y band is the envelope of the 0→1 vibronic transitions induced by mixing of the Q and B transitions [6, 28].

porphyrin ring, and have only slight perturbations due to the presense of the central metal ions' electrons [1].

Metalloporphyrins have electronic spectra which consist of not only Q and B bands but they have as well what are known as N, L, and M bands.

The lowest energy absorption bands, known as the Q bands, consist of two visible bands in the region of 500-600 nm, with the lowest energy band being that of the electronic origin $Q(0,0)$ (i.e., $S_1(Q(0,0)) \leftarrow S_0$). The next highest absorption peak in energy, at relatively constant energy separation from $Q(0,0)$ (higher by about a single mode of vibrational excitation) is called the $Q(1,0)$ (i.e., $S_1(Q(0,0)) \leftarrow S_0$) [1].

Next we come to the Soret B band consisting of an extremely intense band in the region of about 380 to 460 nm. The origin of this band is the $B(0,0)$ second excited singlet state (i.e., $S_2(B(0,0)) \leftarrow S_0$). It has a very large molar extinction coefficient, and is in general the most intense absorption peak in all regular porphyrins. Through the use of high resolution spectra in the vapor phase or a low temperature another band to the blue can be detected in the Soret B band region, and is known as $B(1,0)$ (with a higher energy of one vibrational mode).

Next we come to the N, L, and M bands, which are located in the near-UV region and they have very low intensity. The N band peaks at about 325 nm, the M band peaks at about 215 nm, and the L band peaks anywhere between the N and the M bands. The M bands are difficult to measure as they peak in the deep UV region of the spectrum, and must be corrected for solvent absorption

[1].

Fig. 4. [1] and Fig. 5. [1] illustrate the absorption spectra of free-base, Zn(II), and Fe(III) derivatives of octaethylporphyrin (H_2OEP , $Zn(II)OEP$, $Fe(III)OEP$), and tetratolylporphine (H_2TTP , $Zn(II)TTP$, $Fe(III)TTP$), respectively. They illustrate the most common types of the absorption spectra encountered in most regular porphyrins. The parent, free base porphyrin has two H atoms instead of a metal M, located inside the inner ring, resulting in a lowering of symmetry of the molecule from D_{4h} to D_{2h} . This results in the splitting of the two banded Q regions $Q(0,0)$ and $Q(1,0)$ into the four banded $Q_x(0,0)$, $Q_x(1,0)$, $Q_y(0,0)$ and $Q_y(1,0)$ regions respectively which are x-and y-polarized. The first excited singlet, S_1 , is now made up of $Q_x(0,0)$ and its vibronic overtone $Q_x(1,0)$; and the second excited state singlet, S_2 , is made up of $Q_y(0,0)$ and its vibronic overtone $Q_y(1,0)$ [1]. The system of nomenclature Q, B, N, L, M comes from an early spectroscopic classification system created by Platt where Q and B are read as a quasi-allowed and a blue band, respectively.

Fig. 6 [1] illustrates a Jablonski energy level diagram of a typical porphyrin that has a singlet ground state, S_0 . The singlet (paired spins) manifold S_n is shown on the left-hand side and the triplet (parallel spins) manifold, T_n , is on the right-hand side, and they represent a series of $\pi-\pi^*$ excited states.

Excitation (indicated by the solid arrow and taking place in about 1 femtosecond (fs)) from the ground state S_0 to an excited state in the singlet manifold, S_n , is followed by rapid radiationless decay, called internal conversion,

Fig. 4. Visible Absorption spectra of octaethylporphyrin derivatives of free-base (H_2OEP), $Zn(II)$ ($Zn(II)OEP$), and $Fe(III)$ ($Fe(III)(OEP)(F)$) in CH_2Cl_2 [1].

Fig. 5. Visible Absorption spectra of tetratolylporphine derivatives of free base (H_2TTP), $Zn(II)$, ($Zn(II)TTP$), and $Fe(III)$ ($Fe(III)(TTP)(F)$) in CH_2Cl_2 [1].

Fig. 6. Jablonski energy level diagram for a regular porphyrin [1].

IC, (indicated by the wavy arrow, and taking place in the picosecond (ps) regime) to the lowest excited state in the singlet manifold, S_1 . From S_1 , the molecule may follow several alternate routes of decay. It emits radiation, called fluorescence, fl, (indicated by the dashed arrow, and taking place in the nanosecond (ns) regime) $S_1 \rightarrow S_0$ with a rate constant K_{ph} , or undergoes non-radiative decay, IC, to the ground state S_0 with a rate constant K_1 , or alternatively intersystem cross, ISC, falling into the lowest lying excited state in the triplet manifold, T_1 , $S_1 \sim \sim T_1$, with a rate constant K_{ISC} [1]. From the T_1 , state, the molecule may emit radiation known as phosphorescence, ph, (with a rate constant

K_{ph} , taking place in the nanosecond (ns) to microsecond (ms) regime), $T_1 \rightarrow S_0$; or alternatively non-radiatively IC decay to S_0 with a rate constant K_2 . In certain cases, the molecule in T_n (usually T_1) may repopulate the lowest lying excited state in the singlet manifold, S_1 , leading to what is called delayed fluorescence. Such a repopulation, $T_1 \sim \sim > S_1$, can occur by thermal repopulation (called E-type delayed fluorescence, that has a lifetime the same as the triplet state) or by triplet-triplet annihilation reactions (called P-type delayed fluorescence, that has a lifetime of about half of the triplet state) [1]. As well, a triplet-triplet absorption $T_n \leftarrow T_1$, followed by a reverse ISC, $T_n \sim \sim > S_j$, then a rapid IC, $S_j \sim \sim > S_1$ may repopulate S_1 . If the energy difference between T_n and S_j , $\Delta E(S_j - T_n)$ is relatively small then the reverse ISC process can occur fast enough to compete with IC processes [51]. Fig. 7. [1] illustrates the singlet-singlet, $S_n \leftarrow S_0$, and triplet-triplet, $T_n \leftarrow T_1$, spectra of Zn(II)(TPP). The appearance of the triplet-triplet absorption spectrum is quite different, and has noticeable peaks of about 400 and 460 nm in the blue region, a peak at about 860 nm in the red to near-infrared region. Fig. 8. [1] illustrates schematically the orbitals and possible transitions in metalloporphyrins. In free-base porphyrins and closed shell metalloporphyrins, possible transitions are limited to the delocalized π -system of the porphyrin ring, i.e. ligand centred, LC, $\pi \rightarrow \pi^*$ transitions [1]. However, the presence of open shell transition metal ions in the porphyrin core can produce additional transitions. They can be, metal to ligand charge transfer, MLCT, from the highest occupied metal d orbital to the unoccupied $e_g(\pi^*)$ orbital on the porphyrin

Fig. 7. Absorption spectra corresponding to the ground S_0 (dashed line) and triplet T_1 (solid line) excited states of $Zn(II)(TPP)$ in toluene, corrected for 100% conversion [1].

Fig. 8. Schematic presentation of the porphyrin ring and metal d orbitals and associated transitions in a metalloporphyrin with a d^6 metal ion [1].

ligand, i.e., $d-\pi^*$; the metal centred, MC, promotion of electrons within the metal d orbital manifold, i.e. d-d; and ligand to metal charge transfer, LMCT, from the highest occupied porphyrin ligand orbital, either $a_{1u}(\pi)$ and/or $a_{2u}(\pi)$, to the unoccupied metal d orbitals i.e., $\pi-d$ [1]. The LC $\pi-\pi^*$ transitions being the most allowed, have the highest intensity, and dominate porphyrin absorption spectra, generally masking the much lower intensity of the MLCT $d-\pi^*$

LMCT $\pi-d$, and MC d-d transition that are only weakly allowed. However, the presence of low lying MLCT, LMCT, and MC energy states can have a profound effect on excited state lifetimes, sometimes shortening them dramatically by providing an alternate relaxation pathway back to the ground states [1].

1.2.2 Emission Properties of Porphyrins

The important experimental parameters in fluorescence, fl, and phosphorescence, ph, spectra are the lifetimes $\tau_n(S_n)$, $\tau_{ph}(T_n)$ and the quantum yields $\phi_n(S_n)$, $\phi_{ph}(T_n)$. Porphyrins may be placed into groups known as fluorescent, phosphorescent, luminescent, and radiationless based on their emission behaviour [1].

Fluorescent porphyrins are those that show moderate fluorescence and possess quantum yields in the order of $(0.2) \times 10^{-3}$. Singlet excited state lifetimes are usually in the nanosecond (ns) regime. The observed phosphorescent lifetimes are usually in the microsecond (ms) regime, with a low quantum yield of phosphorescence, ϕ_{ph} , whose value is influenced by the degree of the heavy

atom effect [1].

Phosphorescent porphyrins are those that show moderate phosphorescence and have quantum yields, ϕ_{ph} , in the order of $(10^{-4}-10^0)$. Triplet excited state lifetimes are usually in the ns to ms regime, and less than 3 ms. There is almost no observed (prompt) fluorescence [1].

Luminescent porphyrins have paramagnetic metal ions in the centre of the porphyrin core. This causes the observed emission cannot to be classified as not pure fluorescence, nor pure phosphorescence, as a result of their molecular states being not pure singlet or pure triplet. The emission yields, $\phi_{em} = \phi_f + \phi_{ph}$, are usually above the order of 10^{-4} , and the emission parameters manifest a strong temperature dependance [1].

Radiationless porphyrins are very unusual experimentally. At best they show only a weak emission, and their total emission yields, ϕ_{em} , are less than the order of 10^{-4} . Through the use of high laser power excitation techniques, the actual detected emission can be as low as 10^{-6} . These very low quantum yields of luminescence, ϕ_{em} , of the order of $10^{-4}-10^{-6}$, imply that the excited state lifetimes are extremely short lived, in the order of 60-6000 fs. In these circumstances, any observed (prompt) fluorescence could come from vibrational non-relaxed excited states [1].

Closed shell metal ions cause only weak perturbations (very little π -backbonding), and their porphyrin derivatives absorption spectra are of the normal type. Thus empirically, the emission spectra of regular porphyrins are

characterized as being fluorescent. As well, inspite of differences in symmetry and spectral appearance, free-base porphyrins are also regular, as are the similar N-methyl substituted complexes such as meso-tetrakis (N-methyl-4-pyridyl)porphine, $H_2TMpyP(4)$ [1].

Fig. 9. [50] and Fig. 10. [50] illustrate the emission spectra of the water-soluble meso-tetrakis(4-suphantophenyl)porphine, $H_2TPPS(4)$, and meso-tetrakis(N-methyl-4-pyridyl)porphine, $H_2TMpyP(4)$, and their Zn(II) and Pd(II) metallated derivatives, respectively. The emission spectra for the free-base and Zn(II) complexes are fluorescent, and the Pd(II) complexes are phosphorescent. On inspection of Fig. 9 and Fig. 10., one can see that they consist of two separate emission bands, which are easily visible in the $H_2TPPS(4)$ complexes but are strongly overlapped in the $H_2TMpyP(4)$ complexes. Practically, this is seen in the case of the free base $H_2TMpyP(4)$ which has two bearily resolved emission bands at 675 and 705nm [50].

Its known that water-soluble porphyrins with negatively charged peripheral substituents (e.g. $H_2TPPS(4)$) have a strong tendancy toward dimerization and aggregation at concentrations greater than 10^{-5} M and those with positively changed peripheral substituents (eg. $H_2TMpyP(4)$) do not have this tendancy [53]. Upon dimeration and/or aggregation, there is only weak electronic, but strong physical (Coulombic, Vander Waals) interaction between monomers. The transition dipole moments $(M_{\sigma})_{mn}$ between monmer states $|n\rangle$ and $|m\rangle$ (where $\sigma=x,y$, or z) can interact and cause the excitation energy to

Fig. 9. Emission spectra of meso-tetrakis(4-sulphonatophenyl)porphines in aqueous solutions at room temperature. Shown are the fluorescence spectra for H_2TPPS and $ZnTPPS$ and phosphorescence spectra for $PdTPPS$ [50].

Fig. 10. Emission spectra of meso-tetrakis(N-methyl-4-pyridyl)porphines in aqueous solutions at room temperature. Shown are the fluorescence spectra for H_2TMpyP and $ZnTMpyP$ and phosphorescence spectra for $PdTMpyP$ [50].

jump from molecule to molecule. This is known as "exciton-coupling" and is conveyed by an "exciton" [54-56]. These exciton effects manifest themselves in experimental absorption spectra in three ways; hypochromic (reduced intensity) and band width widening effects, and in shifting of the peak maximum to the blue or to the red. This results from changes in excited state lifetimes and orbital energies. Some lifetimes are increased because their radiative transition probability is decreased (and/or non-radiative transition probability), and some are decreased as a result of their radiative transition probability increasing. Typically all these changes result from the dimer forming two different energy exciton levels, one strongly allowed with a strong intensity and shortened lifetime and one weakly allowed with a weak intensity and lengthened lifetime. Whether the blue shifted or red shifted components are involved with the strongly or weakly allowed exciton transitions depends on the orientation of the two molecules relative to the other [55].

Irregular porphyrins are different from regular porphyrins not only in their emission spectra but in most cases in the absorption spectra as well. The usual absorption types are known as hyper or hypsochromic. Hypsochromic absorption spectra are similar to normal absorption spectra but are blue shifted. These spectra are manifested by metalloporphyrins with strong metal perturbations(π -backbonding) on the π - π^* porphyrin ring transitions, that have their $e_g(d_x)$ orbitals more than half filled [1]. Hyperchromic absorption spectra manifest prominent additional absorption bands other than the Q, B, N bands in the region of

wavelengths shorter than 320 nm (UV-region), due to holes in their less than half filled $e_g(d_\pi)$ orbitals.

1.2.3. Vibrational Modes Infrared and Resonance Raman Spectra of Porphyrins

A three-dimensional N-polyatomic molecule has $3N-6$ degrees of vibrational freedom. In such molecules, the bonds between the atoms define the internal coordinates. The internal coordinates of the metalloporphyrin shown in Fig. 1 are defined by the bonds M-N, N-C $_\alpha$, C $_\alpha$ -C $_\beta$, C $_\beta$ -C $_\beta$, C $_\alpha$ -C $_m$, C $_m$ -X, and C $_\beta$ -Y (X and Y are the peripheral substituents). The three most studied porphyrin types are porphine, octaethylporphyrin, and tetraphenylporphine, where X=H, Y=H; X=H, Y=CH₂CH₃; and X=C₆H₅, Y=H, respectively. For a transition from state $|n\rangle$ to $|m\rangle$ to be IR active the induced transition moment $(M_e)_{mn}$ must change and transform as X, Y, or Z. In contrast, for a transition from $|n\rangle$ to $|m\rangle$ to be Raman active, the polarizability of the induced transition moment $(\alpha_e)_{mn}$ must change, and transform as the quadratic X^2 , Y^2 , Z^2 , XZ , ZY , etc.

As seen in the inset of Fig. 3. both the D_{4h} metalloporphyrin $e_g(\pi^*) \leftarrow a_{2u}(\pi)$ and $e_g(\pi^*) \leftarrow a_{1u}(\pi)$ transitions are E_u and thus IR active. For Raman activity, the direct product of the transitions is $E_u \times E_u = A_{1g} + A_{2g} + B_{1g} + B_{2g}$ and they are therefore also Raman active [6,28]. When substituent atoms or groups X and Y are considered to be point masses, the 37-atom model has 71 in-plane

Table 1. Local Mode Contributions to the In-Plane Normal modes of Metalloporphyrins [6].

Symmetry Species Local Modes ^b		A_{1g}	A_{2g}	B_{1g}	B_{2g}	E_u
ν_{N-C_1}	sym. ^c	1		1		1
	asym. ^c		1		1	1
$\nu_{C_1-C_2}$	sym.	1		1		1
	asym.		1		1	1
$\nu_{C_1-C_m}$	sym.	1			1	1
	asym.		1	1		1
C_2-C_3		1		1		1
ν_{C_m-X}		1			1	1
ν_{C_3-Y}	sym.	1		1		1
	asym.		1		1	1
ν_{M-N}		1 ^d		1		1
δ_{C_3-Y}	sym.	1		1		1
	asym.		1		1	1
δ_{C_m-X}			1	1		1
pyrrole def. (sym.)		1		1		1
porphyrin def.		1	2		3	3
Total		9	8	9	9	18

^aSee Figure 1 for structure and atom labeling.

^b ν_{A-B} = A-B bond stretching; δ_{A-B} = A-B bond bending; def = ring deformation.

^csym. = symmetric, asym. = asymmetric, with respect to the local 2-fold axis.

^dThese contributions are redundant.

vibrational modes (2N-3) and 34 out-of-plane vibrational modes (N-3). The in-plane vibration modes are expressed as follows

[6, 28]:

$$\Gamma_{in-plane} = 9A_{1g} + 8A_{2g} + 9B_{1g} + 9B_{2g} + 18E_u \quad (1)$$

Since the symmetry of these porphyrins includes inversion, the "g" modes are Raman active and the E_u modes are infrared-active, assuring mutual exclusion. Table 1. [6] illustrates how the in-plane bond stretching ν , and bending δ , internal coordinates contribute to the various normal modes [6, 28]. Several exhaustive

Table 2. In-plane skeletal mode frequencies (cm⁻¹) and local mode assignments for Ni(II)P, Ni(II)OEP, and Ni(II)TPP [6, 28].

Symmetry	ν_i	Description ^b	NiOEP	NiP	NiTPP
A_{1g}	ν_1	$\nu(C_m-X)$	(3041) ^c	(3043)	1235
	ν_2	$\nu(C_2-C_2)$	1602	1575	1572
	ν_3	$\nu(C_a-C_m)_{sym}$	1520	1459	1470
	ν_4	$\nu(\text{Pyr. half-ring})_{sym}$	1353	1376	1374
	ν_5	$\nu(C_2-Y)_{sym}$	1138	(3097)	(3097)
	ν_6	$\nu(\text{Pyr. breathing})$	804	995	1004
	ν_7	$\delta(\text{Pyr. def.})_{sym}$	674	731	859
	ν_8	$\nu(\text{Ni-N})$	350/343	369	402
	ν_9	$\delta(C_2-Y)_{sym}$	265/274	1066	1079
B_{1g}	ν_{10}	$\nu(C_a-C_m)_{asym}$	1655	1650	1594
	ν_{11}	$\nu(C_2-C_2)$	1577	1505	1504
	ν_{12}	$\nu(\text{Pyr. half-ring})_{asym}$	1351 ^c	1319 ^c	1302
	ν_{13}	$\delta(C_m-X)$	1250	1155	238
	ν_{14}	$\nu(C_2-Y)_{asym}$	1131	(3079)	(3097)
	ν_{15}	$\nu(\text{Pyr. breathing})$	751	1003	1009
	ν_{16}	$\delta(\text{Pyr. def.})_{asym}$	745 ^c	730	846
	ν_{17}	$\delta(C_2-Y)_{asym}$	305	1060	1054
	ν_{18}	$\nu(\text{Ni-N})$	163	237	277
A_{2g}	ν_{19}	$\nu(C_a-C_m)_{asym}$	1603	1611	1550
	ν_{20}	$\nu(\text{Pyr. quarter-ring})$	1394	1354	1341
	ν_{21}	$\delta(C_m-X)$	1307	1139	(257)
	ν_{22}	$\nu(\text{Pyr. half-ring})_{asym}$	1121	1005	1016
	ν_{23}	$\nu(C_2-Y)_{asym}$	1058	(3057)	(3037)
	ν_{24}	$\delta(\text{Pyr. def.})_{asym}$	597	806	828
	ν_{25}	$\delta(\text{Pyr. rot.})$	551	429	560
	ν_{26}	$\delta(C_2-Y)_{asym}$	(243)	1317	1230
B_{2g}	ν_{27}	$\nu(C_m-X)$	(3041)	(3041)	1269
	ν_{28}	$\nu(C_a-C_m)_{sym}$	1453	1504	(1451)
	ν_{29}	$\nu(\text{Pyr. quarter-ring})$	1407	1365	1377
	ν_{30}	$\nu(\text{Pyr. half-ring})_{asym}$	1160	1003	1004
	ν_{31}	$\nu(C_2-Y)_{asym}$	1015	(3055)	(3057)
	ν_{32}	$\delta(\text{Pyr. def.})_{asym}$	935	819	869
	ν_{33}	$\delta(\text{Pyr. rot.})$	493	435	450
	ν_{34}	$\delta(C_2-Y)_{asym}$	197	1193	1191
	ν_{35}	$\delta(\text{Pyr. transl.})$	144	197	109
E_g	ν_{36}	$\nu(C_m-X)$	(3040)	(3042)	(1254)
	ν_{37}	$\nu(C_a-C_m)_{asym}$	(1637)	1624	(1556)
	ν_{38}	$\nu(C_2-C_2)$	1604	1547	(1552)
	ν_{39}	$\nu(C_a-C_m)_{sym}$	1501	1462	(1473)
	ν_{40}	$\nu(\text{Pyr. quarter-ring})$	1396	1355	(1403)
	ν_{41}	$\nu(\text{Pyr. half-ring})_{sym}$	(1346)	1319	(1331)

Table 2. (continued) [6, 28].

