ADDIS ABABA UNIVERSITY
SCHOOL OF GRADUATE STUDIES
DEPARTMENT OF CHEMISTRY

Tuning Fluorescence Properties of 2,2’-Bipyridine Using Metal Cations

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By
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July, 2010

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Declaration

This project is my original work, has not been presented for a degree in this and other university and all resources and materials used for this project have been duly acknowledged.

Name: Aklilu Guale

Signature ________________

The project has been submitted for examination with my approval as project advisor.

Name Signature

Place and date of submission:

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Addis Ababa University
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Acronyms and abbreviations

DFT	Density Functional Theory
B3LYP	Becke, three-parameter, Lee-Yang-Parr
CPCM	Conductor-like Polarizable Continuum Model
STO-3G	Slater-Type Orbital approximated by 3 Gaussians
UV-Vis	Ultraviolet-Visible

Abstract

The steady state absorption, fluorescence excitation and emission spectra of 2,2’-bipyridine have been studied in polar solvents. Absorption spectra are almost independent of solvent polarity. The dependence of fluorescence emission on excitation wavelength was observed in acetonitrile and p-dioxane but independent of excitation energy in tetrahydrofuran. Addition of metal ion and varying pH of the solution shifted the absorption band to the red-edge and intensity change was observed in fluorescence excitation spectra. Corresponding excitation bands of the metal free conformers were compared to the stable conformers of metal-chelated and protonated 2,2’-bipyridine.

Study of the rotational profile of 2,2’-bipyridine at DFT/B3LYP/STO-3G level shows two energy minima corresponding to two conformations of different energy, cis/trans structures, with barrier to rotation greater than the room temperature thermal energy.

It is shown that distributions of energetically different conformers of the molecule (cis/trans) in the ground state are responsible for the dual fluorescence behavior of the systems.

1. INTRODUCTION

Spectroscopic studies of dual fluorescent organic systems and their photophysics and photochemistry have been an engaging field of research for the past decade. Heteroaromatic compounds containing oxygen and nitrogen chromophores exhibit various properties in their excited states, such as twisted intramolecular charge transfer, intramolecular excimer formation, intramolecular exciplex formation and other different intramolecular phenomena. It is well documented that the rotation of the more flexible part of a bichromophoric molecule acts as a main fluorescence quencher of the system [1]. Presence of free rotation about a sigma bond between chromophores leads to the adoption of several different three-dimensional shapes called conformations. Because each of the various conformations of a molecule has different properties, the conformation the molecule normally adopts has a profound influence on its physical and chemical properties. 2,2'-bipyridine has electronic structure similar to those groups of compounds, which have been proved to have the above-mentioned properties. Attributed to its tendency to exist in cis/trans forms in equilibrium, 2,2'-bipyridine is expected to have dual emission bands from the different conformation forms both in the ground and in excited states.

Among the different isomers of bipyridines, 2,2'-bipyridine has been most extensively studied azaaromatic system having a wide range of applications. The photophysics and photochemistry of metal-2,2'-bipyridine complexes attracted researchers, with particular interest in their application to solar energy conversion, and a modeling intermediates in transition-metal catalyzed reactions. It is also widely used in preparative and analytical chemistry owing to its ability to form metal complexes. In general, bipyridines are of interest in connection with their appliccations as the basis of herbicides, their use in obtaining biologically active materials, their use in medicine and as a building block in supra-molecular chemistry [2, 3]. Therefore, 2,2'-bipyridine has a rich chemistry in which interest is currently growing. 2,2-Bipyridine can be prepared by the dehydrogenation of pyridine using Raney nickel as catalyst [4].

The compound is investigated experimentally by optical spectroscopic techniques and theoretically using computational methods.

1.1. Literature Review

2,2’-bipyridine strongly absorbs in the ultraviolet region of electromagnetic radiation in most polar and non-polar solvents exhibiting two intense bands. Reports on the luminescence in pyridine and its derivatives have been a subject of controversy for many years. As it was reviewed by Castellucci and his co-workers most of the earlier papers reported that 2,2’-bipyridine shows very weak fluorescence emission in nonpolar solvents which can be hardly detected due to low quantum yield and subnanosecond lifetime whereas phosphorescence occurs at low temperature with a rather large quantum yield [5, 6]. Further investigations carried out by Castellucci and his co-workers shows that 2,2’-bipyridine fluoresces both in nonpolar (cyclohexane) and polar (methanol) solvents at 298 K and also accounted for the low quantum yield observed.

Although all molecules are capable of absorption, fluorescence is not observed in large number of compounds. Fluorescence is generally observed in those organic molecules that have rigid framework and not many loosely coupled substituents through which vibronic energy can flow out. From structural point of view, 2,2’-bipyridine presents two important features that reflect its elusive photophysical properties. The first one is the presence of relatively close-lying $n\rightarrow\pi^*$ and $\pi\rightarrow\pi^*$ excited states which can be coupled vibronically out-of-plane vibrations with subsequent modification of the potential energy surface and mixed spectral and photophysical properties. It is well-known that the solvent plays an important role in these effect by influencing the energy gap between the states involved and largely differentiating 2,2`-bipyridine from the parent molecule, biphenyl. The second important feature is the presence of a single bond connecting two azaaromatic moieties, with the possibility of rotation of the rings to reach the minimum-energy conformation with relatively small barriers to be overcome. Related to this feature is the presence of rotamers (rotational isomers) with different possible spectral and photophysical properties. The presence of rotational isomers has recently been evidenced for many ethylene-substituted molecules [5].

The change in geometry results when 2,2`-bipyridine and other azaaromatics coordinate with metal ions like $Zn^{2+}$ changes the photophysical properties of the ligand [3, 7-11]. A red shifted absorbance spectrum has been observed for the $Zn^{+2}$ complexes with 2,2’-bipyridine [7]. Furthermore, titration of the 2,2`-bipyridine with acid resulted in the pKa values of various ground state forms [12]. It is also reported that addition of water to the solutions of 2,2’-bipyridine in acetonitrile (or dimethylformaldehyde) produces the covalent hydrate, 2,2’-bipyridine H_2O that has its own characteristic spectra [12].

