# LASER RAMAN SPECTROSCOPIC AND DIELECTRIC STUDIES OF SOME MOLECULES OF BIOLOGICAL SIGNIFICANCE

## ABSTRACT

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# SYNOPSIS

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This thesis deals with the Laser Raman spectroscopic and dielectric studies of substituted aromatic Schiff bases. The structural and behavioural properties of aromatic Schiff bases make them unique model system to investigate a wide variety of biological phenomena. Our knowledge of these systems may be applied towards better understanding of bilogical processes involving membranes and visual systems. The visual pigments, rhodopsin is composed of the chromophore 11-cis-retinal linked by a Schiff base[1] to the membrane glycoprotein opsin. The absorption bands of the resulting pigment are considerably red-shifted from those of the free chromophore and from protonated and unprotonated Schiff bases formed with retinal in organic solvents. An understanding of the retinal-opsin interactions responsible for the colour of the visual pigments requires information about the retinal-opsin complex. The photoreceptors respond to electrical stimulation in a similar fashion as liquid crystals and are sensitive to changes in the environment. The understanding of the changes due to the variation in environment, thus, becomes important in interpreting biological structures at the molecular level. The understanding of the structural chemistry operating at the biological sites such as the C=N linkage may come through the application of physical and chemical structure probes. Vibrational studies of the protonated and unprotonated Schiff bases reveal that the C=N linkage plays a significant role in controlling biological processes. In several such molecular systems, vibrational spectroscopic studies of the C=N stretching mode was attempted by earlier workers.[2-4] Many Schiff bases are known to behave as liquid crystals[5-8], the present spectroscopic studies may provide basic spectral data for the liquid crystal studies. In addition, it has been proposed by some research workers[9,10] that the Schiff base linkage may be playing role in charge-transfer interactions with a neighbouring peptide unit by creating mobile holes within protein valence bands. The monitoring of the C=N linkage is therefore important for such studies as well. In an attempt to provide more structural information and spectroscopic data on molecules containing the C=N linkage, we have investigated the aromatic Schiff bases having substituent groups of varying chemical character. The molecules studied in the present investigation, essentially contain benzylidene and aniline moiety on either side of the Schiff base linkage. Raman spectroscopic studies assisted by the infrared and electronic absorption spectroscopic techniques have been used to provide information about the conformation of the aromatic Schiff bases and the extent of conjugation in these complex molecules. The benzylidene and the aniline rings of aromatic Schiff bases have been substituted by groups of varying electron donating and accepting properties. The dimethylamino (-$NMe_2$), hydroxy (-OH) and methoxy (-$OCH_3$) groups have been chosen as electron donors whereas the nitro (-$NO_2$)group is used as an acceptor group. By correlating the spectral changes in the electronic absorption band as well as in the relevent Raman bands it has been inferred that the aniline moiety of the aromatic Schiff bases is playing the central role. The benzylidene ring seems to have comparatively less contribution in controlling the position and intensity of the electronic absorption bands in these molecules. These results suggest non-coplanar configuration for the aniline and the benzylidene rings in the aromatic Schiff base molecules.