Symmetry	ν_i	Description ^b	NiOEP ^c	NiP	NiTPP
E_g	ν_{42}	$\delta(C_\alpha-X)$	1231	1150	(233)
	ν_{43}	$\nu(C_\beta-Y)_{sym}$	1153	(3097)	(3097)
	ν_{44}	$\nu(\text{Pyr. half-ring})_{asym}$	1133	1033	(1003)
	ν_{45}	$\nu(C_\beta-Y)_{asym}$	996	(3067)	(3100)
	ν_{46}	$\delta(\text{pyr.})_{asym}$	927	806	(864)
	ν_{47}	$\nu(\text{Pyr. breathing})$	766 ^d	955	(1023)
	ν_{48}	$\delta(\text{pyr.})_{sym}$	605	745	(895)
	ν_{49}	$\delta(\text{Pyr. rot.})$	544	366	(512)
	ν_{50}	$\nu(\text{Ni-N})$	(355)	420	(436)
	ν_{51}	$\delta(C_\alpha-Y)_{asym}$	328 ^e	1064	(1093)
	ν_{52}	$\delta(C_\beta-Y)_{asym}$	263 ^e	1250	(1213)
	ν_{53}	$\delta(\text{Pyr. transl.})$	212 ^e	252	(306)

^a A and B mode frequencies from CS_2 solution (NiOEP, NiP) and crystal (NiTPP) RR spectra at room temperature. E mode frequencies from matrix-isolated (NiOEP) and room temperature KBr pellet (NiP) IR spectra.

^b Local coordinates descriptions in Fig. 5. X, Y = H. H(NiP): H, C_2H_5 , (NiOEP) and C_4H_9 , H (NiTPP).

^c (), calculated frequencies: not observed.

^d Observed only in the *meso-d*₄ isotopomers.

^e These frequencies from 12 K RR spectra of tetragonal crystals

works [6, 28, 45-49] have assigned the skeletal ground state vibrational modes of Ni(II)P, Ni(II)OEP, and Ni(II)TPP in D_{4h} symmetry. All resonance Raman spectra of these metalloporphyrins (as well as D_{2h} free-base porphyrins) are correlated to these D_{4h} vibrational assignments and these in-plane frequencies are listed in Table 2 [6, 28]. The totally symmetric A_{1g} modes ν_1 to ν_9 are the strongest resonance Raman scatterers on Soret B band excitation. Some of the most characteristic resonance Raman shifts $\Delta\bar{\nu}(\text{cm}^{-1})$ are $\nu_1(\text{C}_m-X)$, $\nu_2(\text{C}_\beta-\text{C}_\beta)$, $\nu_3(\text{C}_\alpha-\text{C}_m)$, $\nu_4(\text{C}_\alpha-\text{N})$, and $\delta_9(\text{C}_\beta-\text{H})$. The ν_1 , ν_2 , and δ_9 modes are very sensitive to binding interactions in porphyrin-DNA complexes and the ν_4 is very sensitive

to porphyrin core size expansion on going from Fe(II) to Fe(III) in porphyrin-protein interactions in hemoglobin. In these molecules, the vibrational IR and RR are very powerful and complimentary techniques for obtaining important information about the structure and vibrational energetics of the ground state, and the excited state for RR, if it is populated. The vibrational IR region usually covers the wavelengths $\lambda_{\text{IR}}=2500\text{-}15400\text{nm}$ (i.e. $\overline{\nu}_{\text{IR}}=4000\text{-}650\text{cm}^{-1}$) and thus can only excite the ground state vibrations. Fig. 11. [8] illustrates the IR spectra of porphine, H_2P , and its zinc, copper, and nickel complexes, over 600 to 2000 cm^{-1} . It can be seen that most of the IR peaks are in the $900\text{-}1700\text{ cm}^{-1}$ region resulting from the in-plane stretching, ν and bending δ , at the bonds the porphyrin core [6, 8, 28].

In resonance Raman spectroscopy, the Raman line activity is correlated with the excited state geometry through a conventional rule-of-thumb that states if there is a change in the molecular geometry in the excited state that resembles a normal mode of the ground state molecule, then that normal mode will be active in the resonance Raman spectrum [5]. Thus, the assignment theory is based on a close connection between the change in molecular geometry (conformation and dimensions) experienced on going from the ground electronic state to an excited electronic state and the normal mode of vibration for a Raman active line in resonance with the electronic transition. Thus, although in the ground state, the peripheral modes of the ethyl group and phenyl group of Ni(II)OEP and Ni(II)TTP are not electronically coupled to the porphyrin ring, the presence of

Fig. 11. Infrared spectra of porphine, and its ^{64}Zn , Cu, and Ni complexes [8].

RR peaks at about 1000-1300 cm^{-1} in Ni(II)OEP, and 1000-1200 cm^{-1} and especially about 1640 cm^{-1} in Ni(II)TTP, is attributed to RR enhancement of the peripheral substituent modes [6, 28]. This indicates that in their respective resonance excited electronic states, their geometries are significantly distorted and that a degree of electronic and/or vibronic coupling of the peripheral substituents to the porphyrin ring exists.

It should be kept in mind that the four-orbital model and the assignment of RR peaks to single vibrational normal modes is an oversimplification since there is extensive configuration interaction resulting in the mixing of states and vibrational modes. Due to the large number atoms in the metalloporphyrin molecule, it is difficult to carry out highly accurate molecular orbital MO and energy state calculations via ab initio computer models, only recently have highly accurate MO ab initio studies of metalloporphyrins been carried out [52]. However, the four-orbital and the assignment of single vibrational normal modes, are very useful qualitatively for rationalization of metalloporphyrin RR spectra and vibrational normal mode "book-keeping" and are widely used.

Chapter 2: Theoretical

2.1 Vibrational Infrared (IR) and Resonance Raman (RR) Spectroscopic Theory

The electrons within the framework of bonds that compose a polyatomic molecule have three different types of degrees of freedom, translation and rotation of the molecule as a whole, and vibrations of the various groups of atoms and electrons that make up its internal coordinates. Thus one has atomic, vibrational, and electronic transitions within a molecule in increasing energy, respectively. These internal vibrational and electronic transitions are of primary interest here. For an allowed transition to occur from an electronic state, $|n\rangle$, to another electronic, $|m\rangle$, the following selection rules apply. The induced transition dipole moment between states $|n\rangle$ and $|m\rangle$, labelled $(M_\sigma)_{mn}$, must change during the transition. This expressed as follows:

$$(M_\sigma)_{mn} = - \int \Psi_m^* e' \sigma \Psi_n d\tau = - \langle m | e' \sigma | n \rangle \quad (2)$$

where $\sigma = x, y, \text{ or } z$ and $(M_\sigma)_{mn}$, e' , Ψ_m^* , Ψ_n , $\langle m |$, and $| n \rangle$ denote the transition dipole moment between states n and m polarized in the σ direction, the elemental charge on an electron, the complex conjugate the wave function for state Ψ_m^* , the

wave function for state Ψ_n , the statevector for conjugate state $\langle m|$, and the state vector for state $|n\rangle$, respectively.

The total state for any number of electrons within a polyatomic molecule can be characterized by two important quantities, total orbital angular momentum L and total spin angular momentum S . Total orbital angular momentum arises from the cooperative motion of electrons within their molecular orbitals and total spin angular momentum arises from the coupling of spins from each of those electrons. These two quantities can couple together to form what is known as total angular momentum J , $J=L+S$. For an electronic transition to be considered allowed, the spin selection rule states the total spin of the system cannot change on transition from $|n\rangle$ to $|m\rangle$ i.e. $\Delta S=0$. However, if there is only a weak spin-orbit interaction between L and S , defined as the Russell-Saunders limit the J-J selection rule is partially relaxed and processes between the singlet (spins paired) manifold and the triplet (spins parallel) manifold may occur. Such processes include intersystem crossing ISC, $S_1 \sim \sim T_1$ and phosphorescence $T_1 \rightarrow S_0$, where S_0 , S_1 , and T_1 denote the singlet ground, first excited singlet, and lowest lying triplet states, respectively. These processes are favoured to occur in large polyatomic molecules and in metal ions with a large atomic number, which exhibit high spin-orbit coupling.

Infrared (IR) vibrational (absorption) transitions involve only the vibrations of the ground state manifold and are denoted by the ground state vibrational wave functions $|v'\rangle$. The IR transition dipole moment in equation (2) is represented

by $(M_\sigma)_{v_0}$. When an IR vibrational transition is allowed, the transition dipole moment change during the transition implies that the direct product of the initial and final vibrational state must equally transform as the dipole moment operator, $-e'\sigma$, i.e. as X, Y, or Z. Each vibrational peak in an IR spectrum can then be assigned to various vibrational modes of the ground state. This is very useful in the determination of the presence of different types of bonds (eg. C-H, C-C, C=C, C-N, M-N, etc.) in the molecule, allowing correlations with their different force constants.

Light can interact with matter in different ways: it can be absorbed, emitted, or scattered. Sir C.V. Raman founded a field of spectroscopy known as Raman Spectroscopy which is based on particular forms of scattering. Consider a three-level system, with levels n , m , and r where $E_n < E_m < E_r$ and with state vectors $|n\rangle$, $|m\rangle$, and $|r\rangle$. On excitation with a photon of energy $E_0 = h\nu_0$, the molecule and the photon interact, and scattered photon is emitted, thus the process is known as a two photon event. If the emitted photon is elastically scattered, its energy is equal to the excitation photon, $h\nu_0$, and is known as Rayleigh scattering [3, 9, 10]. If the emitted photon is inelastically scattered, the emitted photon can have an energy lower $h(\nu_0 - \nu_{mn})$, or higher $h(\nu_0 + \nu_{mn})$ than the exciting photon $h\nu_0$ where $\nu_v = \nu_{nn} = \nu_m - \nu_n$. The former is known as spontaneous Stokes Raman scattering, where the latter is known as spontaneous Anti-Stokes Raman scattering. The scattering event from $|n\rangle$ to $|m\rangle$ via the virtual state $|r\rangle$ is a simultaneous non-classical two-photon event where classically no

population numbers of the virtual $|r\rangle$ state and state $|m\rangle$ are produced [3]. The induced dipole moment in spontaneous Stokes-Raman scattering, scattering from the ground state $|n\rangle$ radiates at a frequency $\nu_0 - \nu_{mn}$ to the excited state $|m\rangle$. The induced dipole moment in spontaneous Anti-Stokes Raman scattering, scatters from the excited state $|m\rangle$ radiates at a frequency $\nu_0 + \nu_{mn}$ to the ground state $|n\rangle$.

The Raman shift, $\Delta\bar{\nu}(\text{cm}^{-1})$, for Stokes Raman scattering is defined as $\Delta\bar{\nu} = \bar{\nu}_0 - \bar{\nu}$ where $\bar{\nu}_0$, and $\bar{\nu}$ denote wavenumbers of the exciting, and Raman emitted lines, respectively. For Anti-Stokes Raman scattering it is defined as $\Delta\bar{\nu} = \bar{\nu} - \bar{\nu}_0$.

A practical consideration in obtaining an experimental Raman spectrum is the efficiency of the scattering process. In Rayleigh scattering, about 10^3 scattered photons $\cdot \text{mol}^{-1}$ are observed in the intensity of the emission peak. In contrast, in Raman scattering only about 10^6 scattered photons $\cdot \text{mol}^{-1}$ are observed in the emission peak. In Raman scattering, one also has to consider population effects. The equilibrium Boltzmann population distribution dictates that the number of molecules in the ground state $|n\rangle$ would be much greater than the number of molecules in the excited state $|m\rangle$. Thus, the emission intensity of the spontaneous Stokes Raman scattered peaks is much greater than that of the spontaneous Anti-Stokes Raman scattered peaks. Thus, the quality of the experimental Raman spectrum depends on the light source one uses to excite the sample and create the induced dipole moment. For good results, one needs a

light source that is (1) coherent (has the same phase and frequency), (2) monochromatic, and (3) very intense. In the early 1960's, such a source was constructed, it is known as the laser [32]. The laser has the advantage as well that the polarization of its electric radiation vector, E , can be easily controlled and one can use the E_x , E_y , and E_z components separately.

The Raman experiment is usually performed by illuminating a sample with this type of high intensity light beam, providing a well defined frequency and a single linear polarization. The scattered light is collected over some solid angle, Ω , and measured to determine the frequency shifts and the emitted intensity and polarization illustrated in Fig. 12 [9]. Fig. 13 [9] illustrates the comparison of the processes of (a) vibration IR absorption, (b) normal (non-resonant) Raman scattering, (c) resonance Raman scattering, and (d) absorption and fluorescence emission. Fig. 14. [9] illustrates the Raman scattering phenomenon of the exciting frequency ν_v (i.e. ν_{mn}). The specific Raman processes are illustrated in Fig.14.. These modulate the induced dipole moment (created by the resulting displacement of electronic charge driven by the incident electric field of frequency ν_0), via the coupling of the nuclear vibrational motion (causing oscillating the electron cloud to oscillate at ν_v) giving rise to the beat oscillating radiating at $(\nu_0 \pm \nu_v)$.

Thus, for a vibronic transition between state $|n\rangle$ and $|m\rangle$ to be Raman active, the induced dipole moment, M , must have its Raman scattering polarizability, α , change during the excitation with the electric field E as

Fig. 12. Raman inelastic light-scattering process showing excitation of a sample with light of intensity $I(\nu_0)$. The incident light has an electric field of magnitude and direction shown by e_0 . Raman scattered light at a frequency $\nu_0 - \nu_v$ (ν_v is the Raman active molecular vibrational frequency) and intensity $I(\nu_0 - \nu_v)$ is collected over the solid angle Ω . A ray of this light is shown with its electric field polarized along e_s parallel to the incident field [9].

expressed by the relationship [3]:

$$M = \alpha E \quad (3)$$

and thus,

$$\begin{bmatrix} M_x \\ M_y \\ M_z \end{bmatrix} = \begin{bmatrix} \alpha_{xx} & \alpha_{xy} & \alpha_{xz} \\ \alpha_{yx} & \alpha_{yy} & \alpha_{yz} \\ \alpha_{zx} & \alpha_{zy} & \alpha_{zz} \end{bmatrix} \begin{bmatrix} E_x \\ E_y \\ E_z \end{bmatrix} \quad (4)$$

where M , α , and E are tensors of rank 1, rank 2 and rank 1, respectively. The elements, $\alpha_{\rho\sigma}$, of the scattering tensor, α , for non-resonant conditions for the transition from $|v' \rangle |n \rangle$ to $|v'' \rangle |m \rangle$ is expressed as follows [3]:

$$(\alpha_{\rho\sigma})_{mn} = \sum_r \left(\frac{\langle m | (M_\sigma)_{v''v'''} | r \rangle \langle r | (M_\rho)_{v''v'} | n \rangle}{E_{rn} - E_0} + \frac{\langle m | (M_\rho)_{v''v'''} | r \rangle \langle r | (M_\sigma)_{v''v'} | n \rangle}{E_{rn} + E_0} \right) \quad (5)$$

where the vibronic state vectors are $|v' \rangle |n \rangle$, $|v'' \rangle |m \rangle$, and $|v''' \rangle |r \rangle$ for the ground, excited, and virtual states, respectively. The intensity of the Raman transition for n to m , $(I_{\rho\sigma})_{mn}$ can be expressed as follows [3, 9, 10]:

$$(I_{\rho\sigma})_{mn} = \sigma^R W(\Omega) N I_0 = \frac{16\pi^4(\nu - \nu_0)^4}{c^4} I_0 \langle n_1^G (\alpha_{\rho\sigma})_{mn}^2 \rangle \quad (6)$$

where σ^R , $W(\Omega)$, N , I_0 , and $\langle n_1^G (\alpha_{\rho\sigma})_{mn}^2 \rangle$ denote the total differential Raman cross-section for a single gas-phase molecule integrated over the Raman peak bandwidth (in $\text{cm}^2 \cdot \text{molecule}^{-1} \cdot \text{steradian}^{-1}$); the parameter that details the optical

Fig. 13. IR absorption and Raman scattering. (a) Vibrational IR absorption. (b) Inelastic scattering of light of frequency ν_0 to a frequency of $\nu_0 - \nu_v$. The shift in frequency corresponds to a vibrational frequency in this case, and the scattering is known as vibrational Raman scattering. Because the excitation occurs at a frequency far removed from any known absorption bands associated with excitation to excited states, the phenomenon is known as normal, or non resonance, Raman scattering. (c) Excitation occurring within an electronic transition produces resonance Raman scattering. (d) Absorption and fluorescence emission in which an excited state is created. Typical relaxed fluorescence occurs subsequent to the population of an excited state. Prior to emission, this excited state quickly relaxes to the lowest vibrational level of the lowest singlet excited state. Emission is broad and the molecule undergoes a transition between different vibronic levels of the ground state [9].

Fig. 14. Raman scattering phenomenon. The presence of a vibration of frequency ν_v is shown by the spring. Light scattered at $\nu_0 - \nu_v$ is known as Stokes Raman scattering and light scattered at $\nu_0 + \nu_v$ is known as Anti-Stokes Raman scattering [9].

geometry and includes factors such as the collected solid angle Ω and spectrometer efficiency; the number of molecules within the scattering volume element; the incident excitation intensity ($\text{photons} \cdot \text{cm}^{-2} \cdot \text{s}^{-1}$), into the scattering volume; and the mean value of the product of the density distribution function of the concentration of ground state molecules and the square of the α_{nm} scattering tensor element for the n to m transition; respectively. In terms of the coordinates of the molecule, equations (4) to (6) show that for a transition from $|n\rangle$ to $|m\rangle$

to be Raman active, the direct product of the initial and final states must transform as the quadratic X^2 , Y^2 , Z^2 , XY , YZ , XZ , etc. [3].

As one approaches excitation frequencies that are within an absorption peak, one encounters the natural frequencies of oscillation of specific electron oscillators of the molecular electron cloud and span the molecular absorption peak bandwidth [9, 10]. Excitation in this range is said to be in "resonance" with these electronic transitions (see Fig. 13.), and therefore, the Raman scattering is said to be "resonance Raman" scattering [9, 10]. However, this resonance excitation at the natural frequency of electron cloud oscillation results in an increasing oscillating charge displacement and corresponding increase in the induced dipole moment. This, in turn, directly results in an increased scattering or reradiation efficiency for Raman scattering. The enhancement factor of resonance Raman scattering compared with that of normal Raman scattering is about 10^6 [3, 9, 10].