Many of the literatures reviewed propose the cis/trans forms of 2,2’-bipyridine [IA and IB] based on both experimental and theoretical results [10, 2]. The X-ray analysis [13] indicates that the molecule has a trans-planar configuration in the crystalline state. NMR and dipole moment measurements predicted that the molecule is trans in solution. In fact, dipole moment observed has non-zero value which was accounted as either due to the slight non-planarity, i.e., small twisting around the central carbon-carbon bond of the trans conformation or due to the mixture of trans and cis conformations in solution [2].

Due to the more stability of the trans form, there are little information on the properties of the metal-free cis conformer. There are few reports on the properties of the metal-free cis conformer. Luminescence and electron magnetic resonance (EPR) spectra of metal free cis conformers of 2,2’-bipyridine and methyl substituted 2,2’-bipyridines were observed in mixtures of water with 2-propanol at 77 K [5]. Ikemoto et. al. have also shown the absorption, luminescence and luminescence-excitation spectra and fluorescence lifetime originating from the metal free cis conformer of 6,6’-dimethyl-2,2`-bipyridine at 77 K by using 2-propanol-water mixture as a host.

(IA)

(IB)

Scheme 1: Trans (IA) and cis (IB) conformers of 2,2`-bipyridine

Though there are a theoretical indications based on ab-initio calculations and semi-empirical methods that predict the existence of two minimum energy conformers, cis-like and trans, for both neutral molecule and its protonated cations [2, 14], there are few experimental results on the emission properties of the metal free cis conformer of 2,2’-bipyridine. The ultimate goal of this work is therefore, to investigate the existence of the ground state cis conformer and its fluorescence properties in polar solvents.

1.2. Optical Absorption Spectra

Absorption spectra are graphs of intensity of absorbed radiation versus the wavelength of radiation. Electromagnetic radiation in the UV-Vis region enables one to analyze electronic structure of compounds because the energy of electromagnetic radiations in this region is able to cause electronic transition. The energy of photon absorbed is related to the extent of conjugation and other functional groups of the compound.

The visible region of the spectrum comprises photon energies of 36 to 72 kcal/mole, and the near ultraviolet region, extends this energy range to 143 kcal/mole. Ultraviolet radiation having wavelengths less than 200 nm is difficult to handle, and is seldom used as a routine tool for structural analysis. The energies noted above are sufficient to promote or excite a molecular electron to a higher energy orbital. Consequently, absorption spectroscopy carried out in this region is sometimes called "electronic spectroscopy". An absorption spectrum will show a number of absorption bands corresponding to structural groups within the molecule [15].

Types of electronic transitions in polyatomic molecules

Generally, energetically favored electron promotion will be from the highest occupied molecular orbital (HOMO) to the lowest unoccupied molecular orbital (LUMO), and the resulting species is called an excited state. Different molecules absorb radiation of different wavelengths. The kinds of transition that occurs in order of increasing energy are $n \rightarrow \pi^*$, $\pi \rightarrow \pi^*$, $n \rightarrow \sigma^*$, $\sigma \rightarrow \pi^*$ and $\sigma \rightarrow \sigma^*$ [1, 16].

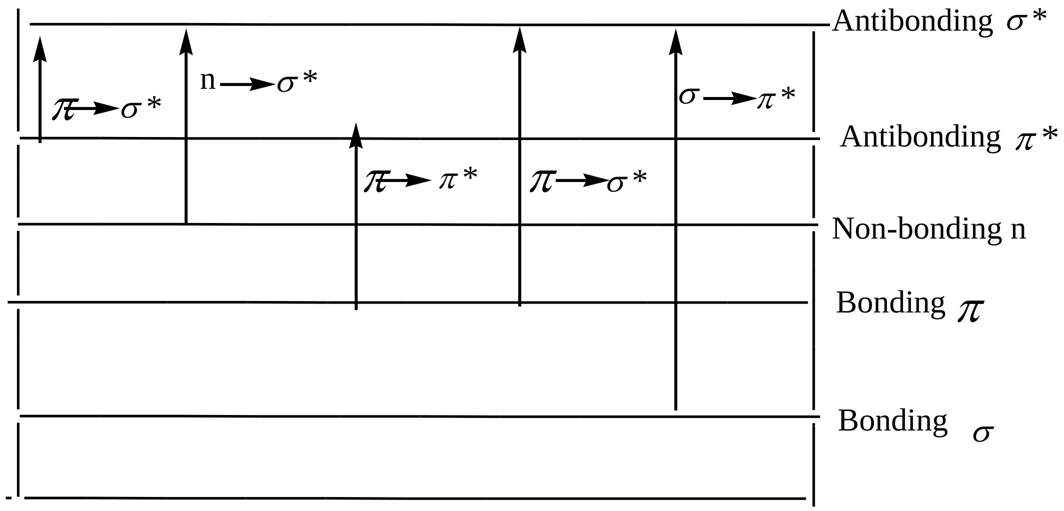


Figure 1: Diagram showing possible electronic transitions of n, π and σ electrons.