The normal Raman spectrum is obtained by excitation in a transparent region of the electronic absorption spectrum and in such a case, the Raman-allowed vibrational modes of the scatterers appear, with variable intensity. When the exciting wavelength approaches an electronic absorption band of the sample, intensity of the specific bands may be enhanced. This is pre-resonance Raman effect. The specific site of a complex molecular system can be detected with the needed sensitivity and selectivity via the pre-resonance Raman effect[11-13], provided that the site gives rise to an electronic absorption band whose wavelength can be approached by available excitation sources. Although the potential of the pre-resonance technique is easy to appreciate in general terms, the particular manifestation of the pre-resonance Raman effect varies from one kind of chromophore to another. The intensity variation of the structurally sensitive Raman bands as a function of the exciting wavelength in pre-resonance region can reveal geometric changes and vibronic couplings of the excited molecular states. The significant terms which contribute to the polarizability tensor under pre-resonance conditions are the terms which involve the change in the internuclear distance during electronic transition ($F_A^2$-term) and the vibronic coupling between two electronic excited states ($F_B^2$-term). Raman intensity enhancement through $F_A^2$-term contains contribution from the Franck-Condon factor which depends on the extent to which the excited electronic state potential is displaced along the normal co-ordinate. On the other hand, the contribution of the $F_B^2$-term towards the pre-resonance Raman intensity, depends on the magnitude of the vibronic coupling. The pre-resonance Raman studies on several conjugated molecular systems reveal that the enhancement in Raman intensity is better determined by the $F_B^2$-term rather than the $F_A^2$-term.[11-16] In aromatic Schiff bases, due to the non-coplanar conformation of the benzylidene and the aniline rings, the extent of conjugation is not expected to be high. The vibrational modes involved in the coupling of the electronic states gain intensity as the exciting wavelength progressively approaches the wavelength corresponding to the excited electronic states. The Raman excitation profiles of the selected three bands [ $\nu_{8a}$, $\nu_{8b}$ (Wilson's notation)[17] and $\nu_{C=N}$ mode] in four Schiff bases namely Benzylidene-p-nitroaniline (BPNA), p-dimethylaminobenzylidene-p'-methoxyaniline (DABPMA), p-dimethylaminobenzylideneaniline (DABA) and Benzylidene-O-hydroxyaniline (BOHA) reveal that the C-C stretching vibrational modes of benzenoid moiety $\nu_{8a}$ and $\nu_{8b}$ are dominant in coupling the excited electronic states. The observed low intensity of the Raman band corresponding to the C=N stretching mode indicates weak conjugation in these systems. For all the three modes, theoretical calculations for the intensity variations were done using Albrecht and Hutley's[15] approach. It is observed that in all the four molecules intensity enhancement of the three bands corresponding to $\nu_{8a}$, $\nu_{8b}$ and $\nu_{C=N}$ modes is better determined by the B-term compared to A-term. Therefore, it can be inferred that the vibronic coupling plays a major role in controlling the pre-resonance Raman intensity of bands corresponding to $\nu_{8a}$, $\nu_{8b}$ and $\nu_{C=N}$ vibrations. The vibrational modes in the aniline moiety seem to be dominant in coupling the charge-transfer and $n-\pi^*$ electronic transitions. The Franck-Condon factors, on the other hand, appear to be less important in the pre-resonance region of these systems.

The dielectric behaviour of complex biological molecules in living organism is substantially influenced by the effects associated with dissolved ionic contents and other chemicals whose concentration varies in a wide range. Frequency-dependent polarization and dielectric losses in complex molecular systems embedded in ionic matrix are capable of providing information needed for the understanding of the behaviour of these complex molecules when surrounded by charges. In an attempt to get deeper insight into the dielectric behaviour of molecules which have biological significance, we have studied the frequency-dependent dielectric behaviour of four aromatic Schiff bases namely Benzylidene-p-nitroaniline (BPNA), Benzylidene-m-hydroxyaniline (BMHA), p-dimethylaminobenzylidene-p'-hydroxyaniline (DABPHA) and p-methoxybenzylidene-p'-nitroaniline (PMBPNA), dispersed in KBr-matrix. The solid phase studies were preferred for the present investigation to avoid possible complications of liquid state.

This thesis consists of seven Chapters. Chapter I introduces the problem and also outlines the interpretations and limitations by earlier workers working on relevent and related systems.

Chapter II presents the basic theoretical background of the Raman spectroscopic and dielectric relaxation techniques. The theoretical aspects of these studies have been presented in two parts. The first part of this Chapter deals with the Raman theories with special emphasis on the intensity variation of structurally sensitive vibrational bands as a function of the exciting wavelength under the pre-resonance conditions. The treatment, is based on Albrecht and Hutley's theoretical approach for the calculation of Raman intensity under pre-resonance conditions. The second part of this Chapter is concerned mainly with the theoretical aspects of dielectric relaxation and related physical processes. Brief theoretical treatments of interactions such as charge-dipole and dipole-dipole interactions are also presented in this part of Chapter II.

A short description of the instrumentation and experimental techniques used in the present work is presented in Chapter III. Among the instruments, the Raman spectrometer and the laser sources have been dealt with in detail. In addition, this chapter also contains a brief introduction to the instrumentation for recording the electronic absorption spectra and the IR spectra of the samples. Some of the important specifications of these instruments are presented in tabular form at the end of this chapter. The salient features of the instrument used for dielectric measurements are also presented in this chapter.

The aromatic Schiff bases with which the present investigation is concerned, were synthesized in our laboratory. The purification of the synthesized compounds is of utmost importance in spectroscopic and dielectric studies. The procedure followed for the synthesis and purification of the aromatic Schiff bases, used in present studies, has been outlined in Chapter IV. The H'NMR spectral data were used for confirming the formation of the compounds. The microanalytical technique was applied for estimating the extent of purity in the synthesized samples.