As one approaches resonance, however, E_m approaches E_0 , a so-called damping factor, Γ_r , dependent on the lifetime τ , must be added to the denominator of equation (5) and the non-resonant second term is dropped to yield the resonance Raman case formula for the scattering tensor element expressed as follows [3-6, 9, 10]:

$$(\alpha_{\rho\sigma})_{mn} \approx A_{FC}(\nu_0) + B_{nFC}(\nu_0) \quad (7)$$

where,

$$A_{FC}(\nu_0) = \sum_r \left(\frac{\langle m | (M_o)_{v''v'''} | r \rangle \langle r | (M_p)_{v''v'''} | n \rangle}{(E_{rn} - E_0) + i\Gamma_r} \right) \quad (8)$$

$$B_{nFC}(\nu_0) = \sum_r \left(\frac{\langle m | \frac{\partial(M_o)_{v''v'''}}{\partial Q} Q | r \rangle \langle r | v''' | v' \rangle | n \rangle}{(E_{rn} - E_0) + i\Gamma_r} + \right. \\ \left. \frac{\langle m | \langle v'' | v''' \rangle | r \rangle \langle r | \frac{\partial(M_p)_{v''v'''}}{\partial Q} Q | n \rangle}{(E_{rn} - E_0) + i\Gamma_r} \right) \quad (9)$$

$$\Gamma_r = \frac{i}{4\pi\tau_r} \quad (10)$$

where i , Q , $A_{FC}(\nu_0)$, and $B_{nFC}(\nu_0)$ denote the imaginary unit, the normal mode displacement, Albrechts A-term for Franck-Condon effects, and the B-term for non Franck-Condon effects, respectively. In the Herzberg-Teller formalism, if the resonant state $|v''' \rangle |r \rangle$ is not strongly allowed and there is another strongly allowed state $|v''' \rangle |k \rangle$ close in energy, then strong interaction between them can occur expressed as follows [6],

$$\frac{\partial(M_o)_{v''v'''}}{\partial Q} = \frac{\langle k | (M_o)_{v''v'''} | r \rangle \langle k | \langle v''' | \frac{\partial H}{\partial Q} | v''' \rangle | r \rangle}{E_{kn} - E_{rn}} \quad (11)$$

where E_{kn} , and $\partial H/\partial Q$ denote the energy of the mixing state, and the derivative of the Hamiltonian with respect to the normal mode displacement. As well, the first term of equation (11) represents the transition dipole moment of the mixing state. The magnitude of $\partial(M_{0n})/\partial Q$ diminishes as the energy separation between the two states increases. The active vibrations are those that are effective in mixing the two states and depends on their geometries. The allowed symmetries are given by the cross (direct) product of the electronic transition representations [6]. A useful visualization of the resonance Raman equations (7)-(11) is as follows: if the transition dipole moment is dependant almost entirely on the ground state normal mode coordinate displacement, Q_0 , then Albrechts A-term dominates at resonance and the B-term is negligible, i.e. Franck-Condon Effects dominate. However if the transition dipole moment is significantly dependant on the normal mode coordinate displacements of the resonant excited state then both the A- and B-terms are active at resonance.

It must be borne in mind, that as one approaches resonance, the incident photons are being absorbed rather than scattered and thus the scattering volume decreases, making absolute changes in intensity which are non-linear with respect to the resonance Raman effect. To correct for this an internal standard is used, one whose non-resonant Raman scattering cross-section is constant over the frequency range of excitation. In aqueous media for example the Raman band of water, $\Delta\bar{\nu}(\text{H}_2\text{O})=3450 \text{ cm}^{-1}$, is used to normalize the intensity of the RR peaks of the analyte molecules in solution.

Fig. 15. Selectivity available from resonance Raman spectroscopy. The absorption spectrum is shown for a sample with two absorption bands. These bands could be two different analytes, two different chromophoric segments of a macromolecule, or two different electronic transitions of a single molecule. Three different Raman spectra are shown that derive from resonance Raman excitation within either of the two absorption bands (a) and (b), or from excitation away from the absorption (c). Excitation within resonance leads to much more intense Raman spectra. The most intense feature is the Rayleigh scattering band (broken curve) [9].

Fig. 16. Resonance Raman protein landscape of sperm whale myoglobin. (a) Myoglobin structure. (b) Absorption spectrum. (c) UV excitation at about 225 nm enhances the tyrosine and tryptophan bands. (d) Excitation further in the UV region enhances amide vibrations whose intensities and frequencies depend on protein secondary structure. Excitation within the heme absorption bands in the visible spectral region (400-600 nm) enhances vibrations of only (e) the heme ring and (f) those between the heme iron and its axial ligands. No protein vibrational modes are observed [10].

The utility of resonance Raman is its selective enhancement of only those RR peaks which are in resonance with the electronic transition, illustrated in Fig. 15 [9]. This selectivity is eloquently demonstrated in Fig. 16 [10], illustrating the various regions of the biomacromolecule myoglobin, which can be selectively excited and their vibrational structure studied via the RR effect.

2.2 The Raman Depolarization Ratio: Isotropic and Anisotropic Effects

At the basis of anisotropic absorption and the Laser-Induced Dichorism Effect (LIDE) is the premise that if a polarized light beam is incident on a medium containing an ensemble of randomly oriented molecules, the molecules that have their transition dipole moments coincidentally parallel to this exciting light beam will absorb the light more than those with their transition moments at various other angles. Let such an aligned molecule be situated at the origin of the coordinate system. Let the rotational relaxation time of the molecule, τ_{rot} , be longer and/or of comparable magnitude to the length of the excitation pulse, τ_{pulse} . As well, let the pump and probe beams arrive at the same time in the origin of the coordinate system [31]. This means that the previously isotropic (spherical) density distribution function of the concentration ground state molecules, n_1^0 , is perturbed and changed to one that is anisotropic with the distortion along the photon incident direction. Now, at large pumping intensities, effective population saturation (EPS), i.e. population numbers, will be induced and excited state absorption cannot be neglected [31]. The photons of the probe beam E_2 are then

absorbed into electronic transitions that originate in the excited state created by the pump E_1 .

For large pump intensity $I_1 \approx (E_1)^2$, conditions can exist at which EPS of the $S_1 \leftarrow S_0$ transitions take place. Thus, it is convenient to normalize the intensity of the pump light to that of the saturation intensity, expressed as follows [31]:

$$f = \frac{I_1}{I_{sat}} \quad (12)$$

where the saturation intensity can be expressed as follows [31]:

$$I_{sat} = \frac{h \nu_{pump}}{\phi_{fl}(S_n) \sigma_1^G \tau_{fl}(S_n)} \quad (13)$$

where $h \nu_{pump}$, $\phi_n(S_n)$, σ_1^G , and $\tau_n(S_n)$ denote the energy of the pump photons, the quantum yield of fluorescence of state S_n (usually $n=1$) terminating in the ground state S_0 , the absorption cross-section of the ground state for the pump photons, and the fluorescence lifetime of state S_n (usually $n=1$) terminating in the ground state S_0 , respectively.

Thus, the spatial anisotropy introduced into the system by the pump photons E_1 , changes the previously isotropic (spherical) density distribution function of the concentration of ground state molecules n_1^G , to an anisotropic (non-spherical) density distribution function of the concentration of ground state molecules $n_1^G(\theta, f)$ and $n_1^G(\phi, \theta, f)$ in the parallel pump-probe, and perpendicular

pump-probe configurations, respectively.

Now to have a basis in which to visualize and express the effects of changing the density distribution function of the concentration of ground state molecules from an isotropic one, n_1^0 , to that of the anisotropic ones; excited in the parallel (Z-polarization) excitation configuration, $n_1^0(\theta, f)$, and excited in the perpendicular (Y-polarization) excitation configuration, $n_1^0(\phi, \theta, f)$; we must use an explicit Euler angle description. This provides a basis for transforming from the coordinates of the space fixed set of axes, XYZ, to the body set of axes, $x'y'z'$ [58]. This can be used to lay down the framework of the expressions and measurement of the Raman depolarization ratio, ρ , via the isotropic α^2 and anisotropic β^2 components of the polarization ellipsoid.

One can carry out the transformation from any given cartesian coordinate system to another via three successive rotations. The Euler angles are then defined as the three successive angles of rotation [57, 58]. The sequence employed here is stated by rotating the initial system of axes, XYZ, by an angle ϕ counter clockwise about the Z-axis, and the resultant coordinate system is labelled the $\xi\eta\zeta$ axes. In the second stage, the intermediate axes, $\xi\eta\zeta$, are rotated about the ξ -axis counter clockwise by an angle θ to produce another intermediate set, the $\xi'\eta'\zeta'$ axes. Now, the ξ' -axis is located at the intersection of the XY- and $\xi'\eta'\zeta'$ -planes and is known as the "line of nodes". Finally, the $\xi'\eta'\zeta'$ axes are rotated counter clockwise by an angle Ψ about the ζ' -axis to yield the desired $x'y'z'$ axes. Fig. 17 [58] illustrates the various steps of the process. Thus, the

Euler angles , (ϕ, θ, Ψ) , defined by this sequence of operations completely specify the orientation at the $x'y'z'$ system relative to the XYZ system and can therefore act as the three needed generalized coordinates [58].

Now, the elements of the complete three-dimensional Eulerian rotational transformation matrix, $R(\phi, \theta, \Psi)$, can be obtained by expressing the matrix as the triple product of the separate rotations, each of which can be expressed in a relatively simple rotational matrix form. Therefore, the initial rotation about Z can be described by the matrix $R_z(\phi)$ where [58]

$$\xi = R_z(\theta)x \quad (14)$$

where ξ and x stand for column matrices, similarly the transformation from $\xi\eta\zeta$ to $\xi'\eta'\zeta'$ can be described by matrix $R_\xi(\theta)$ where [58]

$$\xi' = R_\xi(\theta)\xi \quad (15)$$

and the last rotation to $x'y'z'$ by a matrix $R_{\zeta'}(\Psi)$ where [58]

$$x' = R_{\zeta'}(\Psi)\xi' \quad (16)$$

Therefore, the complete three-dimensional Euler rotational matrix of the transformation is denoted by $R(\phi, \theta, \Psi)$ where [58],

Fig. 17. The rotations defining the Eulerian angles [58].

$$x' = R(\phi, \theta, \Psi)x \quad (17)$$

is the product of the successive rotational matrices

$$R(\phi, \theta, \Psi) = R_{\zeta'}(\Psi)R_{\xi}(\theta)R_z(\phi) \quad (18)$$

Now, the $R_z(\theta)$ transformation is the rotation about Z, and thus the matrix can be expressed as follows [58]:

$$R_z(\phi) = \begin{bmatrix} \cos\phi & \sin\phi & 0 \\ -\sin\phi & \cos\phi & 0 \\ 0 & 0 & 1 \end{bmatrix} \quad (19)$$

Similarly, the $R_{\xi}(\theta)$ corresponds to a rotation about ξ , and can be expressed as follows [58]:

$$R_{\xi}(\theta) = \begin{bmatrix} 1 & 0 & 0 \\ 0 & \cos\theta & \sin\theta \\ 0 & -\sin\theta & \cos\theta \end{bmatrix} \quad (20)$$

Finally, the $R_{\zeta'}(\Psi)$ corresponds to a rotation at ζ' , and can be expressed as follows [58]:

$$R_{\zeta'}(\Psi) = \begin{bmatrix} \cos \Psi & \sin \Psi & 0 \\ -\sin \Psi & \cos \Psi & 0 \\ 0 & 0 & 1 \end{bmatrix} \quad (21)$$

The product matrix of equations (19) to (21) yielding the complete three-dimensional Euler rotational matrix $R(\phi, \theta, \Psi)$ can be expressed as follows [58]:

$$R(\phi, \theta, \Psi) = \begin{bmatrix} R_{XX} & R_{XY} & R_{XZ} \\ R_{YX} & R_{YY} & R_{YZ} \\ R_{ZX} & R_{ZY} & R_{ZZ} \end{bmatrix} \quad (22)$$

$$R_{XX} = \cos \Psi \cos \phi - \sin \Psi \sin \phi \cos \theta \quad (23)$$

$$R_{XY} = \cos \Psi \sin \phi + \sin \Psi \cos \phi \cos \theta \quad (24)$$

$$R_{XZ} = \sin \Psi \sin \theta \quad (25)$$

$$R_{YX} = -\sin \Psi \cos \phi - \cos \Psi \sin \phi \cos \theta \quad (26)$$

$$R_{YY} = -\sin \Psi \sin \phi + \cos \Psi \cos \phi \cos \theta \quad (27)$$

$$R_{yz} = \cos \Psi \sin \theta \quad (28)$$

$$R_{zx} = \sin \theta \sin \phi \quad (29)$$

$$R_{zy} = -\sin \theta \cos \phi \quad (30)$$

$$R_{zz} = \cos \theta \quad (31)$$

Thus, with the complete three-dimensional Euler rotational matrix expressed in equations (22) to (31), one can describe the transformation from XYZ to $x'y'z'$ unambiguously. Since the second rotation is about the ξ -axis (intermediate X-axis), it is known as the X-convention [58].

It is possible using the complete three-dimensional Euler rotational matrix and proper vectors, to transform the scattering matrix α into the standard diagonal form, expressed as follows [3]:

$$\alpha = \begin{bmatrix} \alpha_1 & 0 & 0 \\ 0 & \alpha_2 & 0 \\ 0 & 0 & \alpha_3 \end{bmatrix} \quad (32)$$

where α_1, α_2 , and α_3 are known as the principle values of the polarizability

scattering tensor α . If $\alpha_1 = \alpha_2 = \alpha_3$, the scattering tensors are said to be isotropic (spherical), otherwise they are said to be anisotropic (ellipsoidal) [3]. The standard Raman experiment for the 90° -configuration, where the molecule is located at the origin of the coordinate system, the exciting light beam comes in along the X-axis, is scattered, and detected along the Y-axis of the laboratory coordinate system is illustrated in Fig. 18 [3] and Fig. 19 [3].

A basic parameter, implicit in all Raman spectra, is the depolarization ratio, ρ , which can be expressed as follows [3]:

$$\rho = \frac{I_{\perp}}{I_{\parallel}} \quad (33)$$

where ρ , $I_{\perp}$, and $I_{\parallel}$ denote the depolarization ratio, the observed Raman scattered intensity in the perpendicular and parallel conditions, respectively [26]. There are two experimental configurations which can be used to measure the depolarization ratio. In the first case, Fig. 18 [3] illustrates the measurement of the depolarization ratio for circularly polarized light ρ_c ; in the second case, Fig. 19 [3] illustrates the measurement of the depolarization ratio for linearly polarized light, ρ_l . The convention for measuring ρ_c , illustrated in Fig. 18., employs a polarizer placed between the exciting laser beam travelling along the X-axis on the sample located at the origin. The polarizer is adjusted to let only the Z-polarized and then Y-polarized exciting light. The Raman scattered intensity for each is collected and measured and can be expressed as follows [3]:

Fig. 18. The depolarization ratio of a system of randomly oriented scattering particles for naturally polarized light, ρ_n [3].

Fig. 19. The depolarization ratio of a system of randomly oriented scattering particles for linearly polarized light, ρ_1 [3].

$$\rho_n^0 = \frac{I_{\perp}^0}{I_{\parallel}^0} = \frac{I_{YZ}^0 + I_{YX}^0}{I_{ZZ}^0 + I_{ZX}^0} = \frac{\langle \alpha_{YZ}^2 \rangle + \langle \alpha_{YX}^2 \rangle}{\langle \alpha_{ZZ}^2 \rangle + \langle \alpha_{ZX}^2 \rangle} \quad (34)$$

where ρ_n^0 , $I_{\perp}^0$, $I_{\parallel}^0$, $I_{\sigma\sigma}$, and $\langle \alpha_{\sigma\sigma}^2 \rangle$ denote isotropic depolarization ratio for naturally (circularly) polarized light, the observed isotropic Raman scattered intensity on perpendicular excitation, the observed isotropic Raman scattered intensity on parallel excitation; the isotropic observed intensity on excitation in the ρ -polarization (along the X-axis) and then scattered in the σ -polarization (along the Y-axis); and the mean square average of the $\alpha_{\sigma\sigma}$ scattering tensor element averaged over the complete three-dimensional Euler rotational matrix $R(\phi, \theta, \Psi)$, respectively. The stipulation isotropic, originates from the fact that the randomly oriented scattering particles have an isotropic density distribution function of the concentration of ground state molecules, n_1^G , that can be expressed as follows [3, 31, 57]:

$$n_1^G = \frac{N}{4\pi} \quad (35)$$

where N denotes the total number of molecules with the scattering volume.

In contrast, Fig. 19. [3] one sees the convention for measuring, $\rho_{\parallel}$. First, a polarizer is placed between the exciting laser beam travelling along the X-axis and the sample of the origin, such that only the Z-polarized component of the exciting light beam interacts with the sample and scatters. Then a second

polarizer is placed between the scattered light travelling along the Y-axis and the detector. This second polarizer is oriented first to let the parallel (Z-polarized) and then the perpendicular (X-polarized) components of the scattered light to reach the detector. Thus, the isotropic depolarization ratio can be expressed as follows [3]:

$$\rho_I^0 = \frac{I_{\perp}^0}{I_{\parallel}^0} = \frac{I_{ZX}^0}{I_{ZZ}^0} = \frac{\langle \alpha_{ZX}^2 \rangle}{\langle \alpha_{ZZ}^2 \rangle} \quad (36)$$

To evaluate either expression for the isotropic depolarization ratios for naturally and linearly polarized light respectively one must evaluate the values of the Raman intensities expressed as follows [3, 57]:

$$I_{\rho\sigma} = \frac{16\pi^4(\nu - \nu_0)^4}{c^4} I_0 \langle n_1^G \alpha_{\rho\sigma}^2 \rangle \quad (37)$$

To evaluate the $\langle \alpha_{\rho\sigma}^2 \rangle$ terms, one needs the so called similarity transformation $\alpha' = R(\phi, \theta, \Psi) \alpha R^{-1}(\phi, \theta, \Psi)$ where α' is transformed to the $x'y'z'$ coordinates, and $R^{-1}(\phi, \theta, \Psi)$ is the transpose of $R(\phi, \theta, \Psi)$. This can be expressed as follows:

$$R(\phi, \theta, \Psi) \alpha R^{-1}(\phi, \theta, \Psi) = \begin{bmatrix} (R\alpha R^{-1})_{XX} & (R\alpha R^{-1})_{XY} & (R\alpha R^{-1})_{XZ} \\ (R\alpha R^{-1})_{YX} & (R\alpha R^{-1})_{YY} & (R\alpha R^{-1})_{YZ} \\ (R\alpha R^{-1})_{ZX} & (R\alpha R^{-1})_{ZY} & (R\alpha R^{-1})_{ZZ} \end{bmatrix} \quad (38)$$

$$(R\alpha R^{-1})_{XX} = \alpha_1 R_{XX}^2 + \alpha_2 R_{XY}^2 + \alpha_3 R_{XZ}^2 \quad (39)$$

$$(R\alpha R^{-1})_{XY} = \alpha_1 R_{YX} R_{XX} + \alpha_2 R_{YY} R_{XY} + \alpha_3 R_{YZ} R_{XZ} \quad (40)$$

$$(R\alpha R^{-1})_{YZ} = \alpha_1 R_{ZX} R_{YX} + \alpha_2 R_{ZY} R_{XY} + \alpha_3 R_{ZZ} R_{XZ} \quad (41)$$

$$(R\alpha R^{-1})_{YX} = \alpha_1 R_{XX} R_{YX} + \alpha_2 R_{XY} R_{YY} + \alpha_3 R_{XZ} R_{YZ} \quad (42)$$

$$(R\alpha R^{-1})_{YY} = \alpha_1 R_{YX}^2 + \alpha_2 R_{YY}^2 + \alpha_3 R_{YZ}^2 \quad (43)$$

$$(R\alpha R^{-1})_{YZ} = \alpha_1 R_{ZX} R_{YX} + \alpha_2 R_{ZY} R_{YY} + \alpha_3 R_{ZZ} R_{YZ} \quad (44)$$

$$(R\alpha R^{-1})_{ZX} = \alpha_1 R_{XX} R_{ZX} + \alpha_2 R_{XY} R_{ZY} + \alpha_3 R_{XZ} R_{ZZ} \quad (45)$$

$$(R\alpha R^{-1})_{ZY} = \alpha_1 R_{YX} R_{ZX} + \alpha_2 R_{YY} R_{ZY} + \alpha_3 R_{YZ} R_{ZZ} \quad (46)$$

$$(R\alpha R^{-1})_{zz} = \alpha_1 R_{zx}^2 + \alpha_2 R_{zy}^2 + \alpha_3 R_{zz}^2 \quad (47)$$