Beer–Lambert Law and Oscillator Strength

Experimentally, the efficiency of light absorption at a wavelength λ by an absorbing medium is characterized by the absorbance A(λ) or the transmittance T(λ), defined as

$$A(\lambda) = \log\frac{I_\lambda^o}{I_\lambda}=-\log T(\lambda) \qquad \text{and} \qquad T(\lambda) = \frac{I_\lambda}{I_\lambda^o} \qquad (1)$$

Where I_λ^o and I_λ are the light intensities of the beams entering and leaving the absorbing medium, respectively. In many cases, the absorbance of a sample follows the Beer–Lambert Law

$$A(\lambda) = \log\frac{I_\lambda^o}{I_\lambda} = \varepsilon(\lambda)\ell c \qquad (2)$$

where ε(λ) is the molar (decadic) absorption coefficient (commonly expressed in m^2mol^{-1}), c is the concentration (in $molm^{-3}$) of absorbing species and ℓ is the optical pathlength (in m). Failure to obey the linear dependence of the absorbance on concentration, according to the Beer–Lambert Law, may be due to aggregate formation at high concentrations or to the presence of other absorbing species [16].

The (decadic) absorption coefficient α(λ) is the absorbance divided by the optical pathlength, ℓ:

$$\alpha(\lambda) = \frac{A(\lambda)}{l} = \frac{1}{l}\log\frac{I_\lambda^o}{I_\lambda} \quad \text{or} \quad I_\lambda = I_\lambda^o 10^{-\alpha(\lambda)l} \qquad (3)$$

Physicists usually prefer to use the Napierian absorption coefficient α(λ)

$$\alpha(\lambda) = \frac{1}{l}\ln\frac{I_\lambda^o}{I_\lambda} = a(\lambda)\ln 10 \quad \text{or} \quad I_\lambda = I_\lambda^o e^{-\alpha(\lambda)l} \qquad (4)$$

Because absorbance is a dimensionless quantity, the SI unit for a and α is m^{-1}, but cm^{-1} is often used.

Finally, the molecular absorption cross-section σ(λ) characterizes the photon-capture area of a molecule. Operationally, it can be calculated as the (Napierian) absorption coefficient divided by the number N of molecular entities contained in a unit volume of the absorbing medium along the light path:

$$\sigma(\lambda) = \frac{\alpha(\lambda)}{N} \qquad (5)$$

The molecular absorption cross-section can also be calculated from the experimental value of ε using the following relation:

$$\sigma = \frac{2.303\varepsilon}{N_a} = 3.825\text{x}10^{-19}\varepsilon \text{ (in cm}^2\text{)} \qquad (6)$$

The molar absorption coefficient, ε (λ), expresses the ability of a molecule to absorb light in a given solvent. In the classical theory, molecular absorption of light can be described by considering the molecule as an oscillating dipole, which allows us to introduce a quantity called the oscillator strength, which is directly related to the integral of the absorption band as follows:

$$f = \frac{2303(mc_0^2)}{N_a \pi e^2 n}\int \varepsilon(\bar{\nu})\, d\bar{\nu} = \frac{4.32\text{x } 10^{-9}}{h}\int \varepsilon(\bar{\nu})\, d\bar{\nu} \qquad (7)$$

where m and e are the mass and the charge of an electron, respectively, c_0 is the speed of light, n is the index of refraction, and $\bar{\nu}$ is the wavenumber (in cm^{-1}). f is a dimensionless quantity and values of f are normalized so that its maximum value is 1

The Franck–Condon principle

According to the Born–Oppenheimer approximation, the motions of electrons are much more rapid than those of the nuclei (i.e. the molecular vibrations). Promotion of an electron to an antibonding molecular orbital upon excitation takes about 10^{-15} s, which is very quick compared to the characteristic time for molecular vibrations (10^{-10} –10^{-12} s). This observation is the basis of the Franck–Condon principle: an electronic transition is most likely to occur without changes in the positions of the nuclei in the molecular entity and its environment. The resulting state is called a Franck–Condon state, and the transition is called vertical transition

At room temperature, most of the molecules are in the lowest vibrational level of the ground state. In addition to the ‘pure’ electronic transition called the 0–0 transition, there are several vibronic transitions whose intensities depend on the relative position and shape of the potential energy curves (Figure 2)