Vibrational assignments are necessary for providing information regarding structural features and are also needed for carrying out systematic vibrational studies. Assignment of the vibrational bands associated with the Schiff base linkage is of special value in the sense that the site containing the Schiff base linkage is of biological importance since the linkage is found to be present in biological systems. The assignment of the C=N stretching vibration of some biological systems like rhodopsin are reported in literature.[3,4] The vibrational assignments for the substituted aromatic Schiff bases containing donor and acceptor groups may throw light on the environmental effects on Raman bands as the substituted aromatic Schiff bases could be treated as model systems for complex biological sites. The details of the band assignments for the four aromatic Schiff bases, are presented in Chapter V.

Chapter VI contains the theoretically calculated Raman intensity factors $F_A^2$ and $F_B^2$ of Albrecht and Hutley's theory, for three well-resolved Raman bands corresponding to $\nu_{8a}$, $\nu_{8b}$ and $\nu_{C=N}$ modes of vibration. The experimentally measured values of relative Raman intensities of the bands corresponding to the above mentioned vibrational modes are also presented at various exciting wavelegths. Both the theoretically calculated values and the experimentally measured values of the intensity factors are normalized with respect to the exciting wavelength $\lambda_o = 4880$ Å and the normalized Raman ointensity factors $R_A$, $R_B$ and $R_I$

corresponding to the $F_A^2$, $F_B^2$ terms and the experimentally observed values are compared in Tables VI-1-4. In addition, the electronic absorption spectra of these molecules are presented in this chapter as they are required for plotting the intensity variation as a function of wavelength which is known as excitation profile. The interpretation of the electronic absorption spectra has also been given in order to identify electronic transitions which are likely to be mixed. The Raman spectra in conjunction with electronic absorption spectra indicate that in these four aromatic Schiff bases the benzylidene and the aniline rings do not have coplanar configurations. X-ray crystallographic analysis shows that trans-azobenzene and trans-stilbene molecules which are iso-$\pi$-electronic with the aromatic Schiff bases have a planar or nearly planar structure.[18,19] Comparative study with these molecules clearly indicates that the Schiff base molecules are not highly conjugated systems and suggests that the molecule is twisted around the C=N bond thus leading to a non-coplanar conformation. The non-coplanarity of the benzylidene and the aniline rings, therefore, divides the molecule into two weakly interacting conjugated fragments. One of the $\pi$-systems is probably localized at the aniline moiety containing the nitrogen lone pair whereas the other $\pi$-system is localized at the benzylidene moiety. It has been observed that the substitution in the aniline ring by hydroxyl group in the ortho-position enhances the relative Raman intensity of the band corresponding to the C=N stretching mode whereas substitution in the para-position appears to be less effective, in enhancing such conjugation in the aromatic Schiff base molecules. The non-coplanarity in the aromatic Schiff base molecules is also found to be in agreement with the X-ray and electron-diffraction studies.[20,21]

Chapter VII presents the experimental dielectric data of four Schiff bases i.e. BPNA, BMHA, DABPHA and PMBPNA in solid phase, dispersed in the strong ionic matrix of Potassium Bromide (KBr). All the four Schiff bases are observed to exhibit dielectric relaxation at frequencies below 5KHz. The frequency-dependent data of these molecular systems, reveal that mainly two physical processes are prominent in governing the dielectric behaviour in these complex molecules. This behaviour is, however, a function of the molecular structure and the relaxation frequency varies from molecule to molecule. The relaxation frequencies are observed around 1KHz and 4KHz for BMHA and DABPHA respectively. However, for BPNA and PMBPNA, the relaxation frequency is around 100Hz. The variation of the dissipation factor as a function of frequency reveals that in all the four systems the frequency region below the relaxation frequency is dominated by the electronic type of nomadic polarization which arises mainly due to the long range delocalization of the $\pi$-electrons in these systems. In the frequency-region above the observed relaxation frequency the time allowed is not sufficient for delocalization of $\pi$-electrons and hence, the nomadic polarization ceases to respond to the applied electric field. In this frequency region, the motion of molecular fragments seems to play role towards the dielectric properties of these dispersed systems. Due to the randomly distributed ionic environment around the Schiff base molecules, the applied electric field suffers spatial non-uniformity leading to the electrostatic strain in sensitive molecular fragments. The motion of these molecular fragments with respect to the applied electric field seems to be important in controlling the high frequency dielectric behaviour. The chemical nature and the position of the substituent-groups in these molecules appeared to contribute significantly towards the dielectric properties in the higher frequency region, presently investigated.

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