Now, to evaluate $\langle \alpha_{\rho\sigma}^2 \rangle$ one must use the mean value equation expressed as follows:

$$\langle \alpha_{\rho\sigma}^2 \rangle = \frac{\int_0^{2\pi} \int_0^{2\pi} \int_0^\pi (R\alpha R^{-1})_{\rho\sigma}^2 \sin\theta d\theta d\phi d\Psi}{\int_0^{2\pi} \int_0^{2\pi} \int_0^\pi \sin\theta d\theta d\phi d\Psi} \quad (48)$$

It can be shown that the integration over the Euler angles yields the following expression [3]:

$$\langle \alpha_{\sigma\sigma}^2 \rangle = \frac{1}{15} \left(3 \sum_{i=1}^3 \alpha_i^2 + 2 \sum_{i<j} \alpha_i \alpha_j \right) \quad (49)$$

and

$$\langle \alpha_{\rho\sigma}^2 \rangle = \frac{1}{15} \left(\sum_{i=1}^3 \alpha_i^2 - \sum_{i<j} \alpha_i \alpha_j \right) \quad (50)$$

Thus, for the case of the isotropic Raman depolarization ratio for naturally (circularly) polarized light, ρ_n^0 , one obtains the following expressions [3]:

$$I_1^0 = B \frac{N}{4\pi} I_0 \left(4 \sum_{i=1}^3 \alpha_i^2 + \sum_{i<j} \alpha_i \alpha_j \right) = B \frac{N}{4\pi} I_0 \frac{45\alpha^2 + 7\beta^2}{3} \quad (51)$$

and

$$I_1^0 = B \frac{N}{4\pi} I_0 \left(2 \sum_{i=1}^3 \alpha_i^2 - 2 \sum_{i < j} \alpha_i \alpha_j \right) = B \frac{N}{4\pi} I_0 2 \beta^2 \quad (52)$$

where;

$$\alpha = \frac{1}{3}(\alpha_1 + \alpha_2 + \alpha_3) \quad (53)$$

and

$$\beta^2 = \frac{1}{2}[(\alpha_1 - \alpha_2)^2 + (\alpha_2 - \alpha_3)^2 + (\alpha_1 - \alpha_3)^2] \quad (54)$$

and

$$B = \frac{16 \pi^4 (v - v_0)^4}{c^4} \frac{1}{15} \quad (55)$$

where α , and β denote the isotropic (spherical) and anisotropic (non-spherical) components of the polarizability ellipsoid, respectively [3]. Therefore, the isotropic depolarization ratio for naturally polarized light, ρ_n^0 , can be expressed as follows [3]:

$$\rho_n^0 = \frac{I_{\perp}^0}{I_{\parallel}^0} = \frac{6\beta^2}{45\alpha^2 + 7\beta^2} \quad (56)$$

Similarly, for the case of the isotropic Raman depolarization ratio for linearly polarized light, ρ_l^0 , one obtains the following expressions [3]:

$$I_{\parallel}^0 = B \frac{N}{4\pi} I_0 \left(3 \sum_{i=1}^3 \alpha_i^2 + 2 \sum_{i<j} \alpha_i \alpha_j \right) = B \frac{N}{4\pi} I_0 \frac{45\alpha^2 + 4\beta^2}{3} \quad (57)$$

and

$$I_{\perp}^0 = B \frac{N}{4\pi} I_0 \left(\sum_{i=1}^3 \alpha_i^2 - \sum_{i<j} \alpha_i \alpha_j \right) = B \frac{N}{4\pi} I_0 \beta^2 \quad (58)$$

where

$$\rho_l^0 = \frac{I_{\perp}^0}{I_{\parallel}^0} = \frac{3\beta^2}{45\alpha^2 + 4\beta^2} \quad (59)$$

On inspection of the expressions for ρ_n^0 , and ρ_l^0 , one can see that for the Raman scattering tensors of spectroscopic transitions, it is important to have the values of α and β . If these tensors, or their derivatives, belong to the totally symmetric representations of the cubic point group, the spherical part α , is non-zero and the anisotropic (ellipsoidal) part β is zero. Therefore, the depolarization ratios ρ_n^0 and

ρ_i^0 are both zero.

Now, for Raman tensors or their derivatives, belonging to the totally symmetric representations of all other point groups, both the isotropic (spherical) α and anisotropic (ellipsoidal) β terms are non-zero. Therefore, depending on the isotropy or anisotropy of the Raman transitions, the depolarization ratios vary between $0 < \rho_n^0 < 6/7$ and $0 < \rho_i^0 < 3/4$ for the natural and linear polarization respectively. Thus, a RR peak with depolarization ratio of zero, is said to be completely polarized, whereas a RR peak is said to be completely depolarized if it has a depolarization ratio of 6/7 or 3/4 for naturally or linearly polarized light, respectively [3].

In addition, one must consider non-linear the consequences of the pump beam E_i perturbing the previously isotropic density distribution function of the concentration of ground state molecules, n_i^G , (see equation (35)). An anisotropic density distribution function for the concentration of ground state molecules is created in the parallel excitation (Z-polarization) pump-probe configuration, and can be expressed as follows [31, 59] (see Appendix):

$$n_i^G(\theta, f) = \frac{N}{4\pi} [1 - f \cos^2\theta] \quad (60)$$

Similarly, in the perpendicular excitation (Y-polarization) pump-probe configuration, the anisotropic density distribution function of the concentration of ground state molecules, can be expressed as follows (see Appendix):

$$n_1^G(\phi, \theta, f) = \frac{N}{4\pi} [1 - f \sin^2 \theta \sin^2 \phi] \quad (61)$$

These induced anisotropies, will manifest themselves in changes in the intensities of RR peaks that are created by the probe beam E_2 . The total (isotropic I^0 , and anisotropic I^1 intensities) depolarization ratios for naturally and linearly polarized light can be expressed as follows [31] (see Appendix):

$$\rho_n = \frac{I_{\perp}^0 - f I_{\perp}^1}{I_{\parallel}^0 - f I_{\parallel}^1} = \frac{(I_{YZ}^0 + I_{YX}^0) - f(I_{YZ}^1 + I_{YX}^1)}{(I_{ZZ}^0 + I_{ZX}^0) - f(I_{ZZ}^1 + I_{ZX}^1)} \quad (62)$$

and thus,

$$\rho_n = \frac{(\langle \alpha_{YZ}^2 \rangle + \langle \alpha_{ZX}^2 \rangle) - f(\langle \alpha_{YZ}^2 \sin^2 \theta \sin^2 \phi \rangle + \langle \alpha_{YX}^2 \sin^2 \theta \sin^2 \phi \rangle)}{(\langle \alpha_{ZZ}^2 \rangle + \langle \alpha_{ZX}^2 \rangle) - f(\langle \alpha_{ZZ}^2 \cos^2 \theta \rangle + \langle \alpha_{ZX}^2 \cos^2 \theta \rangle)} \quad (63)$$

Similarly,

$$\rho_I = \frac{I_{\perp}^0 - f I_{\perp}^1}{I_{\parallel}^0 - f I_{\parallel}^1} = \frac{I_{ZX}^0 - f I_{ZX}^1}{I_{ZZ}^0 - f I_{ZZ}^1} \quad (64)$$

$$\rho_I = \frac{\langle \alpha_{ZX}^2 \rangle - f \langle \alpha_{ZX}^2 \cos^2 \theta \rangle}{\langle \alpha_{ZX}^2 \rangle - f \langle \alpha_{ZZ}^2 \cos^2 \theta \rangle} \quad (65)$$

Thus, to evaluate the expressions for ρ_n and ρ_I one must calculate the various $\langle \alpha_{\rho\sigma}^2 \sin^2 \theta \cos^2 \phi \rangle$ and $\langle \alpha_{\rho\sigma}^2 \cos^2 \theta \rangle$ expressions as follows (see Appendix),

$$\langle \alpha_{\rho\sigma}^2 \cos^2 \theta \rangle = \frac{\int_0^{2\pi} \int_0^{2\pi} \int_0^\pi ((R\alpha R^{-1})_{\rho\sigma}^2 \cos^2 \theta) \sin \theta d\theta d\phi d\Psi}{\int_0^{2\pi} \int_0^{2\pi} \int_0^\pi \sin \theta d\theta d\psi d\Psi} \quad (66)$$

and

$$\langle \alpha_{\rho\sigma}^2 \sin^2 \theta \sin^2 \phi \rangle = \frac{\int_0^{2\pi} \int_0^{2\pi} \int_0^\pi ((R\alpha R^{-1})_{\rho\sigma}^2 \sin^2 \theta \sin^2 \phi) \sin \theta d\theta d\phi d\Psi}{\int_0^{2\pi} \int_0^{2\pi} \int_0^\pi \sin \theta d\theta d\phi d\Psi} \quad (67)$$

On evaluation of these terms, the anisotropic correction factors for the parallel excitation (Z-polarization) pump-probe configuration can be expressed as follows [31] (see Appendix),

$$I_{ZZ}^1 = \frac{B}{7} \frac{N}{4\pi} I_0 \left[(3\alpha_1^2 + 3\alpha_2^2 + 15\alpha_3^2) + (2\alpha_1\alpha_2 + 6\alpha_2\alpha_3 + 6\alpha_1\alpha_3) \right] \quad (68)$$

and

$$I_{zx}^1 = \frac{B}{7} \frac{N}{4\pi} I_0 [(2\alpha_1^2 + 2\alpha_2^2 + 3\alpha_3^2) + (-\alpha_1\alpha_2 - 3\alpha_2\alpha_3 - 3\alpha_1\alpha_3)] \quad (69)$$

Similarly, the anisotropic correction factors for the perpendicular excitation (Y-polarization) pump-probe configuration, derived in this work, can be expressed as follows (see Appendix):

$$I_{yz}^1 = \frac{B}{7} \frac{N}{4\pi} I_0 [(3\alpha_1^2 + 2\alpha_2^2 + 2\alpha_3^2) + (-3\alpha_1\alpha_2 - \alpha_2\alpha_3 - 3\alpha_1\alpha_3)] \quad (70)$$

and

$$I_{yx}^1 = \frac{B}{7} \frac{N}{4\pi} I_0 [(\alpha_1^2 + 3\alpha_2^2 + 3\alpha_3^2) + (-\alpha_1\alpha_2 - 5\alpha_2\alpha_3 - \alpha_1\alpha_3)] \quad (71)$$

Table 3. [31] and Table 4. show the results of the calculated scattering light intensities for transitions governed by different tensors for the case when the pump is on and off. The tensors are presented according to their principal axes of polarizability. By rotation around specific axes, some of these transform into those given in the character table of 32 point groups, and may be used experimentally to assign RR peaks, for example, to different asymmetric vibrations of the D_2 point groups (tensors V-VII) [31].

However, in the analysis of the behaviour of ρ_a and/or ρ_l as a function of

Table 3. The relationship of polarized Raman intensities related to different tensors, excited in the parallel pump-probe configuration [31]

	Tensor ^a	$I_{zz}^2 / (B\alpha^2)$	$I_{zz}^1 / (B\alpha^2)$	$I_{zx}^0 / (B\alpha^2)$	$I_{zx}^1 / (B\alpha^2)$
I	$\begin{bmatrix} \alpha_1 & 0 & 0 \\ 0 & \alpha_2 & 0 \\ 0 & 0 & \alpha_3 \end{bmatrix}$	15	5	0	0
II	$\begin{bmatrix} \alpha_1 & 0 & 0 \\ 0 & \alpha_2 & 0 \\ 0 & 0 & 0 \end{bmatrix}$	8	8/7	1	3/7
III	$\begin{bmatrix} 0 & 0 & 0 \\ 0 & \alpha_2 & 0 \\ 0 & 0 & \alpha_3 \end{bmatrix}$	8	24/7	1	2/7
IV	$\begin{bmatrix} \alpha_1 & 0 & 0 \\ 0 & 0 & 0 \\ 0 & 0 & \alpha_3 \end{bmatrix}$	8	24/7	1	2/7
V	$\begin{bmatrix} \alpha_1 & 0 & 0 \\ 0 & -\alpha_2 & 0 \\ 0 & 0 & 0 \end{bmatrix}$	4	4/7	3	5/7
VI	$\begin{bmatrix} \alpha_1 & 0 & 0 \\ 0 & 0 & 0 \\ 0 & 0 & -\alpha_3 \end{bmatrix}$	4	12/7	3	8/7
VII	$\begin{bmatrix} 0 & 0 & 0 \\ 0 & \alpha_2 & 0 \\ 0 & 0 & -\alpha_3 \end{bmatrix}$	4	12/7	3	8/7

^aFor all tensors $\alpha_1 = \alpha_2 = \alpha_3 = \alpha$.

Table 4. The relationship of polarized Raman intensities related to different tensors, excited in the perpendicular pump-probe configuration.

	Tensor ^a	$I_{yz}^0 / (B\alpha^2)$	$I_{yz}^1 / (B\alpha^2)$	$I_{yx}^0 / (B\alpha^2)$	$I_{yx}^1 / (B\alpha^2)$
I	$\begin{bmatrix} \alpha_1 & 0 & 0 \\ 0 & \alpha_2 & 0 \\ 0 & 0 & \alpha_3 \end{bmatrix}$	0	0	0	0
II	$\begin{bmatrix} \alpha_1 & 0 & 0 \\ 0 & \alpha_2 & 0 \\ 0 & 0 & 0 \end{bmatrix}$	1	2/7	1	3/7
III	$\begin{bmatrix} 0 & 0 & 0 \\ 0 & \alpha_2 & 0 \\ 0 & 0 & \alpha_3 \end{bmatrix}$	1	3/7	1	1/7
IV	$\begin{bmatrix} \alpha_1 & 0 & 0 \\ 0 & 0 & 0 \\ 0 & 0 & \alpha_3 \end{bmatrix}$	1	2/7	1	3/7
V	$\begin{bmatrix} \alpha_1 & 0 & 0 \\ 0 & -\alpha_2 & 0 \\ 0 & 0 & 0 \end{bmatrix}$	3	8/7	3	5/7
VI	$\begin{bmatrix} \alpha_1 & 0 & 0 \\ 0 & 0 & 0 \\ 0 & 0 & -\alpha_3 \end{bmatrix}$	3	8/7	3	5/7
VII	$\begin{bmatrix} 0 & 0 & 0 \\ 0 & \alpha_2 & 0 \\ 0 & 0 & -\alpha_3 \end{bmatrix}$	3	5/7	3	11/7

^aFor all tensors $\alpha_1 = \alpha_2 = \alpha_3 = \alpha$.

pump intensity, I_1 , one must consider one more factor known as the anisotropic polarized excited state absorption spectrum effect [11, 12]. In a mono or two-beam resonance Raman experiment, situations exist where the wavelength of the probe laser beam, λ_{probe} , can be tuned to another absorption band of the molecule, and one must consider the effect the possible presence of an excited state absorption spectrum, with a significantly low transmission, coincident with the wavelength of Raman scattered light. Due to spatial anisotropy which is created in the distribution of the ground state S_0 , there will be spatial anisotropy in the created excited states S_n , and therefore, their excited state spectra are polarized [11, 12]. Thus, as the Raman scattered probe light transverses these regions of low transmission in the excited state absorption spectra, it will be absorbed and therefore, attenuate the observed Raman intensity in a polarized manner [11, 12].

Now, consider the situation for the case when the excited state absorption has a polarization in the YZ-plane. Consider first-order anisotropic effects on the isotropic components (i.e., effects on I^0) for the pump-probe configuration. In the case of naturally polarized light, the subsequent behaviour of ρ_a^0 is described as follows: if pump E_1 and the probe E_2 are placed in the perpendicular excitation (i.e., Y-polarization) configuration, the observed Raman emission $I_1^0 \approx 6\beta^2$, is split such that $I_{YZ}^0 \approx 3\beta^2$ and $I_{YX}^0 \approx 3\beta^2$ (the $B(N/4\pi)I_0$ term divides out). This would result in the $I_{YZ}^0 \approx 3\beta^2$ component being absorbed (attenuated) leaving the $I_{YX}^0 \approx 3\beta^2$ unaffected [11, 12]. In contrast, if E_1 and E_2 are placed in the parallel excitation (i.e. Z-polarization) configuration, the observed Raman

emission $I_1^0 \approx 45\alpha^2 + 7\beta^2$, is split such that $I_{zz}^0 \approx 45\alpha^2 + 4\beta^2$ and $I_{zx}^0 \approx 3\beta^2$. This would result in the $I_{zz}^0 \approx 45\alpha^2 + 4\beta^2$ component being absorbed (attenuated), leaving the $I_{zx}^0 \approx 3\beta^2$ component unaffected. However, it can be seen that I_1^0 would be more attenuated than I_1^0 , which would result in an increase in ρ_a^0 [11, 12]. Thus, in the first-order (i.e. I^0) approximation, one can propose an expression for ρ_n expressed as follows:

$$\rho_n = \frac{[T_{yz}^{ans}(f)(3\beta^2) + (3\beta^2)] - f[I_1^1]}{[T_{zz}^{ans}(f)(45\alpha^2 + 4\beta^2) + (3\beta^2)] - f[I_1^1]} \quad (72)$$

where both the anisotropic polarized excited state transmission attenuation factor excited in the Z-polarization (along the X-axis), and scattered in the Z-polarization (along the Y-axis), $T_{zz}^{ans}(f)$, and that which is excited in the Y-polarization (along the X-axis), and scattered in Z-polarization (along the Y-axis), $T_{yz}^{ans}(f)$, (i.e., in the YZ-plane), can be expressed as follows:

$$T_{\rho\sigma}^{ans}(f) = e^{-\left(\frac{N}{4\pi} f \sigma_E(\rho\sigma)\right)L} \quad (73)$$

Here $\rho\sigma = ZZ$ and YZ , and $\sigma_E(\rho\sigma)$ denotes the prepared polarized absorption cross-section of the created excited state, respectively (i.e., $T_{zz}^{ans}(f)$ and $T_{yz}^{ans}(f)$ are active, whereas $T_{zx}^{ans}(f)$ and $T_{yx}^{ans}(f)$ are not). On inspection of equations (72) and (73), one can see that in the situation where $\sigma_E(\rho\sigma)/\sigma_A \gg 1$, the transmission of the polarized excited state absorption spectrum is much lower than

that of the ground state absorption spectrum, σ_A (i.e., $\sigma_E(\rho\sigma) \gg 0$ and/or $f \gg 1$). This results in the behaviour $\rho_n^0 \rightarrow 1$, as $T_{xx}^{ans}(f) \rightarrow 0$. At the other limit, in the case where $\sigma_E(\rho\sigma)/\sigma_A \approx 0$, the transmission of the polarized excited state absorption spectrum is much higher than that of the ground state absorption spectrum (i.e. $\sigma_E(\rho\sigma) \approx 0$), this results in the behaviour $\rho_n^0 \rightarrow 6/7$, as $T_{xx}^{ans}(f) \rightarrow 1$.