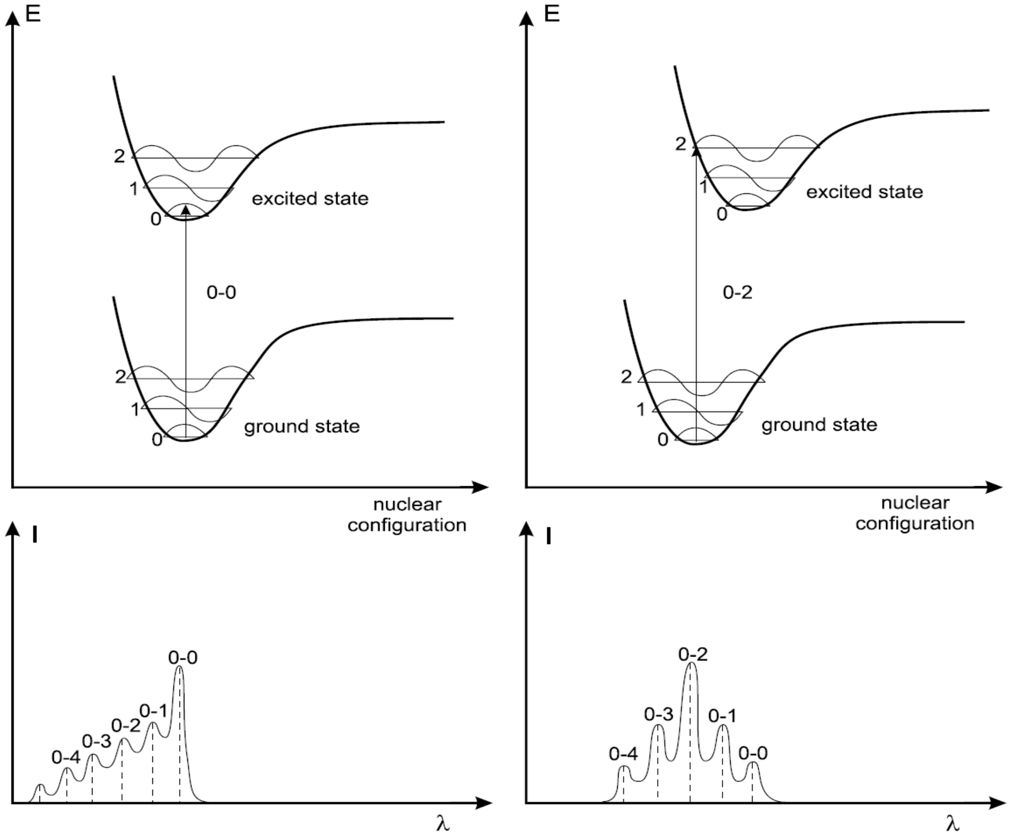


Figure 2: Top: Potential energy diagrams with vertical transitions (Franck–Condon principle).Bottom: shape of the absorption bands; the vertical broken lines represent the absorption lines that are observed for a vapor, whereas broadening of the spectra is expected in solution (solid line)

Isosbestic Points

In spectroscopy, an isosbestic point is a specific wavelength at which two chemical species have the same molar absorptivity (ε) or more generally are linearly related. This occurs because the two substances absorb light of that specific wavelength to the same extent, and the analytical concentration remains constant. Isosbestic points are commonly met when electronic spectra are taken (a) on a solution in which a chemical reaction is in progress (in which case the two absorbing components concerned are a reactant and a product, A + B), or (b) on a solution in which the two absorbing components are in equilibrium and their relative proportions are controlled by the concentration of some other component, typically the concentration of hydrogen ions, e.g., an acid-base indicator equilibrium. The effect may also appear (c) in the spectra of a set of solutions of two unrelated non-interacting components having the same total concentration.

A pair of substances can have several isosbestic points in their spectra. When a 1-to-1 (one mole of reactant gives one mole of product) chemical reaction (including equilibria) involves a pair of substances with an isosbestic point, the absorbance of the reaction mixture at this wavelength remains invariant, regardless of the extent of reaction (or the position of the chemical equilibrium). The requirement for an isosbestic point to occur is that the two species involved are related linearly by stoichiometry, such that the absorbance is invariant for one particular wavelength. Thus other ratios than one to one are possible. The presence of an isosbestic point typically does indicate that only two species that vary in concentration contribute to the absorption around the isosbestic point [16].

1.2.1. Factors Affecting Absorption Spectra

Different factors affect the absorption spectra of compounds. Intensities, position and shape of absorption bands are affected by temperature, solvent, PH of the media. Under this heading the effect of solvent will be treated briefly.

Effect of Solvent

When absorption spectra are measured in solvents of different polarity, it is found that these solvents usually modify the position, intensities, and shapes of the absorption bands [15]. Since solvent-solute interactions can change the geometry, the electronic structure, and the dipole moment of a solute, UV-Vis absorption or/and emission (fluorescence) band positions of solvent-sensitive dyes will vary with the polarity of the medium. This phenomena is called solvatochromism.[17] These changes are a result of physical intermolecular solute-solvent interaction forces (such as ion-dipole, dipole-dipole, dipole-induced dipole, hydrogen bonding, etc.), which above all tend to alter the energy difference between ground and excited state of the absorbing species containing chromophore. The medium influence on absorption spectra can be considered by comparing the spectral changes observed (a) on going from the gas phase to solution, or (b) simply by changing the nature of the solvents. Because of the difficulty in measuring the absorption spectrum in the gas phase, the second method is used most of the times.

The term solvatochromism is used to describe the pronounced changes in position (and sometimes intensity) of a UV-Vis absorption band that accompanies a change in the polarity of the medium. The band shift observed with increasing solvent polarity can be either to the higher energy band called a hypsochromic (or blue) or to a lower energy side called a bathochromic (or red) shift. Solvent affect absorption band by altering the energy difference between the ground and excited states of the absorbing species. In most cases the dipole moment of the molecule in the ground state is different from that of the excited state. If the dipole moment of the molecule in the ground state is greater than that of the excited state, polar solvents stabilize the molecule in the ground state than they do in the excited state resulting in hypsochromic shift. However, if the dipole moment of the excited state is greater than that of the ground state, more polar solvents stabilize the excited state more and consequently bathochromic shift is observed.

Solvents also affect absorption spectra of molecules, which are able to exist in different resonance structures by preferentially stabilizing one kind of structure over the other so that it predominates and its characteristic spectra will be observed [18]. Changing solvent polarity also causes a dramatic change in molecular geometry. This can be shown by calculating the changes in bond length and net π-electron charges with varying solvent polarity. In general, with changing the polarity of solvents the electronic structure of compounds is changed and consequently the absorption bands are changed

1.3. Fluorescence Spectra

Generation of luminescence through excitation of a molecule by ultraviolet or visible light photons is a phenomenon termed photoluminescence, which is formally divided into two categories, fluorescence and phosphorescence, depending upon the electronic configuration of the excited state and the emission pathway [16]. Fluorescence is a luminescence, in which absorption of a photon of radiation by molecular causes the emission of photon at a longer wavelength. The energy difference between the absorbed and emitted photon ends up as molecular vibrations or heat or other excited state processes. Attributed to the energy loss in the excited state by the processes mentioned above the absorbed and emitted photon by the molecule are in different region of electromagnetic radiation. The energy gap between the two processes depends on the nature of the fluorophore, temperature, solvent, PH of the media, etc. At room temperature, most molecules occupy the lowest vibrational level of the ground electronic state, and on absorption of light they are elevated to produce excited states.

The simplified Perrin–Jablonski diagram below shows summarizes the photophysical events in a molecule which is convenient for visualizing in a simple way the possible processes.

The vertical arrows corresponding to absorption start from the 0 (lowest) vibrational energy level of S_0 because the majority of molecules are in this level at room temperature. Fluorescence and phosphorescence always originate from thermally equilibrated, electronically excited molecules. Thermal equilibration corresponds to all molecules being in the lowest vibrational level (v=0) [12].

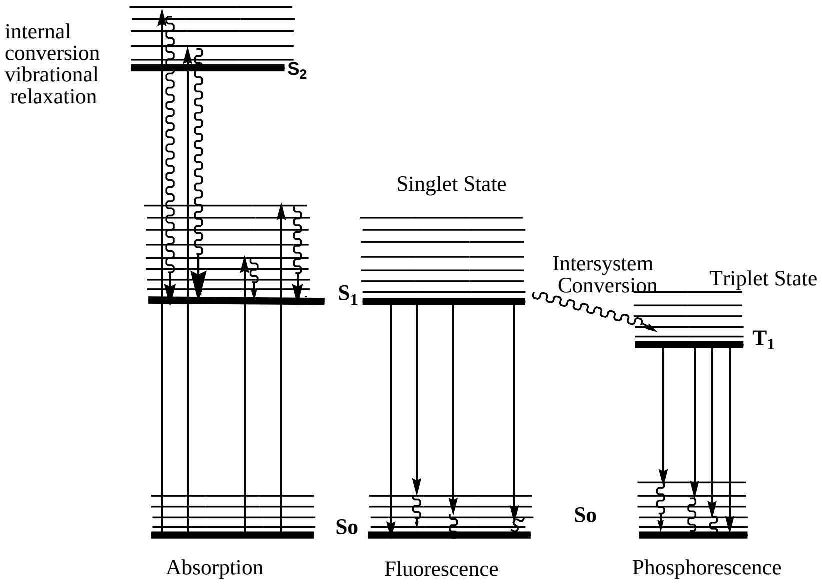


Figure 3: Schematic diagram of transitions giving rise to absorption and fluorescence emission spectra.

Two basic types of spectra can be produced by a fluorescence spectrometer [18, 19]. In a fluorescence spectrum, or emission spectrum, the wavelength of the exciting radiation is held constant (at a wavelength at which the analyte absorbs) and the spectral distribution of the emitted radiation is measured. In an excitation spectrum, the fluorescence signal, at a fixed emission wavelength, is measured as the wavelength of the exciting radiation is varied. Because an analyte can fluoresce only after it has absorbed radiation, an excitation spectrum identifies the wavelengths of light that the analyte is able to absorb. Thus, subject to certain constraints, the excitation spectrum of a molecule should be the same as its UV-Vis absorption spectrum. The excitation spectrum for a compound should not change as the emission wavelength is varied. Whenever the excitation spectrum varies with choice of emission wavelength, there is good reason to believe that two or more different substances are responsible for the observed fluorescence. Fluorescence occurs when a molecule excited by absorption of radiation relaxes to its ground state.

Excitaion: $S_o + h\nu \rightarrow S_1$

Emission: $S_1 \rightarrow S_o + h\nu`$

where hν is photon energy. State S_0 is called the ground state of the fluorophore (fluorescent molecule) and S_1 is its first (electronically) excited state.

The specific frequencies of exciting and emitted light are dependent on the particular system. The excited electron can revert to the ground state in several. It can undergo 'non-radiative relaxation' in which the excitation energy is dissipated as heat (vibrations) to the solvent. Excited organic molecules can also relax via conversion to a triplet state which may subsequently relax via phosphorescence or by a secondary non-radiative relaxation step. Relaxation of an S1 state can also occur through interaction with a second molecule through fluorescence quenching. Molecules that are excited through light absorption or via a different process (e.g. as the product of a reaction) can transfer energy to a second 'sensitizer' molecule, which is converted to its excited state and can then fluoresce [20, 22].

The correspondence of emission and excitation wavelength can be shown in contour plot. A contour plot is a graphical technique for representing a 3-dimensional surface by plotting constant z slices, called contours, on a 2-dimensional (x,y) format. Contours are lines connecting points of equal intensity. The contour plot is an alternative to a 3-D surface plot. The contour plot is formed by:emission wavelength as vertical axis (independent variable 2), excitation wavelength as horizontal axis (independent variable 1) and contour lines (iso-response values) connecting the excitation and emission wavelengths.

Isoemissive point

Isoemissive point is defined as the wavelength or frequency at which all the emission spectra observed with a change in the parameter (time, temperature, pH, concentration etc.) have the same intensity. Isoemissive point in fluorescence spectroscopy is analogous to the isosbestic point in the absorption spectroscopy except that isosbestic point refers to two absorptive species in the ground state whereas isoemissive point refers to two emissive species in the excited state

1.3.1. Factors Affecting Fluorescence Spectra

A variety of environmental and intramolecular factors affect fluorescence emission, including interactions between the fluorophore and surrounding solvent molecules, other dissolved inorganic and organic compounds, temperature, pH, and the localized concentration of the fluorescent species [18, 19, 22]. The effects of these parameters vary widely from one fluorophore to another, but the absorption and emission spectra, as well as quantum yields, can be heavily influenced by environmental variables. In fact, the high degree of sensitivity in fluorescence is primarily due to interactions that occur in the local environment during the excited state lifetime.

Intramolecular Effects

The molecular structure of a fluorophore can have an effect on the intensity of fluorescence. For example, structural rigidity plays a key role in the fluorescence intensity of a chromophore with a more rigid structure typically resulting in enhanced fluorescence, whereas lack of rigidity results in enhanced internal conversion and an increase in radiationless decay [22]. If a molecule has a large number of modes of vibration in the lower state, then the lower vibrational modes of the higher electronic state will be able to excite higher vibrational modes of the lower electronic state with high probability. Hence, vibrational dissipation of excitation energy and thus internal conversion will be favored [12]. Structural modifications such as the addition of substituents may influence the fluorescence properties of the compound due to enhanced rates of non-radiative processes, which depend upon the nature and position of the substituent, can alter both the emission profile as well as the fluorescence intensity. Observed changes include additional bands due to vibrational transitions and bathochromic shifts in the absorption and emission spectrum because of an increase in the extent of the π-electron configuration.