Similarly, consider the opposite situation where the polarization of the excited state absorption spectrum is in the YX-plane (i.e., only the $T_{zx}^{ans}(f)$ and $T_{yx}^{ans}(f)$ terms are active). In the first order (i.e. I^0) approximation, ρ_n^0 would now be decreased, and ρ_n can be expressed as follows:

$$\rho_n = \frac{[(3\beta^2) + T_{yx}^{ans}(f)(3\beta^2)] - f[I_1^1]}{[(45\alpha^2 + 4\beta^2) + T_{zx}^{ans}(f)(3\beta^2)] - f[I_1^1]} \quad (74)$$

where the decrease of $\rho_n^0 \rightarrow 6/7$ to that of $\rho_n^0 \rightarrow 3/4$, occurs for the case of $\sigma_E(\rho\sigma) \gg 0$ and/or $f \gg 1$, for polarized vibrational modes.

On inspection of equations (72) and (74), one can see that only factors (ii) and (iii) are explicitly expressed in the model. Qualitatively, factor (i), ground state depletion will reduce the population numbers of the ground state and thus reduce the intensity of the ground state RR lines. Future studies should incorporate factor (i) explicitly into the above model, via the use of Boltzman equations to express the effect of the distribution of ground and excited state population numbers on ρ_n .

Chapter 3: Experimental

3.1 Sample Handling

The meso-tetrakis(N-methyl-4-pyridyl)porphine, $H_2TMpyP(4)$, was purchased from Porphyrin Products Inc. (Logan, Utah, U.S.A.), as the tosylate salt, and used without further purification.

The phosphate buffer solution (0.01M phosphate; 0.3M NaCl; $pH=7.0\pm0.1$) was used as the solvent for the $H_2TMpyP(4)$ solutions. The phosphate buffer was made up from analytical (Aldrich) grade reagents (KH_2PO_4 , K_2HPO_4 , and NaCl) by dissolving them in ultra-pure deionized water (18 M Ω). The pH value was measured via a Fischer Accumet pH Meter (Model 825 MP).

An $H_2TMpyP(4)$, (M.W. 1363.63g.mol⁻¹) stock solution of concentration 1.47×10^{-3} M was prepared by dissolving 20 mg of the compound, in a 10mL solution, made up to the mark with the $pH=7$ phosphate buffer solution.

The phosphate buffer solution, and $H_2TMpyP(4)$ stock solution were stored in the dark at 5°C. All solutions used in the UV-visible absorption, monobeam transmission, resonance Raman experiments were made up fresh 24 hours before the experiments sealed in $l=1$ cm quartz optical sample cells performed at room temperature (20°C).

3.2 UV-visible Absorption and IR spectra

The UV-visible absorption spectra were run on a Lambda 4B UV-vis spectrophotometer using $l=1\text{cm}$ quartz sample cells. The IR spectra were run on a Perkin-Elmer 1600 series FTIR using a solid KBr pellet.

3.3 Lasers and Optics

The tunable UV-visible pulsed laser source was an XeCl excimer laser (Lumonics Model TE320-2 with a wavelength of 308nm, repetition rate 25 Hz, and a pulse duration $\tau_{\text{pulse}}=5\text{ns}$) pumping a tunable dye laser (Lumonics Model hyperDYE-300). The wavelength ranges of laser excitation were 394-445nm (using DPS and Stiben 3), and 460-600nm (using Coumarin 2, Coumarin 102, Coumarin 152, and Rhodamin 6G) corresponding to the blue Soret B band region, and the green-yellow Q band region. As well, a continuous wave (CW) Ar^+ laser (CR-4 SUPERGRAPHITE, COHERENT RADIATION) was used as an excitation pump source at the 476.5nm (20986cm^{-1}) and 514.5nm (19436cm^{-1}) laser lines, corresponding to the Soret B band and the Q_y band, of $\text{H}_2\text{TMpyP}(4)$ respectively. On exciting the laser cavity, the laser beam was passed through a 90° (right angle) constant deviation Pellin-Broca dispersing prism to separate the super radiance from the lasing region of the emitted beam. Mirrors, neutral density filters, Glan-Thompson polarizers, lenses and irises, on adjustable mounts, arranged on a series of fixed optical rails, were used to guide the laser beam through the sample and to the double slit monochromator (Jobin-Yvon) detector.

3.4 Detection Systems

For the monobeam transmission experiments, the laser beam, after tranversing the quartz sample cell, was collected at 180° , focussed through an iris onto an optical power meter (Optikon) set in the power mode.

For the resonance Raman experiments, after the laser beam transversed the quartz sample, scattered light was collected at 90° . The scattered light passed first through a scrambler (producing the circularly polarized light) mounted in contact with the quartz sample cell, and was then collected via a large spherical lense of focal length $f=10$ cm, which focussed the scattered light into the entrance slit of the double slit monochrometer DSM (Jobin-Yvon). The signal was detected by a cooled RCA 31034 photomultiplier with a nanosecond response and then amplified by a fast preamplifier (Stanford Research Associates Model SR445). The amplified signal was sent to a boxcar operating with a 3ns gate (Standard Research Associates Models SR 250, 235, 275, 245). The scanning DSM, as well as the gate of the boxcar, were either controlled by a Raven PC-XT computer or recorded by a recorder. The wavenumber, cm^{-1} , of the scanning DSM, were calibrated via the 435.8 nm (22946 cm^{-1}) line of an Hg lamp.

3.5 Monobeam Transmission and Resonance Raman Experiments

Fig. 20 illustrates the monobeam transmission experimental set up for the $\text{H}_2\text{TMpyP}(4)$ monomer studies. Neutral density filters (Kodak-Eastman Inc.) were used to vary the excitation pump (intensity) power $I_i(\text{mW})$

with a range of about 0-5 mW. A spherical lens with a focal length of, $f=10$ cm, was used to focus the pump beam into the $l=1$ cm quartz sample cell. The pump beam, on exiting the quartz sample cell, was focussed onto the detector head of the power meter (Optikon) and a reading of the power was obtained in milliwatts, mW. The transmission, $T=I/I_0$, of the sample was measured by recording the transmitted power with the sample in, I , and out, I_0 (i.e., I_0), of the sample holder. Numerous sets of T vs I_0 monobeam transmission curves were recorded over wavelengths of 394-445nm in the Soret B band region, and 460-600nm in the Q band region for $H_2TMpyP(4)$ in a pH=7 phosphate buffer solution. From the monobeam transmission curves [33], the value of the plateau of the curve at very low power, T_0 , and the value of the plateau of the curve at very high power

, T_{eff} , the excited state absorption spectra for $S_m \leftarrow S_3(B)$ in the Soret B band region, and for $S_k \leftarrow S_1(Q_x)$ in the Q band region were calculated [11, 12, 33]. As well, a monobeam transmission effective population saturation (EPS) study was carried out for $H_2TMpyP(4)$ in a pH=7 phosphate buffer at an excitation wavelength of 422 nm.

Fig. 21. illustrates the experimental setup for the resonance Raman experiments. A Glan-Thompson polarizer was positioned between the exciting pump beam and the sample and was used to change the polarization of the exciting pump beam from the parallel to the perpendicular excitation configuration for the measurement of the Raman depolarization ratio for naturally (circularly)

Fig. 20. Monobeam transmission experimental setup for $H_2TMpyP(4)$ monomer studies. Pr = Pellin-Broca prism; Ir = iris; NF = neutral density filter; L = lens; S = sample cell; PM = power meter.

Fig. 21. Resonance Raman experimental setup for H₂TMpyP(4) monomer studies. Pr = Pellin Broca prism; Ir = iris; NF = neutral density filter; P = Glan-Thompson polarizer; L₁ = normal size, $f = 10$ cm, spherical lens; S = sample cell; PM = power meter; Sc = scrambler; L₂ = large size, $f = 10$ cm, spherical lens; DSM = double slit monochromator, detection system.

polarized light, ρ_n . Resonance Raman spectra of $H_2TMpyP(4)$ in a pH=7 phosphate buffer solution were recorded over the typical Raman vibrational shift range of about 900-1700 cm^{-1} , excited at 422 and 460nm using the pulsed XeCl excimer driven tunable dye laser; and excited at 476.5 and 514.5nm using the CW Ar^+ laser. The depolarization ratio for naturally polarized light, ρ_n of RR peaks at $\nu_2(C_\beta-C_\beta)$, $\Delta\bar{\nu}=1558\text{ cm}^{-1}$ and $\delta(pyr)$, $\Delta\bar{\nu}=1643\text{ cm}^{-1}$ excited at 422, 476.5, and 514.5 nm were calculated and scattering tensors were proposed [11].

The relative Raman cross-sections in the perpendicular $\sigma_R^\perp$ and parallel $\sigma_R^\parallel$ directions for naturally polarized light were measured for $H_2TMpyP(4)$, in a pH=7 phosphate buffer solution. From these values for $\sigma_R^\perp$ and $\sigma_R^\parallel$, the behaviour of the depolarization ratio for naturally polarized light ρ_n was plotted as a function of pump intensity, $I_i(\text{mW})$, over a range of 0-5 mW, for the RR peaks $\nu_2(C_\beta-C_\beta)$, $\Delta\bar{\nu}=1558\text{ cm}^{-1}$, and $\delta(pyr)$ $\Delta\bar{\nu}=1643\text{ cm}^{-1}$ of the $H_2TMpyP(4)$ monomer.

In both the monobeam transmission and the resonance Raman experiments, UV-vis absorption spectra were run before and after each experiment, to ensure that the spectra were the same and no photooxidation products were formed. As well, all RR spectra were normalized to the Raman band water $\Delta\bar{\nu}(H_2O)=3450\text{ cm}^{-1}$. Triplet-triplet pump-probe resonance Raman experiments were attempted with the pump beam at 422 nm and the probe beam at 460 nm. The resonance Raman spectra, within experimental error, were identical in the presence and absence of the 460 nm pump, and thus no triplet state, T_1 could be detected.

Chapter 4: Results and Discussion

4.1 Ground and Excited State Absorption Spectra

For the porphyrin concentrations (1.47×10^{-4} M to 1.47×10^{-5} M, for the monobeam transmission and the resonance Raman studies) used in these experiments, it is known that water-soluble porphyrins with negative charges at the periphery, e.g. meso-tetrakis(4-carboxylphenyl)porphine, $H_2TCPP(4)$, have a strong tendency to form aggregates, whereas water-soluble porphyrins with positive charges at the periphery, e.g. $H_2TMpyP(4)$, show no tendency for aggregation under the same conditions [53]. Fig. 22 [11] illustrates the structure of the monomer $H_2TMpyP(4)$, ground state Soret B band, $S_3(B) \leftarrow S_0$, (1), and the excited state, $S_m \leftarrow S_3(B)$, absorption spectra of meso-tetrakis(N-methyl-4-pyridyl)porphine, $H_2TMpyP(4)$, 1.47×10^{-5} M, in a 1 cm quartz sample cell, in a pH=7 phosphate buffer solution. Fig. 23., illustrates the ground state Q band, $S_n(Q) \leftarrow S_0$ (1), and excited state $S_k \leftarrow S_1(Q_n)$ (2), absorption spectra of meso-tetrakis(N-methyl-4-pyridyl)porphine, $H_2TMpyP(4)$, 1.47×10^{-4} M, in a 1 cm quartz sample cell, in a pH=7 phosphate buffer solution. Both Fig. 22 and 23, were obtained via monobeam transmission experiments [11, 33] using a spherical lens, of focal length $f=10$ cm (see Fig. 20 of section 3.5). The appearance of the ground state $S_3(B) \leftarrow S_0$ (1) absorption spectrum is quite different compared to that of the excited state $S_m \leftarrow S_3(B)$ (2) absorption spectrum of Fig. 22 [11]. The absorbance, A , and hence the absorption cross-section of the ground state S_0 ,

Fig. 22. Ground state (1), and excited state (2) absorption spectra of meso-tetrakis(N-methyl-4-pyridyl)porphine, $H_2TMpyP(4)$, 1.47×10^{-5} M, in a 1 cm cell, in a pH=7 phosphate buffer solution (see text) in the Soret B band region [11].

Fig. 23. Ground state (1), and excited state (2) absorption spectra of meso-tetrakis(N-methyl-4-pyridyl)porphine, $H_2TMpyP(4)$, 1.47×10^{-4} M in a 1 cm cell, in a pH-7 phosphate buffer solution (see text), in the Q band region.

Fig. 24. Jablonski energy level diagram for meso-tetrakis(N-methyl-4-pyridyl)porphine, $\text{H}_2\text{TMpyP}(4)$ (adapted from [51]).

, $\sigma_A(S_0)$, of the ground state (1) absorption curve is much higher than that of the excited state $S_3(B)$, $\sigma_E(S_3(B))$, over the range of 394-440 nm. At wavelengths greater than 440 nm, the situation changes where $\sigma_E(S_3(B)) > \sigma_A(S_0)$. The smooth excited state (2) absorption curve increases to 445 nm, later probably flattening out and decreasing in absorbance, A [11].

In Fig. 23. the appearance of the excited state $S_k \leftarrow S_1(Q_x)$ (2), absorption spectrum is very similar to that of the ground state $S_n(Q) \leftarrow S_0$ (1) absorption spectrum. On the other hand, the absorbance, A and hence, the absorption cross-section of the excited state (2) $S_1(Q_x(0,0))$, $\sigma_E(S_1(Q_x))$, absorption curve is much larger than that of the ground state S_0 , $\sigma_A(S_0)$. The two prominent absorption peaks at 505 nm and 578 nm in the excited state $S_k \leftarrow S_1(Q_x)$ (2) absorption spectrum, are blue shifted, relative to the absorption peaks at 518 nm and 584 nm for the $S_2(Q_y(1,0)) \leftarrow S_0$ and $S_1(Q_x(1,0)) \leftarrow S_0$ transitions, respectively of the ground state $S_n(Q) \leftarrow S_0$ (1) absorption spectrum.

Fig. 24. illustrates the Jablonski energy level diagram for meso-tetrakis (N-methyl-4-pyridyl)porphine, $H_2HMPyP(4)$. The first excited singlet $S_1(Q_x(0,0))$, and the lowest-lying triplet, T_1 , state absorb at wavelengths of 640 nm and 860 nm; and have fluorescence $\tau_n(S_1(Q_x(0,0))) = 5.3$ ns and phosphorescence $\tau_{ph}(T_1) = 175$ ns lifetimes, respectively [1, 51]. The second excited singlet Q band $S_2(Q_y)$, and third excited singlet Soret B band $S_3(B)$ states have lifetimes in the picosecond (ps) regime, with the lifetime of the Soret B band being the shorter [1, 51].

4.2 Monobeam Transmission, Effective Population Saturation, EPS, Study of the H₂TMpyP(4) monomer

Fig. 25. illustrates the monobeam transmission effective population saturation, EPS, study of the H₂TMpyP(4) monomer, excited at 422 nm. The H₂TMpyP(4) solution had a concentration of 1.47×10^{-5} M, in a pH=7 phosphate buffer solution.

This transmission curve shows the transmission rising steeply between 0-1 mW, and then at higher pump power, the transmission curve flattens out, by $I_i=5$ mW, to a limiting value of $T_{eff}=0.192$. One can estimate the maximum lifetime of $\tau(S_3(B))$ from the H₂TMpyP(4) monomer transmission curve using the equation [33],

$$\tau_E \approx \frac{N_{molecule}}{N_{photons}} \tau_{pulse}$$

where τ_E , $N_{molecule}$, $N_{photons}$, and τ_{pulse} denote the maximum value of the excited state saturated at I_{eff} , the number of molecules in the irradiated volume of the exciting laser beam, the number of photons delivered per pulse into the irradiated volume, and the duration of the exciting laser pulse, respectively. The pulse duration of the excimer pump tunable dye laser is $\tau_{pulse}=5$ ns.

First, one must calculate the energy of the 5 ns pulse at the value of $I_{eff}=5$ mW. This is done by dividing the average power (intensity) at effective

Fig. 25. Monobeam transmission, effective population saturation, EPS, study of the $H_2TMpyP(4)$ monomer, 1.4×10^{-5} M, excited at 422 nm (23697 cm^{-1}); in a pH=7 phosphate buffer solution (see text), in a 1 cm cell.

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population saturation, I_{eff} , by the repetition rate of the laser as follows:

$$\frac{(5 \text{ mW})}{(25 \text{ Hz})} = 2 \times 10^{-1} \text{ J} \cdot \text{pulse}^{-1}$$

Thus, the energy of $I_{\text{eff}} = 5 \text{ mW}$ of the 5 ns pulse is $2 \times 10^{-4} \text{ J pulse}^{-1}$.

Next, one must calculate the energy per photon at the excitation wavelength of 422 nm. This is done as follows:

$$\frac{hc}{\lambda_l} = \frac{(6.63 \times 10^{-34} \text{ J} \cdot \text{s})(3 \times 10^8 \text{ m} \cdot \text{s}^{-1})}{(4.22 \times 10^{-7} \text{ m})} = 4.71 \times 10^{-19} \text{ J} \cdot \text{photon}^{-1}$$

Thus, the calculation of the number of photons supplied at 422 nm per 5 ns pulse, N_{photon} , is as follows:

$$N_{\text{photon}} = \frac{(2 \times 10^{-4} \text{ J} \cdot \text{pulse}^{-1})}{(4.71 \times 10^{-19} \text{ J} \cdot \text{photon}^{-1})} = 4.25 \times 10^{14} \text{ photon} \cdot \text{pulse}^{-1}$$

Next, one must calculate the irradiated volume V_{irr} . Using the spherical lens to focus the exciting laser beam through the quartz sample cell described in Chapter 3, the irradiated volume is approximately that of a cylinder of radius r and length L ,

$$V_{\text{irr}} = \pi r^2 L$$

Typical radius of irradiation values are $r = (75-150) \mu\text{m}$ [32, 33]. In the case of a sample cell of length $L = 1 \text{ cm}$, one obtains values of the range of the irradiated volume as,

$$V_{irr} = (1.8 - 7.1) \times 10^{-7} \text{ mL}$$

The next parameter one must calculate is the number of molecules that are present inside the irradiated volume, $N_{molecule}$. This can be expressed as,

$$N_{molecule} = N_A V_{irr} C$$

where N_A and C denote Avogadro's number, and the concentration of the analyte molecule in solution. For the concentration of $H_2TMpyP(4)$, $1.47 \times 10^{-5} \text{ M}$, one obtains the value of the range of the number of analyte molecules in the irradiated volume as,

$$N_{molecule} = (1.6 - 6.3) \times 10^{12} \text{ molecule}$$

Thus, for a pulse of duration, $\tau_{pulse} = 5 \text{ ns}$, at a wavelength of 422 nm, with an effective saturation intensity of 5 mW, supplying $N_{photon} = 4.25 \times 10^{24} \text{ photon pulse}^{-1}$ exciting $N_{molecules} = (1.6 - 6.3) \times 10^{12}$ molecules, the value of the maximum lifetime of the saturated excited state $S_3(B)$ one obtains is,

$$\tau_E = \frac{N_{pulse}}{N_{photon}} \tau_{pulse} = \tau(S_3(B)) = (19 - 74) \text{ ps}$$

Within the accuracy of the approximation made in these calculations, it can be stated that the lifetime of the $S_3(B)$ state in the $H_2TMpyP(4)$ monomer is in the picosecond regime.

4.3 Ground State IR and Vibration Resonance Raman Spectra

Fig. 26. and Fig. 27. illustrate the IR spectra of meso-tetrakis(N-methyl-4-pyridyl)porphine, $H_2TMpyP(4)$ in a KBr pellet, over a range of 4000-650 cm^{-1} , and 900-1700 cm^{-1} , respectively. The IR peaks for $H_2TMpyP(4)$ are difficult to assign unambiguously, [8], and there are several prominent IR peaks in the 900-1700 cm^{-1} region of Fig. 27. The IR peaks at 1010, 1188, and 1640 cm^{-1} are conventionally assigned to the stretching ν and bending δ modes of the N-methyl-4-pyridyl groups of E_u symmetry, and the in plane vibrational modes of the porphyrin are not observed [8].