Solvent Effect

Similarly, to absorption spectroscopy, there are solvent effects on the spectral properties of fluorescent molecules. Changes in the fluorescence emission of a molecule can be caused by solvents of different polarity, of different dielectric constant or of different polarizability. Once the excited state of a molecule is formed in solution, the excited state molecule interacts to varying degrees with surrounding solvent molecules, depending on their polarity, before returning to the ground state. These excited-state solute-solvent interactions of fluorescent molecules are often reflected in the spectral position and shape of the emission bands as well as in the lifetimes of the excited state molecules [15].

Solvent dependence of fluorescence emission position is sometimes called solvatofluorochromism. The 0 → 0 transition is generally the same for absorption and emission. According to stoke`s rule, the fluorescence maximum is always located at lower wave numbers (higher wavelength) than absorption maximum because of the loss in energy due to vibrational relaxation. The gap between the maximum of the first absorption band and the maximum of the corresponding fluorescence band is called stokes shift, and is usually expressed in wave numbers as $\Delta\bar{\nu} = \bar{\nu}_a - \bar{\nu}_f$. This stokes shift provides valuable information on the excited state. When the dipole moment of a fluorescent molecule is larger in the excited state than in the ground state i.e., $\mu_e - \mu_g$, the differential solvation of the two states by solvents of varying polarity gives rise to an increase in stokes shift with increasing solvent polarity. When considering the solvent dependence of the position of emission bands, the finite relaxation time, τ_R for the rearrangement of the solvent molecules surrounding the solute molecule in the Franck-Condon excited state and the finite lifetime, of the molecule in the excited state need to be considered. In the case of $\tau_R > \tau_{ex,}$, the emission will occur before any arrangement of solvent molecules in the solvation shell takes place. The initial state of emission process is the Franck-Condon excited state and the final state is the equilibrium ground state. Hence, the wave number of emission will be equal to the wave number of corresponding absorption. In the case of $\tau_R < \tau_e$, reorientation of the solvent molecules can take place after electronic excitation and a relaxed excited state is obtained in which another solvation equilibrium has been established. It is from this equilibrium state that fluorescence occurs at room temperature.

After the fluorophore has been excited to higher vibrational levels of the first excited singlet state S_1, excess vibrational energy is rapidly lost to surrounding solvent molecules as the fluorophore slowly relaxes to the lowest vibrational energy level (occurring in the Pico second time scale). Solvent molecules assist in stabilizing and further lowering the energy level of the excited state by re-orienting (termed solvent relaxation) around the excited fluorophore in a slower process that requires between 10 and 100 Pico seconds. This has the effect of reducing the energy separation between the ground and excited states, which results in a red shift (to longer wavelengths) of the fluorescence emission. Increasing the solvent polarity produces a correspondingly larger reduction in the energy level of the excited state, while decreasing the solvent polarity reduces the solvent effect on the excited state energy level. The polarity of the fluorophore also determines the sensitivity of the excited state to solvent effects. Polar and charged fluorophores exhibit a far stronger effect than non-polar fluorophores [15].

A general explanation of solvent effects on fluorescence spectra is based on the differential solvation of the fluorescent molecules in their ground and excited states, mediated by various non-specific and specific intermolecular forces acting between the solute and solvent. The strength of such a force depends up on the similarity in the polarity of the solvent and the fluorophore.

Temperature Effects

Changes in temperature affect the viscosity of the medium and hence the number of collisions of the molecules of the fluorophore with solvent molecules and the strength of hydrogen bonding. Fluorescence intensity is sensitive to such changes and the fluorescence of certain fluorophores shows temperature dependence. In such cases, the use of thermostatted cell holders is to be recommended. Normally, it is sufficient to work at room temperature with the provision that any sample procedure involving heating or cooling must also allow sufficient time for the final solution to reach ambient before measurement [22, 23].

pH Effects

Relatively small changes in pH will sometimes radically affect the intensity and spectral characteristics of fluorescence. Most phenols are fluorescent in neutral or acidic media, but the

presence of a base leads to the formation of nonfluorescent phenate ions. 5-hydoxyindoles for, example, show a shift in fluorescence emission maximum from 330 nm at neutral pH to 550 nm in strong acid without any change in the absorption spectrum [19, 24].

Coordination with Metal Ions

Coordination with a metal ion can also have a profound influence on the fluorescence characteristics of the molecule. Many transition metal ions have electrons that can be promoted to an orbital of the chromophore causing mixing of the electronic states that result in the appearance of charge transfer bands in the fluorescence spectrum. Coordination of fluorescing or phosphorescing aromatic ligand by metal ion is actually an acid base reaction, with the metal ion acting as a Lewis acid and the ligand acting as a Lewis base. In this regard, the coordination of ligand by metal ion is analogous to the protonation of the ligand, in which case, the hydrogen ion functions as the Lewis acid. The coordination of aromatic ligands by non-transition metal ions with closed shell diamagnetic metal ions (e.g. Zn^{2+}, Cd^{2+}, Al^{3+}, Ga^{3+}) fluoresce strongly, with fluorescence spectra similar to the chromophore. Coordination with these metal ions has also the effect of producing positive polarization at the coordination sites on the ligand and the spectral shifts are produced by coordination with these ions are similar to shifts produced by protonating the ligand at the coordination sites. Paramagnetic metal ions however, are found to interact strongly with the atomic orbitals of the molecules, resulting in quenching of the fluorescence due to increased efficiency of intersystem crossing, a direct consequence of unpaired electrons in the d-orbitals [3, 7-10,12].