Fig. 28. and Fig. 29. illustrate the vibrational resonance Raman spectra of the ground state of 1.47×10^{-4} M meso-tetrakis(N-methyl-4-pyridyl)porphine, $H_2TMpyP(4)$, in a 1 cm cell in a pH=7 phosphate buffer solution, excited at 422 and 460 nm, respectively. Table. 5 lists the assignment of the resonance Raman spectrum of meso-tetrakis(N-methyl-4-pyridyl)porphine monomer, in a pH=7 phosphate buffer excited at 422 nm. On inspection of Fig. 28. and Fig. 29, one can see that they exhibit a similar resonance Raman peak intensity pattern except that the $\nu_4(C_\alpha-N)$, $\Delta\bar{\nu}=1365$ cm^{-1} and $\delta(pyr)$, $\Delta\bar{\nu}=1643$ cm^{-1} RR peaks are of much lower intensity when excited at 460 nm, than when excited at 422 nm. The vibrational modes that are Raman active are those that mimic the geometric distortions present in the resonant excited state. Both the excitations are in resonance with the Soret B band excited state $S_3(B)$. The Soret B band is composed equally of two components: one x-polarized, known as B_x , and one y-

Fig. 26. IR spectrum of meso-tetrakis(N-methyl-4-pyridyl)porphine, $H_2TMpyP(4)$, in a KBr pellet, over 4000-650 cm^{-1} .

Fig. 27. IR spectrum of meso-tetrakis(N-methyl-4-pyridyl)porphine, $\text{H}_2\text{TMpyP}(4)$, in a KBr, over 900-1700 cm^{-1} .

Fig. 28. Vibrational resonance Raman spectrum of meso-tetrakis(N-methyl-4-pyridyl)porphine, $\text{H}_2\text{TMPyP}(4)$, monomer, 1.47×10^{-4} M, in a pH=7 phosphate buffer solution (see text), in a 1 cm cell, excited at 422 nm (23697 cm^{-1}).

Fig. 29. Vibrational resonance Raman spectrum of meso-tetrakis(N-methyl-4-pyridyl)porphine, $\text{H}_2\text{TMpyP}(4)$ monomer, 1.47×10^{-4} , in a pH=7 phosphate buffer solution (see text), in a 1 cm cell, excited at 460 nm (21739 cm^{-1}).

Table 5. Assignment of part of the resonance Raman spectrum of the meso-tetrakis(N-methyl-4-pyridyl)porphine, $H_2TMpyP(4)$, monomer, excited at 422 nm.

Raman shift $\Delta\bar{\nu}(cm^{-1})$	assignment ^a
968 m ^b	A_{1g} (in D_{4h})
1001 m	$\nu_6(C_{\alpha}-C_{\beta})$, A_{1g}
1056 w	pyr $\delta(C-H)$, A_{1g}
1095 m	$\delta_3(C_{\beta}-H)$, A_{1g}
1188 m	$\delta(pyr)$, $\nu(N^+-CH_3)$, A_{1g}
1218 s	$\delta(pyr)$, A_{1g}
1251 vs	$\nu_1(C_{\equiv}pyr)$, A_{1g}
1293 s	$\delta(pyr)$, A_{1g}
1365 s	$\nu_4(C_{\alpha}-N)$, A_{1g}
1558 vs	$\nu_2(C_{\beta}-C_{\beta})$, A_{1g}
1643 vs	$\delta(pyr)$, A_{1g}

^a ν , stretching; δ , bending; pyr, pyridine mode.

^b w, weak; m, medium; s, strong; vs, very strong intensity.

polarized, known as B_y . For $H_2TMpyP(4)$, the Soret B band ranges over about 390-470 nm (see Fig. 22. [11]) and has a band maximum at about 422 nm. The B_y component is associated with the shorter wavelength of the Soret B band, and the B_x component is associated with the longer wavelength component. Since we are dealing with ground state S_0 RR scattering, therefore, an excitation at 422 nm would result in an equal resonance enhancement of the B_y , B_x RR peaks (i.e. both α_1 , and α_2 equally) and their respective intensities. On the other hand, as the excitation is shifted to the longer wavelength region, an excitation at 460 nm would result in an unequal resonance enhancement of B_y , B_x , enhancing mainly B_x (i.e. α_1 mostly), and thus, RR peaks whose intensity had a large B_y component would experience some degree of hypochromicity.

On comparison of Fig. 27. and Fig. 28. illustrating respectively the IR spectra over the vibrational absorption range of $\bar{\nu}_{IR}=900-1700\text{ cm}^{-1}$, and the ground state vibration RR spectrum over the Raman shift range of $\Delta\bar{\nu}=900-1700\text{ cm}^{-1}$ for $H_2TMpyP(4)$, one can see that the band intensity patterns are quite different. These differences are a result of the different processes and selection rules that govern the IR and RR phenomena. An IR peak results from an absorption from one vibrational level to another within the electronic ground state that causes a change in the dipole moment and transforms as X, Y, Z, of E_u symmetry for D_{4h} . On the other hand, a RR peak results from a scattering event from one vibrational level to another between electronic states, but causes a change in the polarizability and transforms as X^2 , Y^2 , Z^2 , XY , YZ , XZ , of A_{1g} .

symmetry for D_{4h} on Soret B band excitation. Since resonance Raman peaks derive their intensity from the distortion in the geometry of the resonant excited state, this vividly illustrates the infrared (IR) E_u (N-methyl-4-pyridyl) modes at 1188 and 1640 cm^{-1} (which are not coupled to the porphyrin ring vibration in the ground state) are sufficiently distorted in the resonant excited state, such that they are coupled to the vibrations of the porphyrin ring. Thus, they experience RR enhancement on Soret B band excitation and are correlated to $\Delta\bar{\nu}=1188\text{ cm}^{-1}$, $\delta(\text{pyr})$, $\nu(\text{N}^+-\text{CH}_3)$, A_{1g} and $\Delta\bar{\nu}=1643\text{ cm}^{-1}$, $\delta(\text{pyr})$, A_{1g} RR modes, respectively. Therefore, the true power of resonance Raman spectroscopy is its ability to selectively determine vibrational couplings of the resonant excited state to that of the ground state, and thus act as a very sensitive probe of the geometric distortions present within that resonant state via the variation of the excitation wavelength.

4.4 Pulsed Laser-Induced Raman Intensities

Fig. 30. [11] illustrates the depolarization ratio for naturally polarized light ρ_n as a function of pump intensity I_i (mW) over a range of 0 to 5 mW for monomeric $\text{H}_2\text{TMpyP}(4)$, 1.47×10^{-4} M, excited at 422 nm. Table 6 [11] lists the scattering tensors and the experimentally determined depolarization ratio for circularly polarized light ρ_n for the $\nu_2(\text{C}_\beta-\text{C}_\beta)$ and $\delta(\text{pyr})$ RR peaks of monomer $\text{H}_2\text{TMpyP}(4)$ excited at 422, 476.5 and 514.5 nm.

To analyze the behaviour of ρ_n versus I_i illustrated in Fig. 30, one must

consider three factors as follows: (i) ground state depletion, (ii) induced anisotropy in the density distribution function n_1^a , and (iii) the anisotropic polarized excited state absorption spectrum effect.

On inspection of Fig. 30 [11] illustrating the ρ_a versus I_1 curves of monomer $H_2TMpyP(4)$ RR peaks of 1558 and 1643 cm^{-1} ; one can see that they behave very differently. The ρ_a versus I_1 curve for $\Delta\bar{\nu}=1558\text{ cm}^{-1}$ increases linearly in value from $\rho_a=0.22$, from $I_1=0$ to 5 mW, respectively. On the other hand, the ρ_a versus I_1 curve for $\Delta\bar{\nu}=1643\text{ cm}^{-1}$ remains almost constant in value where $\rho_a=0.37$ to 0.38 from 0 to 5 mW.

The first factor one must consider to explain this behaviour is ground state depletion. For monomeric $H_2TMpyP(4)$, the $S_1(Q_x)$ state absorbs at 640 nm and has a lifetime of $\tau(S_1(Q_x))=5.3\text{ ns}$ [1], whereas, the Soret $S_3(B)$ state absorbs at 422 nm and has an estimated lifetime of $\tau(S_3(B))=(19-74)\text{ps}$ (see section 4.2). The lowest lying triplet T_1 absorbs at 860 nm [51], and has a lifetime of $\tau(T_1)=175\text{ ns}$ [1]. Therefore, after an excitation at 422 nm with a 5 ns pulse, there is enough power in the pulse to saturate the $S_3(B)$ state (by consecutive stepwise absorption of two photons $S_3(B) \leftarrow S_0 + h\nu$, and then $S_m \leftarrow S_3(B) + h\nu$) [1], and therefore, cause ground state depletion [11]. In addition, if some intersystem crossing $S_1 \sim \sim > T_1$ occurs, not all of the population will have returned to the ground state by the time that the next 5 ns pulse comes along, hence, additional ground state depletion will occur (by triplet-triplet absorption $T_k \leftarrow T_1 + h\nu$) [1]. It should be kept in mind that as ground state depletion occurs, more photons,

Raman shift, $\nu_2(\text{C}_\beta\text{-C}_\beta)$, $\Delta\bar{\nu} = 1558 \text{ cm}^{-1}$

Raman shift, $\delta(\text{pyr})$, $\Delta\bar{\nu} = 1643 \text{ cm}^{-1}$

Fig. 30. Plot of relative Raman cross-section, perpendicular; $\sigma_R^{\perp}$, parallel, $\sigma_R^{\parallel}$, and the depolarization ratio of naturally (circularly) polarized light, ρ_n , versus pump intensity, I_i (mW) for (meso-tetrakis(N-methyl-4-pyridyl)porphine, $\text{H}_2\text{TMpyP}(4)$, $1.47 \times 10^{-4} \text{ M}$, in a 1 cm cell, in a pH=7 phosphate buffer (see text), excited at 422 nm (23697 cm^{-1}) [11].

Table 6. Scattering tensors and experimentally determined depolarization ratios for naturally polarized light, ρ_n , for $\nu_2(\text{C}_\beta\text{-C}_\beta)$, $\Delta\bar{\nu}=1558\text{ cm}^{-1}$, A_{1g} , and $\delta(\text{pyr})$, $\Delta\bar{\nu}=1643\text{ cm}^{-1}$, A_{1g} , of the $\text{H}_2\text{TMpyP(4)}$ monomer excited at 422, 476.5, and 514.5 nm [11].

$\lambda_i(\text{nm})$	Raman shift, $\Delta\bar{\nu}=1558\text{ cm}^{-1}$	ρ_n	Raman shift, $\Delta\bar{\nu}=1643\text{ cm}^{-1}$	ρ_n
	Tensor*		Tensor	
422*, $S_3(\text{B})$	$\begin{bmatrix} a & 0 & 0 \\ 0 & a & 0 \\ 0 & 0 & 0 \end{bmatrix}$	0.22	$\begin{bmatrix} 4b & 0 & 0 \\ 0 & b & 0 \\ 0 & 0 & 0 \end{bmatrix}$	0.37
476.5*, $S_3(\text{B})$	$\begin{bmatrix} a & 0 & 0 \\ 0 & 4a & 0 \\ 0 & 0 & 0 \end{bmatrix}$	0.36	$\begin{bmatrix} 4b & 0 & 0 \\ 0 & b & 0 \\ 0 & 0 & 0 \end{bmatrix}$	0.38
514.5*, $S_3(\text{Q}_Y)$	$\begin{bmatrix} 0 & 0 & 0 \\ 0 & 4a & 0 \\ 0 & 0 & 0 \end{bmatrix}$	0.50	$\begin{bmatrix} 0 & 0 & 0 \\ 0 & b & 0 \\ 0 & 0 & 0 \end{bmatrix}$	-

* pulsed dye laser, extrapolated to zero pump intensity, $I_i=0\text{ mW}$.

* continuous wave (CW) Ar^+ laser (ground state S_0 scattering).

* $a > b$.

$h\nu_i$, become available for Raman scattering (an effective increase in the transmission, T) because the transmission increases with increasing laser power I_i . Hence, although fewer molecules are residing in the ground state, more laser photons, $h\nu_i$, are available for Raman scattering, in both the $I_{\perp}$ and $I_{\parallel}$ cases and ρ_n would be unaffected [11].

Next, one must consider the isotropic, I^0 , and anisotropic, I^1 , contributions to the observed Raman intensities, I . The anisotropic correction factors are the perpendicular excitation (Y-polarization) configuration, $I_{\perp}^1 = I_{YZ}^1 + I_{YX}^1$; and in the perpendicular excitation (Z-polarization) configuration, $I_{\parallel}^1 = I_{ZZ}^1 + I_{ZX}^1$, for naturally polarized light can be expressed as follows:

$$I_{\perp}^1 = \frac{B}{7} \frac{N}{4\pi} I_0 [(4\alpha_1^2 + 5\alpha_2^2 + 5\alpha_3^2) + (-4\alpha_1\alpha_2 - 6\alpha_2\alpha_3 - 4\alpha_1\alpha_3)] \quad (75)$$

and

$$I_{\parallel}^1 = \frac{B}{7} \frac{N}{4\pi} I_0 [(5\alpha_1^2 + 5\alpha_2^2 + 18\alpha_3^2) + (\alpha_1\alpha_2 + 3\alpha_2\alpha_3 + 3\alpha_1\alpha_3)] \quad (76)$$

Now, using equations (75) and (76) and the proposed scattering tensors [11] (see the first row of Table 6 [11]), one can express the total depolarization ratio for naturally polarized light, ρ_n , for the $\nu_2(C_\beta-C_\beta)$, $\Delta\bar{\nu} = 1558 \text{ cm}^{-1}$ H₂TMpyP(4) monomer RR peak, as follows:

$$\rho_n = \frac{6 - \frac{5}{7}f}{27 - \frac{11}{7}f} \quad (77)$$

and the $\delta(\text{pyr})$, $\Delta\bar{\nu}=1643 \text{ cm}^{-1}$ $\text{H}_2\text{TMpyP(4)}$ monomer peak,

$$\rho_n = \frac{78 - \frac{53}{7}f}{216 - \frac{89}{7}f} \quad (78)$$

As the normalized pump intensity, f approaches zero, ρ_n^0 approaches $2/9$ and $13/36$, for the $\nu_2(\text{C}_\beta\text{-C}_\beta)$ and $\delta(\text{pyr})$ RR peaks of the $\text{H}_2\text{TMpyP(4)}$ monomer, respectively. On inspection of equations (77) and (78), it can be seen that for $\nu_2(\text{C}_\beta\text{-C}_\beta)$, ρ_n is somewhat dependant on pump intensity I_1 (i.e. f), whereas for $\delta(\text{pyr})$, ρ_n is much more dependant on I_1 (with about an eight times more dependance on I_1). Therefore in both cases, ρ_n is either slightly, or greatly decreased, but not increased. Hence, ground state depletion and anisotropic ground state distribution alone cannot explain the behaviour of ρ_n in Fig. 30, and one must consider a third factor effecting ρ_n .

This third factor is the polarization of the excited state $S_m \leftarrow S_3(\text{B})$ transition, which is known as the anisotropic polarized excited state absorption effect. Consider that the polarization of the $S_m \leftarrow S_3(\text{B})$ transition is in the YZ-plane (i.e., the $T_{ZZ}^{\text{ans}}(f)$ and $T_{YZ}^{\text{ans}}(f)$ terms are active, whereas the $T_{ZX}^{\text{ans}}(f)$ and $T_{YX}^{\text{ans}}(f)$ terms are inactive). If the pump is directed perpendicularly, the intensity

of the Raman emission $I_1^0 \approx 6\beta^2$ is split such that $I_{YZ}^0 \approx 3\beta^2$ and $I_{YX}^0 \approx 3\beta^2$ (the $B(N/4\pi)I_0$ term divides out). This would result in the $I_{YZ}^0 \approx 3\beta^2$ component being absorbed and the $I_{YX}^0 \approx 3\beta^2$ component being unaffected. Therefore, I_1^0 will be reduced by some amount. Now, if the pump comes in the parallel direction, the intensity of the Raman emission, $I_1^0 \approx 45\alpha^2 + 7\beta^2$ is split into $I_{ZZ}^0 \approx 45\alpha^2 + 4\beta^2$ and $I_{ZX}^0 \approx 3\beta^2$. This would result in the $I_{ZZ}^0 \approx 45\alpha^2 + 4\beta^2$ component being absorbed and the $I_{ZX}^0 \approx 3\beta^2$ component being unaffected. However, I_1^0 would be more reduced than I_1^0 , resulting in an increase in ρ_n^0 . Therefore, for $\delta(\text{pyr})$, $\Delta\bar{\nu} = 1643 \text{ cm}^{-1}$, the nearly constant ρ_n curve may be a result of a balance between the second factor, the anisotropic ground state density distribution, n_1^G , (pushing ρ_n down) which cancels the third factor, the anisotropic polarized excited state absorption spectrum effect (pushing ρ_n up by a nearly equal amount). Now for $\nu_2(\text{C}_\beta\text{-C}_\beta)$, $\Delta\bar{\nu} = 1558 \text{ cm}^{-1}$, ρ_n was only slightly affected by the second factor, thereby leaving the third factor, the anisotropic polarized excited state absorption spectrum effect, free to dominate and drive up ρ_n [11].

It is interesting to consider Fig.6. shown in [23]. It shows the resonance Raman spectra of Cu(II)(TMpyP(4)) and $\text{poly(dA-dT)poly(dA-dT)}$ excited at 556 nm. The more intense upper trace A, at $I_1 = 10^4 \text{ mW}$, shows additional bands at $\Delta\bar{\nu} = 1346 \text{ cm}^{-1}$ assigned to the $(\text{Cu(II)(TMpyP(4)))}^{*+} \cdot (\text{dA-dT})^{*-}$ exciplex. In trace B, at $I_1 = 10^5 \text{ mW}$, the bands are more intense than the ground state peaks of free Cu(II)(TMpyP(4)) , at $\nu_4(\text{C}_\alpha\text{-N})$, $\Delta\bar{\nu} = 1366 \text{ cm}^{-1}$ and $\delta(\text{pyr})$, $\Delta\bar{\nu} = 1570 \text{ cm}^{-1}$ [25]. It is tempting to speculate that the weakness of trace B (more excited state)

may not only be due to the ground state depletion cited by Turpin et al [25], but also to the anisotropic polarized excited state absorption spectrum effect. In the $(\text{Cu(II)(TMpyP(4)))}^{*+}-(\text{dA-dT})^{*-}$ exciplex, the absorption spectrum shows a significantly low transmission in this region, and this may manifest itself if ρ_a were calculated [11].

Chapter 5: Conclusion

In this work, excited state $S_m \leftarrow S_3(B)$ and $S_k \leftarrow S_1(Q_x)$ absorption of meso-tetrakis(N-methyl-4-pyridyl)porphine, $H_2TMpyP(4)$ in the Soret B band and the Q band region, respectively were presented. A monobeam transmission, effective population saturation study, EPS, of monomeric $H_2TMpyP(4)$ yielded an effective population saturation transmission value of $T_{eff}=0.192$. This resulted in the maximum calculated lifetime of the $S_3(B), \tau(S_3(B))$ state of the $H_2TMpyP(4)$ monomer to be estimated in the range of (19-74)ps. Within the level of the approximations present in these calculations, it can be stated that the lifetime of the $S_3(B)$ of the $H_2TMpyP(4)$ monomer are in the ps region.