Concentration (Inner-Filter) Effects

Fluorescence intensity will be reduced by the presence of any compound that is capable of absorbing a portion of either the excitation or emission energy [20]. At high concentrations this can be caused by absorption due to the fluorophore itself. More commonly, particularly when working with tissue or urine extracts, it is the presence of relatively large quantities of other absorbing species that is troublesome. The purpose of extraction procedures is usually to eliminate such species so that the final measurement is made upon a solution essentially similar to the standard. At low concentration the emission of light is uniform from the front to the back of sample cuvette. At high concentration more light is emitted from the front than the back. Since emitted light only from the middle of the cuvette is detected the concentration must be low.

Quenching

Decrease of fluorescence intensity by interaction of the excited state of the fluorophore with its surroundings is known as quenching [20]. Quenching is any process in which a sample constituent decreases the fluorescence quantum yield for the analyte. Among the most common fluorescence quenchers is O2; removal of oxygen from a sample before fluorometric analysis is often advisable. One-way that fluorescence quenching can occur is intermolecular electronic energy transfer:

$$A^* + B \rightarrow A + B^*$$

Here an excited analyte molecule (A*) transfers excitation energy to a quencher molecule B, causing de-excitation of A and forming an excited quencher molecule, B*. If B* is a fluorescent species, its fluorescence (called sensitized fluorescence) may then be observed. This phenomenon can allow one to observe fluorescence from a molecule (B) that may be difficult to excite directly. More often, however, these processes are a nuisance. Not only do they cause a decrease in the fluorescence signal observed for a given concentration of analyte (A) in the sample, but also they may produce unwanted background fluorescence signals [25].

Adsorption, Photodecomposition and Oxidation

Loss of organic substances by adsorption onto the walls of the container becomes particularly troublesome at the sub-microgram level. New glass surfaces should be thoroughly cleaned in acid before use and measurements of aromatic substances in nonpolar solvents should be avoided. Often the addition of a small quantity of a polar solvent to a non-polar one will greatly reduce adsorption losses. Fluorimeters employ intense light sources to produce high sensitivity and in some cases the level of incident light may be sufficient to decompose the sample under investigation.

1.4. Computational Method

Potential Energy Surface

A potential energy surface (PES) is a geometric hypersurface on which the potential energy of a set of reactants is plotted as a function of the coordinates representing the molecular geometries of the system. The PES is the most complete description of all the conformers, isomers, and energetically accessible motions of a system. Minima on this surface correspond to optimized geometries. The lowest-energy minimum is called the global minimum. There can be many local minima, such as higher-energy conformers or isomers.

Optimization

The process of locating and computing the structure of an energy minimum or saddle point on the potential energy surface is usually referred to as geometry optimization. Geometry optimization methods start with an initial geometry and then change that geometry to find a lower-energy shape. This usually results in finding a local minimum of the energy. This local minimum corresponds to the conformer that is closest to the starting geometry [26].

2. OBJECTIVE, MATERIALS AND METHOD

2.1. Objectives

The ultimate goal of this project is to give confirmation and evidence on the existence of the ground state isomeric structures in equilibrium. Specifically, determination of absorption spectra, excitation and emission spectra by regulating emission/ excitation energy, use of metal ions, pH effect, and with computational results.

2.2. Materials and Method

2,2’-bipyridine (Sigma-Aldrich) was used without further purification; and cyclohaxane (Riedel-de Haen), tetrahydrofuran (Lancaster), 1,4-dioxane (Aldrich), acetonitrile (Sigma-Aldrich), dichloromethane (Timstar-Laboratory Suppliers), butanol (BDH), ethanol were used as solvent. Acetone was used for cleaning purpose. For complexation anhydride zincchloride and for pH adjustment hydrochloric acid HCl (Riedel-de Haen) were used.

Absorption spectra of the solutions were recorded on a SPECTRONIC GENESYS 2PC UV-Vis spectrophotometer containing deuterium and tungsten lamp as light source for different spectral regions. Before measuring the absorption spectrum of the sample, the base line correction was done by placing solvent in quartz cuvette of 1 cm. The fluorescence emission and excitation spectra were recorded with Fluoromax-4 (Jobin Yvon) spectrofluorometer that has xenon lamp as light source. Both UV-Vis and spectrofluorometer are connected to a personal computer. Quartz cuvettes were used as sample holder in making the measurements. Emission spectrum was recorded by fixing the excitation wavelength and scanning the emission wavelength over the range of interest. Similarly, excitation spectrum was recorded by fixing the emission wavelength and scanning the excitation wavelength. To investigate the effect of pH, dilute HCl was added to solution of 2,2’-bipyridine in acetonitrile and the pH was taken followed by measurement of the spectra. De-ionized water was used for the preparation of the HCl solutions. Post data processing was done by using Origin.7 software. In the data analysis, the Raman and Rayleigh peaks were deleted from the excitation and emission spectra.

Computational Method

Quantum Chemical calculations were performed at the DFT/B3LYP levels of theory with the Gaussian 03 package. Geometries of neutral 2,2’-bipyridine were optimized at DFT/B3LYP/3-21G levels with no symmetry constraints. Potential energy scans were carried out at DFT/B3LYP/STO-3G (Slater-Type Orbital approximated by 3 Gaussians) levels for gas phase. In solvents, additional parameter conductor-like polarized continuum model (CPCM) was used.

3. RESULTS AND DISCUSSION

3.1. Experimental

3.1.1. Absorption Spectra of 2,2’-bipyridine

Optical absorption measurements were used to study the absorption position and the influence of polarity and hydrogen bonding properties of solvents on the position of bands, intensity and shape of the spectra as a whole. The absorption spectra of 2,2`-bipyridine were recorded in solvents of different polarity as depicted in Figure 4, and the values are tabulated in Table 1 for both bands observed in the spectra.