Using the Euler angles (ϕ, θ, Ψ) of the complete three-dimensional Euler rotational matrix, $R(\phi, \theta, \Psi)$, in the standard coordinate system of the right-handed screw using the X-convention, expressions for the first-order anisotropic correction factors to the density distribution function of the concentration of ground state molecule, $n_1^0(\phi, \theta, f)$, in the perpendicular excitation (Y-polarization) configuration, I_{YZ}^1 and I_{YX}^1 , equations (70) and (71), were derived in this thesis. The need for this complete treatment can be seen by comparasion to the use of the two-dimensional Euler rotational matrix, $R(\phi, \theta, 0)$ to derive the expressions for I_{YZ}^1 and I_{YX}^1 . By setting $\Psi=0$, and limiting the variables of integration to only these two-dimensions (ϕ, θ) , incomplete expressions for I_{YZ}^1 and I_{YX}^1 will be obtained (see Appendix). This being the case, not enough of the Raman scattered intensity will be attenuated by these two-dimensional (incomplete) first-order

anisotropic correction factors, I_{YZ}^1 , and I_{YX}^1 (derived from $R(\phi, \theta, 0)$). This will result in the calculated total Raman depolarization ratio for naturally (circularly) polarized light, ρ_n , being too high in value. As well, the sole reason the correct expression for I_{ZZ}^1 is reproduced when using $R(\phi, \theta, 0)$ to derive it, is that the R_{ZX} , R_{ZY} , and R_{ZZ} matrix elements are the same in both $R(\phi, \theta, 0)$ (two-dimensional case), and $R(\phi, \theta, \Psi)$ (three-dimensional case). On utilization of $R(\phi, \theta, 0)$ to derive the expression for I_{ZX}^1 , the correct form of equation (69) (see p. 8696 of reference [31]) is not reproduced (see Appendix). Thus, the complete three-dimensional Euler rotational matrix $R(\phi, \theta, \Psi)$, was utilized to reproduce the complete expressions of equations (68) and (69) for I_{ZZ}^1 and I_{ZX}^1 , (see p. 8696 of reference [31]), and further used to derive in this thesis, the complete expressions for I_{YZ}^1 and I_{YX}^1 , equations (70) and (71). On having derived expressions for I_{YZ}^1 and I_{YX}^1 , one can calculate not only ρ_i using the previously derived expressions for I_{ZX}^1 and I_{ZZ}^1 , but one can now calculate ρ_n using the expressions for $(I_{YZ}^1 + I_{YX}^1)$ and $(I_{ZZ}^1 + I_{ZX}^1)$.

In the analysis of the total depolarization ratio for naturally polarized light, ρ_n , as a function of pump intensity, I_i , curves, for the $H_2TMpyP(4)$ monomer, three factors (i) ground state depletion, (ii) induced anisotropy in the density distribution function of the concentration of the ground state molecules, n_i^0 , and (iii) the anisotropic polarized excited state absorption spectrum effect, were used as a framework to interpret the experimental results. In certain circumstances, the last factor, the anisotropic polarized excited state absorption effect, can be

shown to dominate driving ρ_a up for the $\nu_2(C_\beta-C_\beta)$, $\Delta\bar{\nu}=1558\text{ cm}^{-1}$ RR peak of monomeric $H_2TMpyP(4)$ with its $S_3(B)$ state polarized in the YZ-plane (i.e., the $T_{ZZ}^{ans}(f)$ and $T_{YZ}^{ans}(f)$ terms are active). When used in conjunction with other techniques such as time-resolved isotropic and anisotropic absorption and fluorescence, LIDE, RREP, differential absorption spectroscopy, circular dichroism, viscometry, and X-ray crystallography, the analysis of the total Raman depolarization ratio for naturally polarized light, ρ_a , as a function of pump intensity, I_i , via the three factors (i) ground state depletion, (ii) induced anisotropic density distribution of the concentration ground state molecules, n_i^0 , and (iii) the anisotropic polarized excited state absorption spectrum effect, as presented here in this work, could be used in future work to shed new light on the interactions within $H_2TMpyP(4)$ -DNA complex, towards the goal of determining more precisely its structure and dynamics in various experimental conditions.

Since, the ground breaking work in 1969 by Tao [29], the study of anisotropic effects in absorption, fluorescence and recently, in resonance Coherent Anti-Stokes Raman Scattering rCARS [59] has provided not only important information such as rotation relaxation times, τ_{rot} , for many systems, but as well a framework for visualizing and interpreting the dynamic processes governing the interplay between the quantum photophysics and photochemistry of the solute molecule, and that of the classical continuum of the bulk solvent.

Future studies, proposed in the area of this work are included here as the

Fig. 31. Covalently amide linked, meso-tetrakis(N-methyl-pyridyl)porphine-like, dimer.

characterization of the porphyrin-DNA complex, the lanthanide porphyrin $\text{Eu(III)(TMpyP(4)(Cl))}$, the carotenoporphyrin ($8'$ -apo- β -caroteno) Cu(II)(TMpyP(4)) , and also a covalently amide linked $\text{H}_2\text{TMpyP(4)}$ -like dimer which is illustrated in Fig. 31. Dihematoporphyrin ester/ether DHE, and its purified drug form Photofrin II [18-22] used in photodynamic therapy (PDT), has been a medical reality, used in the treatment of cancer clinically for the past decade and $\text{H}_2\text{TMpyP(4)}$ -like molecules were suggested "next-generation" possible drugs for PDT by T.G. Dougherty [21], and S.B. Brown and T.G. Truscott [22]. The covalently linked amide $\text{H}_2\text{TMpyP(4)}$ -like dimer, proposed in this thesis for future study, illustrated in Fig. 31, may have not only the photosensitizing ability to destroy cell membranes by colloid osmotic lysis, [18], but once inside the cell, it may manifest potential intercalation abilities and intercalate (sandwich in between two adjacent nucleic acid base pairs) into the DNA double-helix, thus making it a possible candidate for a drug to be used in the treatment of HIV (AIDS). Therefore, the synthesis and characterization of the photophysics and photochemistry of new porphyrins, and covalently linked porphyrin dimers in the future may prove helpful in the treatment of cancer and HIV, and the analysis of the ρ_a versus I_1 curves in future studies of these drug-DNA complexes, using factors (i), (ii), and (iii) (the anisotropic polarized excited state absorption spectrum effect) may yield very useful and important information on the interactions and structures of these drug-DNA complexes to this end.

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**Appendix: Note in Added Proof of the Derivation of the First-Order
Anisotropic Correction Factors I_{YZ}^{-1} and I_{YX}^{-1} Utilizing The Complete Three-
Dimensional Euler Rotational Matrix $R(\phi, \theta, \Psi)$**

To transform from one set of Cartesian coordinates, XYZ, into another set, $x'y'z'$, one utilizes what is known as a "similarity transformation", $\alpha' = R\alpha R^{-1}$, where α , R , R^{-1} , and α' denote the diagonal Raman scattering matrix (tensor) in the (space fixed set) XYZ coordinate system, the Euler rotational matrix, the transpose of the Euler Rotational Matrix, and the transformed Raman scattering matrix in the new (body set) $x'y'z'$ coordinate system. As stated in section 2.2, any direction can be defined in terms of three angles ϕ , θ , Ψ known as Euler ("yaw", "pitch", and "roll") angles (see Fig. 17. [58], section 2.2). For this similarity transformation to be carried out without ambiguity, a convention must be agreed upon. The convention accepted in North America and Western Europe (and gaining acceptance in Eastern Europe) is known as the X-convention within the coordinate system of the right-handed screw (see section 2.2), and this convention is adhered to in this thesis.

The transformation from the space fixed set of axes, XYZ, to that of the body set of axes, $x'y'z'$, is facilitated via the complete three-dimensional Euler rotational matrix, $R(\phi, \theta, \Psi)$, such that the standard diagonal form of the Raman scattering matrix α (in XYZ) becomes $\alpha' = R(\phi, \theta, \Psi)\alpha R^{-1}(\phi, \theta, \Psi)$ (in $x'y'z'$) can be expressed as follows [58],

$$\alpha = \begin{bmatrix} \alpha_1 & 0 & 0 \\ 0 & \alpha_2 & 0 \\ 0 & 0 & \alpha_3 \end{bmatrix} \quad (\text{A1})$$

$$R(\phi, \theta, \Psi) = \begin{bmatrix} R_{XX} & R_{XY} & R_{XZ} \\ R_{YX} & R_{YY} & R_{YZ} \\ R_{ZX} & R_{ZY} & R_{ZZ} \end{bmatrix} \quad (\text{A2})$$

where,

$$R_{XX} = \cos \Psi \cos \phi - \sin \Psi \sin \phi \cos \theta \quad (\text{A3})$$

$$R_{XY} = \cos \Psi \sin \phi + \sin \Psi \cos \phi \cos \theta \quad (\text{A4})$$

$$R_{XZ} = \sin \Psi \sin \theta \quad (\text{A5})$$

$$R_{YX} = -\sin \Psi \cos \phi - \cos \Psi \sin \phi \cos \theta \quad (\text{A6})$$

$$R_{YY} = -\sin \Psi \sin \phi + \cos \Psi \cos \phi \cos \theta \quad (\text{A7})$$

$$R_{YZ} = \cos \Psi \sin \theta \quad (\text{A8})$$

$$R_{ZX} = \sin \theta \sin \phi \quad (\text{A9})$$

$$R_{ZY} = -\sin \theta \cos \phi \quad (\text{A10})$$

$$R_{ZZ} = \cos \theta \quad (\text{A11})$$

$$\alpha' = R(\phi, \theta, \Psi) \alpha R^{-1}(\phi, \theta, \Psi) \quad (\text{A12})$$

$$R(\phi, \theta, \Psi) \alpha R^{-1}(\phi, \theta, \Psi) = \begin{bmatrix} (R\alpha R^{-1})_{XX} & (R\alpha R^{-1})_{XY} & (R\alpha R^{-1})_{XZ} \\ (R\alpha R^{-1})_{YX} & (R\alpha R^{-1})_{YY} & (R\alpha R^{-1})_{YZ} \\ (R\alpha R^{-1})_{ZX} & (R\alpha R^{-1})_{ZY} & (R\alpha R^{-1})_{ZZ} \end{bmatrix} \quad (\text{A13})$$

where,

$$(R\alpha R^{-1})_{XX} = \alpha_1 R_{XX}^2 + \alpha_2 R_{XY}^2 + \alpha_3 R_{XZ}^2 \quad (\text{A14})$$

$$(R\alpha R^{-1})_{XY} = \alpha_1 R_{YX} R_{XX} + \alpha_2 R_{YY} R_{XY} + \alpha_3 R_{YZ} R_{XZ} \quad (\text{A15})$$

$$(R\alpha R^{-1})_{xz} = \alpha_1 R_{zx} R_{yx} + \alpha_2 R_{zy} R_{yy} + \alpha_3 R_{zz} R_{yz} \quad (\text{A16})$$

$$(R\alpha R^{-1})_{yx} = \alpha_1 R_{xx} R_{yx} + \alpha_2 R_{xy} R_{yy} + \alpha_3 R_{xz} R_{yz} \quad (\text{A17})$$

$$(R\alpha R^{-1})_{yy} = \alpha_1 R_{yx}^2 + \alpha_2 R_{yy}^2 + \alpha_3 R_{yz}^2 \quad (\text{A18})$$

$$(R\alpha R^{-1})_{yz} = \alpha_1 R_{zx} R_{yx} + \alpha_2 R_{zy} R_{yy} + \alpha_3 R_{zz} R_{yz} \quad (\text{A19})$$

$$(R\alpha R^{-1})_{zx} = \alpha_1 R_{xx} R_{zx} + \alpha_2 R_{xy} R_{zy} + \alpha_3 R_{xz} R_{zz} \quad (\text{A20})$$

$$(R\alpha R^{-1})_{zy} = \alpha_1 R_{yx} R_{zx} + \alpha_2 R_{yy} R_{zy} + \alpha_3 R_{yz} R_{zz} \quad (\text{A21})$$

$$(R\alpha R^{-1})_{zz} = \alpha_1 R_{zx}^2 + \alpha_2 R_{zy}^2 + \alpha_3 R_{zz}^2 \quad (\text{A22})$$

and the relevant squares of these matrix elements,

$$\begin{aligned} (R\alpha R^{-1})_{zz}^2 &= (\alpha_1^2 R_{zx}^4 + \alpha_2^2 R_{zy}^4 + \alpha_3^2 R_{zz}^4) \\ &+ 2(\alpha_1 \alpha_2 R_{zx}^2 R_{zy}^2 + \alpha_2 \alpha_3 R_{zy}^2 R_{zz}^2 + \alpha_1 \alpha_3 R_{zx}^2 R_{zz}^2) \end{aligned} \quad (\text{A23})$$

$$(R\alpha R^{-1})_{ZX}^2 = (\alpha_1^2 R_{XX}^2 R_{ZX}^2 + \alpha_2^2 R_{XY}^2 R_{ZY}^2 + \alpha_3^2 R_{XZ}^2 R_{ZZ}^2) \\ + 2(\alpha_1 \alpha_2 R_{XX} R_{ZX} R_{ZY} R_{XY} + \alpha_2 \alpha_3 R_{XY} R_{ZY} R_{XZ} R_{ZZ} + \alpha_1 \alpha_3 R_{XX} R_{ZX} R_{XZ} R_{ZZ}) \quad (A24)$$

$$(R\alpha R^{-1})_{YZ}^2 = (\alpha_1^2 R_{ZX}^2 R_{YX}^2 + \alpha_2^2 R_{ZY}^2 R_{YY}^2 + \alpha_3^2 R_{ZZ}^2 R_{YZ}^2) \\ + 2(\alpha_1 \alpha_2 R_{ZX} R_{YX} R_{ZY} R_{YY} + \alpha_2 \alpha_3 R_{ZZ} R_{YZ} R_{ZY} R_{YY} + \alpha_1 \alpha_3 R_{ZX} R_{YX} R_{ZZ} R_{YZ}) \quad (A25)$$

$$(R\alpha R^{-1})_{YX}^2 = (\alpha_1^2 R_{XX}^2 R_{YX}^2 + \alpha_2^2 R_{XY}^2 R_{YY}^2 + \alpha_3^2 R_{XZ}^2 R_{YZ}^2) \\ + 2(\alpha_1 \alpha_2 R_{XX} R_{YX} R_{XY} R_{YY} + \alpha_2 \alpha_3 R_{XZ} R_{YZ} R_{XY} R_{YY} + \alpha_1 \alpha_3 R_{XX} R_{YX} R_{XZ} R_{YZ}) \quad (A26)$$

Now we must utilize the above equations to obtain expressions for the Raman scattered intensities that are excited in the ρ -polarization (along the X-axis) and which are scattered in the σ -polarization (along the Y-axis), denoted as $I_{\rho\sigma}$. The expression for $I_{\rho\sigma}$ can be written as follows [3, 57, 59],

$$I_{\rho\sigma} = \frac{16\pi^4(\nu - \nu_0)^4}{c^4} I_0 \langle n_1^G \alpha_{\rho\sigma}^2 \rangle = I_{\rho\sigma}^0 - f I_{\rho\sigma}^1 \quad (A27)$$

where I_0 , n_1^G , $\alpha_{\rho\sigma}^2$, f , $I_{\rho\sigma}^0$, $I_{\rho\sigma}^1$, and $I_{\rho\sigma}$ denote the incident intensity, the density distribution function of the concentration of ground state molecules, the square of

the $\rho\sigma^{\text{th}}$ scattering matrix (tensor) element, the normalized incident intensity, the zeroth-order isotropic Raman scattered intensity, the first-order anisotropic Raman scattered intensity, the total Raman scattered intensity excited in the ρ -polarization (along the X-axis) and scattered in the σ -polarization (along the Y-axis), respectively. The $\langle n_1^G \alpha_{\rho\sigma}^2 \rangle$ term represents the averaging process over all possible orientations.

Here we let the probe beam E_2 (very weak intensity) perform the function of creating the resonance Raman spectrum, and the pump beam E_1 (very strong intensity) perform the function of creating the excited states from the ground state (However, the arguments presented here for the two beam experiment, E_1 and E_2 , are also valid for the monobeam experiment, using only E_1 , where E_1 acts as both pump and probe). In the absence of the pump, the density distribution is randomly oriented and isotropic (spherical) in nature (can be visualized as an undisturbed sphere). The isotropic density distribution function for the concentration of ground state molecules, n_1^G , can be expressed as follows [3, 31],

$$n_1^G = \frac{N}{4\pi} \quad (\text{A29})$$

where N is the total number of molecules in the scattering volume. Therefore equation (A27) can be written as follows,

$$I_{\rho\sigma}^0 = \frac{16\pi^4(\nu - \nu_0)^2}{c^4} \frac{N}{4\pi} I_0 \langle \alpha_{\rho\sigma}^2 \rangle \quad (\text{A30})$$

and thus obtaining an expression for $I_{\rho\sigma}^0$ becomes a matter of evaluating the $\langle \alpha_{\rho\sigma}^2 \rangle$ term. This is accomplished through the use of the mean value theorem expressed as follows,

$$\langle \alpha_{\rho\sigma}^2 \rangle = \frac{\int_0^{2\pi} \int_0^{2\pi} \int_0^\pi ((R\alpha R^{-1})_{\rho\sigma}^2) \sin\theta d\theta d\phi d\Psi}{\int_0^{2\pi} \int_0^{2\pi} \int_0^\pi \sin\theta d\theta d\phi d\Psi} \quad (\text{A31})$$

where $0 \leq \theta \leq \pi$, $0 \leq \phi \leq 2\pi$, and $0 \leq \Psi \leq 2\pi$. Now the isotropic depolarization ratio for naturally polarized light, ρ_n^0 , can be expressed as follows [3],

$$\rho_n^0 = \frac{I_{\perp}^0}{I_{\parallel}^0} = \frac{I_{YZ}^0 + I_{YX}^0}{I_{ZZ}^0 + I_{ZX}^0} = \frac{\langle \alpha_{YZ}^2 \rangle + \langle \alpha_{YX}^2 \rangle}{\langle \alpha_{ZZ}^2 \rangle + \langle \alpha_{ZX}^2 \rangle} \quad (\text{A32})$$

and for the isotropic depolarization ratio for linearly polarized light, ρ_l^0 , we have [3],

$$\rho_l^0 = \frac{I_{\perp}^0}{I_{\parallel}^0} = \frac{I_{ZX}^0}{I_{ZZ}^0} = \frac{\langle \alpha_{ZX}^2 \rangle}{\langle \alpha_{ZZ}^2 \rangle} \quad (\text{A33})$$

Therefore, the task of determining the expression for ρ_n^0 and ρ_l^0 in terms of the coefficients of α_i^2 and $\alpha_i\alpha_j$ becomes that of obtaining expressions for I_{ZZ}^0 , I_{ZX}^0 , I_{YZ}^0 , and I_{YX}^0 through the evaluation of the $\langle \alpha_{ZZ}^2 \rangle$, $\langle \alpha_{ZX}^2 \rangle$, $\langle \alpha_{YZ}^2 \rangle$,

and $\langle \alpha^2_{yx} \rangle$ terms, respectively. This can be facilitated through the sequential substitution of equations (A23) to (A26) for $(R\alpha R^{-1})^2_{zz}$, $(R\alpha R^{-1})^2_{zx}$, $(R\alpha R^{-1})^2_{yz}$, and $(R\alpha R^{-1})^2_{yx}$ into equation (A31). For each case, one must evaluate the six sets of integrals, to obtain each of the six coefficients (i.e., α_1^2 , α_2^2 , α_3^2 , $\alpha_1\alpha_2$, $\alpha_2\alpha_3$, and $\alpha_1\alpha_3$). On carrying out this procedure, one obtains for the isotropic density distribution function of the concentration of ground state molecules, n_1^0 , [3],

$$I_{zz}^0 = B \frac{N}{4\pi} I_0 \left[3 \sum_{i=1}^3 \alpha_i^2 + 2 \sum_{i<j} \alpha_i \alpha_j \right] \quad (\text{A34})$$

$$I_{zx}^0 = B \frac{N}{4\pi} I_0 \left[\sum_{i=1}^3 \alpha_i^2 - \sum_{i<j} \alpha_i \alpha_j \right] \quad (\text{A35})$$

$$I_{yz}^0 = B \frac{N}{4\pi} I_0 \left[\sum_{i=1}^3 \alpha_i^2 - \sum_{i<j} \alpha_i \alpha_j \right] \quad (\text{A36})$$

$$I_{yx}^0 = B \frac{N}{4\pi} I_0 \left[\sum_{i=1}^3 \alpha_i^2 - \sum_{i<j} \alpha_i \alpha_j \right] \quad (\text{A37})$$

Therefore, through the use of the zeroth-order Raman intensities, represented in equations (A34) to (A37), one can evaluate ρ_a^0 through the $(I_{yz}^0 + I_{yx}^0)$ and $(I_{zz}^0 + I_{zx}^0)$ terms; and as well evaluate ρ_i^0 through the I_{zx}^0 and I_{zz}^0 terms.

Now we turn our attention to the situation where the pump E_1 is present. There are two cases to consider; case (1) the pump E_1 (and probe E_2) is in the parallel (Z-polarization) excitation configuration, and case (2) the pump E_1 (and probe E_2) is in the perpendicular (Y-polarization) excitation configuration. First

let us look at case (1), when the pump is in the parallel (Z-polarization) excitation configuration. The pump and probe come in along the X-axis in the Z-polarization, excited states are created, and the population of the ground state is reduced. This will result in a solid cone of molecules centred on the Z-axis being removed from the isotropic density distribution function of the concentration of ground state molecules n_1^G (expressed in equation (A29)). Therefore, the isotropic density distribution is disturbed and an anisotropic density distribution is created, $n_1^G(\theta, f)$, and a representation of this is illustrated in Fig. A1. The created anisotropic density distribution can be visualized as a sphere that has a solid cone "scouped out" along the Z-axis. Thus, the expression for this anisotropic density distribution function for the concentration of ground state molecules changes from the isotropic one ($n_1^G = N/(4\pi)$) to the following expression,

$$n_1^G = \frac{N}{4\pi} [1 - f \cos^2 \Omega] \quad (\text{A38})$$

where Ω is known as the solid angle. To evaluate the $\cos^2 \Omega$ term, we use the following expressions,

$$\mu = \begin{bmatrix} \sin \theta \cos \phi \\ \sin \theta \sin \phi \\ \cos \theta \end{bmatrix} \quad (\text{A39})$$

Fig. A1. Anisotropic Density Distribution Function of the Concentration of Ground State Molecules, $n_i^G(\theta, f)$, Producing I_{zz} and I_{zx} in the Parallel (Z-polarization) Excitation Configuration.

$$\mathbf{Z} = \begin{bmatrix} 0 \\ 0 \\ 1 \end{bmatrix} \quad (\text{A40})$$

where μ defines the edge of the solid cone in spherical coordinates, and $\mathbf{Z}$ defines the Z-axis along which the solid cone is centred, and we have the following,

$$\cos^2 \Omega = |\mu^{-1} \mathbf{Z}|^2 = \cos^2 \theta \quad (\text{A41})$$

Thus, on the substitution of equation (A41) into equation (A38), the anisotropic density distribution function of the concentration of ground state molecules, excited in the parallel (Z-polarization) excitation configuration, $n_1^G(\theta, f)$, can be expressed as follows [31, 59],

$$n_1^G(\theta, f) = \frac{N}{4\pi} [1 - f \cos^2 \theta] \quad (\text{A42})$$

Now, for case (2) of the pump E_1 in the perpendicular (Y-polarization) excitation configuration, the created anisotropic density distribution is illustrated in Fig. A2. Here both the pump and the probed come in along the X-axis in the Y-polarization, excited states are created, and the population of the ground state is reduced. This will result in a solid cone of molecules, centred on the Y-axis, being removed from the isotropic density distribution function of the concentration of ground state molecules, n_1^G (expressed in equation (A29)).

Therefore, the isotropic density distribution is disturbed and an anisotropic density distribution is created $n_1^G(\phi, \theta, f)$ (see Fig. A2.). The created anisotropic density distribution can be visualized as sphere that has a solid cone "scouped out" along the Y-axis. Thus, to obtain an expression for $n_1^G(\phi, \theta, f)$ we use the similiar procedure that was used to obtain equation (A42), using the expressions for μ (equation (A39)) and Y ,

$$Y = \begin{bmatrix} 0 \\ 1 \\ 0 \end{bmatrix} \quad (A43)$$

as follows,

$$\cos^2 \Omega = |\mu^{-1} Y|^2 = \sin^2 \theta \sin^2 \phi \quad (A44)$$

Thus we have the expression for the anisotropic density distribution function for the concentration of ground state molecules, excited in the perpendicular (Y-polarization) excitation configuration, $n_1^G(\phi, \theta, f)$, as follows,

$$n_1^G(\phi, \theta, f) = \frac{N}{4\pi} [1 - f \sin^2 \theta \sin^2 \phi] \quad (A45)$$

Now that one has expressions for $n_1^G(\theta, f)$ and $n_1^G(\phi, \theta, f)$, the anisotropic first-order correction factors I_{zz}^1 , I_{zx}^1 , I_{yz}^1 , and I_{yx}^1 can be obtained utiling the complete three-dimensional Euler rotational matrix $R(\phi, \theta, \Psi)$. To evaluate I_{zz}^1

Fig. A2. Anisotropic Density Distribution Function of the Concentration of Ground State Molecules, $n_1^G(\phi, \theta, f)$, Producing I_{YZ} and I_{YX} in the Perpendicular (Y-polarization) Excitation Configuration.

and I_{zx}^{-1} , through $n_1^G(\theta, f)$, the problem is reduced to deriving expressions for the $\langle \alpha_{zz}^2 \cos^2 \theta \rangle$ and $\langle \alpha_{zx}^2 \cos^2 \theta \rangle$ terms, respectively, that are present in case (1).

This can be accomplished through the mean value equation expressed as follows,

$$\langle \alpha_{\rho\sigma}^2 \cos^2 \theta \rangle = \frac{\int_0^{2\pi} \int_0^{2\pi} \int_0^\pi ((R\alpha R^{-1})_{\rho\sigma}^2 \cos^2 \theta) \sin \theta d\theta d\phi d\Psi}{\int_0^{2\pi} \int_0^{2\pi} \int_0^\pi \sin \theta d\theta d\phi d\Psi} \quad (A46)$$

by successive substitution of equations (A23) and (A24) into equation (A46).

As well, to evaluate I_{yz}^{-1} and I_{yx}^{-1} , through $n_1^G(\phi, \theta, f)$, the problem is reduced to deriving expressions for the $\langle \alpha_{yz}^2 \sin^2 \theta \sin^2 \phi \rangle$ and $\langle \alpha_{yx}^2 \sin^2 \theta \sin^2 \phi \rangle$ terms that are present in case (2). This can be accomplished through the mean value equation expressed as follows,

$$\langle \alpha_{\rho\sigma}^2 \sin^2 \theta \sin^2 \phi \rangle = \frac{\int_0^{2\pi} \int_0^{2\pi} \int_0^\pi ((R\alpha R^{-1})_{\rho\sigma}^2 \sin^2 \theta \sin^2 \phi) \sin \theta d\theta d\phi d\Psi}{\int_0^{2\pi} \int_0^{2\pi} \int_0^\pi \sin \theta d\theta d\phi d\Psi} \quad (A47)$$

by the successive substitution of equations (A25) and (A26) into equation (A47).

Thus, the anisotropic first-order correction factors for the parallel (Z-polarization) excitation configuration be expressed as follows [31],

$$I_{zz}^1 = \frac{B}{7} \frac{N}{4\pi} I_0 [(3\alpha_1^2 + 3\alpha_2^2 + 15\alpha_3^2) + (2\alpha_1\alpha_2 + 6\alpha_2\alpha_3 + 6\alpha_1\alpha_3)] \quad (\text{A48})$$

and

$$I_{zx}^1 = \frac{B}{7} \frac{N}{4\pi} I_0 [(2\alpha_1^2 + 2\alpha_2^2 + 3\alpha_3^2) + (-\alpha_1\alpha_2 - 3\alpha_2\alpha_3 - 3\alpha_1\alpha_3)] \quad (\text{A49})$$

Similarly, the anisotropic first-order correction factors for the perpendicular (Y-polarization) excitation configuration, derived in this thesis, can be expressed as follows,

$$I_{yz}^1 = \frac{B}{7} \frac{N}{4\pi} I_0 [(3\alpha_1^2 + 2\alpha_2^2 + 2\alpha_3^2) + (-3\alpha_1\alpha_2 - \alpha_2\alpha_3 - 3\alpha_1\alpha_3)] \quad (\text{A50})$$

and

$$I_{yx}^1 = \frac{B}{7} \frac{N}{4\pi} I_0 [(\alpha_1^2 + 3\alpha_2^2 + 3\alpha_3^2) + (-\alpha_1\alpha_2 - 5\alpha_2\alpha_3 - \alpha_1\alpha_3)] \quad (\text{A51})$$

Table 3. [31] and Table 4. (see section 2.2) show the results of the calculated Raman scattered light intensities for the transitions governed by different tensors (i.e., tensors I-VII) for the case when the pump is off, I^0 , and on, I^1 . On inspection of equations (A48) to (A51), one notices a certain set of commonalities they possess. All equations have the same divisor of their B term

(i.e., $1/7$), and all coefficients of the α_i^2 and $\alpha_i\alpha_j$ terms are non-zero. When the Raman transition is governed by the symmetric scattering tensor I (i.e., $\alpha_1=\alpha_2=\alpha_3$), the on-diagonal term I_{ZZ}^1 , equation (A48), is non-zero, whereas all the off-diagonal terms I_{ZX}^1 , I_{YZ}^1 , and I_{YX}^1 , equations (A49) to (A51), have a zero value. It must be born in mind that equations (A48) to (A51) are derived through the utilization of the complete three-dimensional Euler rotational matrix, $R(\phi, \theta, \Psi)$, by letting the Euler angles (ϕ, θ, Ψ) vary over all possible angles.

However, if one chooses to limit the variables of integration from the three-dimensional case, (ϕ, θ, Ψ) , to the two-dimensional case, (ϕ, θ) for $\Psi=0$, we have the two-dimensional Euler rotational matrix, $R(\phi, \theta, 0)$, expressed as follows,

$$R(\phi, \theta, 0) = \begin{bmatrix} R_{XX} & R_{XY} & R_{XZ} \\ R_{YX} & R_{YY} & R_{YZ} \\ R_{ZX} & R_{ZY} & R_{ZZ} \end{bmatrix} \quad (A52)$$

where,

$$R_{XX} = \cos \phi \quad (A53)$$

$$R_{XY} = \sin \phi \quad (A54)$$

$$R_{XZ} = 0 \quad (A55)$$

$$R_{YX} = -\sin \phi \cos \theta \quad (\text{A56})$$

$$R_{YY} = \cos \phi \cos \theta \quad (\text{A57})$$

$$R_{YZ} = \sin \theta \quad (\text{A58})$$

$$R_{ZX} = \sin \theta \sin \phi \quad (\text{A59})$$

$$R_{ZY} = -\sin \theta \cos \phi \quad (\text{A60})$$

$$R_{ZZ} = \cos \theta \quad (\text{A61})$$

and the similarity transformation can be expressed as follows,

$$\alpha' = R(\phi, \theta, 0) \alpha R^{-1}(\phi, \theta, 0) \quad (\text{A62})$$

On the reduction of the variables of integration from (ϕ, θ, Ψ) to (ϕ, θ) , the evaluation of the $\langle \alpha_{ZZ}^2 \cos^2 \theta \rangle$, $\langle \alpha_{ZX}^2 \cos^2 \theta \rangle$; and $\langle \alpha_{YZ}^2 \sin^2 \theta \sin^2 \phi \rangle$, $\langle \alpha_{YX}^2 \sin^2 \theta \sin^2 \phi \rangle$ terms can be expressed as follows,

$$\langle \alpha_{p_o}^2 \cos^2 \theta \rangle = \frac{\int_0^{2\pi} \int_0^\pi ((R\alpha R^{-1})_{p_o}^2 \cos^2 \theta) \sin \theta d\theta d\phi}{\int_0^{2\pi} \int_0^\pi \sin \theta d\theta d\phi} \quad (\text{A63})$$

and

$$\langle \alpha_{p_o}^2 \sin^2 \theta \sin^2 \phi \rangle = \frac{\int_0^{2\pi} \int_0^\pi ((R\alpha R^{-1})_{p_o}^2 \sin^2 \theta \sin^2 \phi) \sin \theta d\theta d\phi}{\int_0^{2\pi} \int_0^\pi \sin \theta d\theta d\phi} \quad (\text{A64})$$

where the form of the $(R\alpha R^{-1})_{p_o}^2$ terms are identical to those of equations (A23) to (A26); however the R_{p_o} matrix elements are now defined as those of the two-dimensional Euler rotational matrix, $R(\phi, \theta, 0)$, equations (A53) to (A61), not those of the three-dimensional Euler rotational matrix, $R(\phi, \theta, \Psi)$, equations (A3) to (A11). The evaluation of I_{zz}^{-1} , I_{zx}^{-1} via equations (A23) and (A24) sequentially substituted into equation (A63); and the evaluation of I_{yz}^{-1} , I_{yx}^{-1} via equation (A25) and (A26) sequentially substituted into equation (A64); will be simplified as there are now only two variables of integration (ϕ, θ) and any term with R_{xz} (equation (A55)) in it will be zero.

Following the process involved in the evaluation of the first-order anisotropic correction factors I_{zz}^{-1} , I_{zx}^{-1} , I_{yz}^{-1} , and I_{yx}^{-1} , which utilized the complete three-dimensional Euler rotational matrix, one can now use the (incomplete) two-dimensional Euler rotational matrix, $R(\phi, \theta, 0)$, to derive the expressions for the

first-order anisotropic correction factors over the variables of integration (ϕ, θ). Thus for case (1) of the anisotropic density distribution function of the concentration of ground state molecules, excited in the parallel (Z-polarization) excitation configuration, $n_1^0(\theta, f)$, (expressed by equation (A42)) the first-order anisotropic correction factors to the Raman scattered intensities I_{ZZ}^1 and I_{ZX}^1 , using equations (A23) and (A24), with $R(\phi, \theta, 0)$ matrix elements equations (A53) to (A61), to evaluate equation (A63) (i.e., $\langle \alpha_{ZZ}^2 \cos^2 \theta \rangle$ and $\langle \alpha_{ZX}^2 \cos^2 \theta \rangle$) can be expressed as follows,

$$I_{ZZ}^1 = \frac{B}{7} \frac{N}{4\pi} I_0 \left[(3\alpha_1^2 + 3\alpha_2^2 + 15\alpha_3^2) + (2\alpha_1\alpha_2 + 6\alpha_2\alpha_3 + 6\alpha_1\alpha_3) \right] \quad (A65)$$

and

$$I_{ZX}^1 = \frac{B}{4} \frac{N}{4\pi} I_0 \left[(\alpha_1^2 + \alpha_2^2) + (-2\alpha_1\alpha_2) \right] \quad (A66)$$

Similarly, for case (2) of the anisotropic density distribution function of the concentration of ground state molecules, excited in the perpendicular (Y-polarization) excitation configuration, $n_1^0(\phi, \theta, f)$ (expressed in equation (A45)), the first-order anisotropic correction factors to the Raman scattered intensities I_{YZ}^1 and I_{YX}^1 , using equations (A25) and (A26), with $R(\phi, \theta, 0)$ matrix elements equations (A53) to (A61), to evaluate equation (A64) (i.e., $\langle \alpha_{YZ}^2 \sin^2 \theta \sin^2 \phi \rangle$ and $\langle \alpha_{YX}^2 \sin^2 \theta \sin^2 \phi \rangle$) can be expressed as follows,

$$I_{YZ}^1 = \frac{B}{14} \frac{N}{4\pi} I_0 \left[(5\alpha_1^2 + \alpha_2^2 + 8\alpha_3^2) + (2\alpha_1\alpha_2 - 4\alpha_2\alpha_3 - 12\alpha_1\alpha_3) \right] \quad (A67)$$

and

$$I_{YX}^1 = \frac{B}{8} \frac{N}{4\pi} I_0 \left[(\alpha_1^2 + \alpha_2^2) + (-2\alpha_1\alpha_2) \right] \quad (A68)$$

On comparison of the set of equations for I_{ZZ}^1 , I_{ZX}^1 , I_{YZ}^1 , and I_{YX}^1 which were derived using the complete three-dimensional Euler rotational matrix $R(\phi, \theta, \Psi)$ (equations (A48) to (A51)), with the set of equations which were derived using the (incomplete) two-dimensional Euler rotational matrix $R(\phi, \theta, 0)$ (equations (A65) to (A68)), one can clearly see they are different. Equations (A66) to (A68) of the "two-dimensional" off-diagonal correction factors I_{ZX}^1 , I_{YZ}^1 , and I_{YX}^1 have different coefficients (some zero) for the α_i^2 and $\alpha_i\alpha_j$ terms, and the divisor of the B term, is different for each equation (i.e., 1/4, 1/14, and 1/8) as compared to the corresponding "three-dimensional" off-diagonal correction factors (equations (A49) to (51)), whose α_i^2 and $\alpha_i\alpha_j$ coefficients are non-zero (they span all of α_1 , α_2 , α_3), and whose divisor of the B term is the same throughout (i.e., 1/7).

The root of the differences between the "three-dimensional" first-order anisotropic correction factors I_{ZZ}^1 , I_{ZX}^1 , I_{YZ}^1 , and I_{YX}^1 (equations (A48) to (A51)) and those of the (incomplete) "two-dimensional" set of first-order anisotropic

correction factors (equations (A65) to (A68)) is that in order to achieve complete results one needs to use the Euler rotational matrix that takes into account all three Euler angles (ϕ, θ, Ψ) ("yaw", "pitch", and "roll"), and not to limit the variables of integration to only two Euler angles (ϕ, θ) ("yaw", and "pitch"). If one limits the system to only the two-dimensional (ϕ, θ) case (before carrying out the integrations), and ignoring the "third-dimension" Ψ , "roll" angle, one achieves incomplete results for the expressions of the off-diagonal, first-order anisotropic correction factors I_{ZX}^1 , I_{YZ}^1 , and I_{YX}^1 . If one were to tabulate the "two-dimensional" I_{ZX}^1 , I_{YZ}^1 , and I_{YX}^1 values for the matrices II-VII, and compare them to the corresponding entries in Table 3. and Table 4. that represent the "three-dimensional" case, one would see that they are different, as expected. If one were to use these "two-dimensional" first-order anisotropic correction factors, then the Raman scattered intensities will not be attenuated (reduced) sufficiently, and the calculation of the total depolarization ratio for naturally polarized, ρ_n , and linearly polarized, ρ_l , light will be incomplete (too high).

At this point, the fact that the form of the on-diagonal first-order anisotropic correction factor I_{ZZ}^1 expression in the "two-dimensional" case (equation (A65)) and that of the "three-dimensional" case (equation (A48)) are identical, may on the surface seem confusing, and deserves further explanation. I_{ZZ}^1 depends on the form of $\langle \alpha_{ZZ}^2 \cos^2 \theta \rangle$, which in turn depends on the R_{ZX} , R_{ZY} , and R_{ZZ} matrix elements of the Euler rotational matrix that is used in the similarity transformation $\alpha' = R\alpha R^{-1}$. On inspection of the R_{ZX} , R_{ZY} , and R_{ZZ}

matrix elements of both the three-dimensional Euler rotational matrix $R(\phi, \theta, \Psi)$ (equations (A9) to (A11)), and the two-dimensional Euler rotational matrix $R(\phi, \theta, 0)$ (equations (A59) to (A61)), one can see that their forms are identical. Therefore, due to the fact that their forms are coincident (depend only on (ϕ, θ) and not Ψ), the same coefficients of the α_i^2 and $\alpha_i \alpha_j$ terms, and the divisor of the B term, work out the same in both the $R(\phi, \theta, \Psi)$ and $R(\phi, \theta, 0)$ systems. However, if one were to proceed to derive I_{zx}^1 , I_{yz}^1 , and I_{yx}^1 utilizing $R(\phi, \theta, 0)$ one obtains incomplete expressions, different from the complete expressions derived using the three-dimensional Euler rotational matrix $R(\phi, \theta, \Psi)$ (which takes into account the "yaw", "pitch", and "roll" angles).

END

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