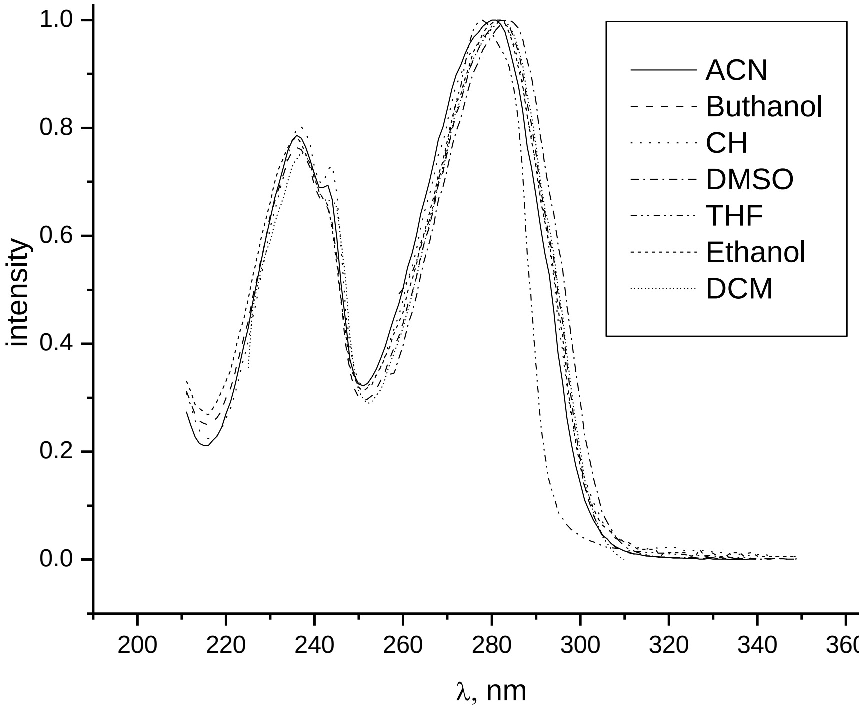


Figure 4: Peak normalized solvent effect on the absorption spectra of 2,2'-bipyridine

The spectra show only a slight shift in the wavelength of maximum absorption (λ_{max}) for both bands relative to that observed for cyclohexane. The shorter wavelength band was shifted to within the range of 236 nm-237 nm and the longer wavelength band was shifted to within the range of 279 nm -284 nm. This small change indicates that the solvent-solute interaction that alters the energy difference between the ground and excite states are relatively small when the polarity of solvents changes.

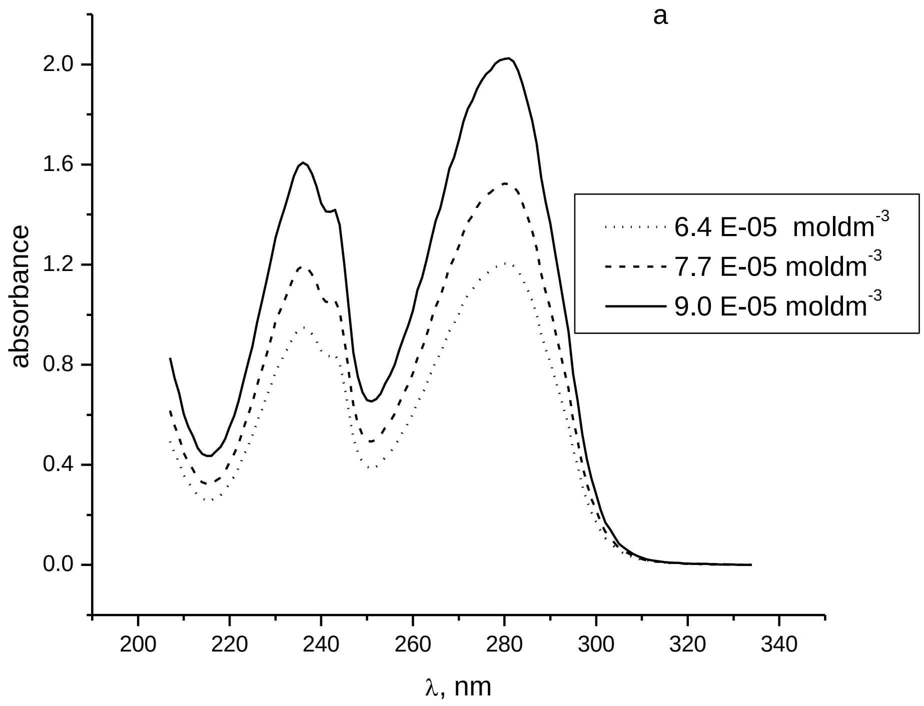
Since the energy differences are the direct consequences of dipole change during transition, therefore, the small change in band maxima in different solvents can be ascribed to small change in the dipole moment of 2,2'-bipyridine in the excited state relative to the ground state compared that observed in cyclohexane.

Table 1: Values of absorption maxima of 2,2'-bipyridine in solvents of different polarity.

Solvent	λ_{max} (nm) higher energy band	λ_{max} (nm) lower energy band
Cyclohexane	237	282
Tetrahydrofuran	-	278

Buthanal	236	282
Ethanol	236	281
Dimethylsulfoxide	-	284
Dichloromethane	237	282
Acetonitrile	236	281

As it was stated in the theoretical section, both cis and trans conformers of 2,2’-bipyridine are expected to exist in equilibrium. Trans structure is expected to be planar and less polar where as the cis structure is polar and has non-zero dipole moment. Therefore, the effect of solvent polarity on both cis/trans conformers cannot be the same for solvents of varying polarity. The small change in absorption band maxima may suggest that the population of the less stable cis conformation of 2,2`-bipyridine is small, even in a polar media [14]. It was reported that the small solvent effects observed in polar and hydrogen bonding solvents indicates the low lying excited state, $S_1$, of 2,2`-bipyridine is a π→π* state [6, 11].



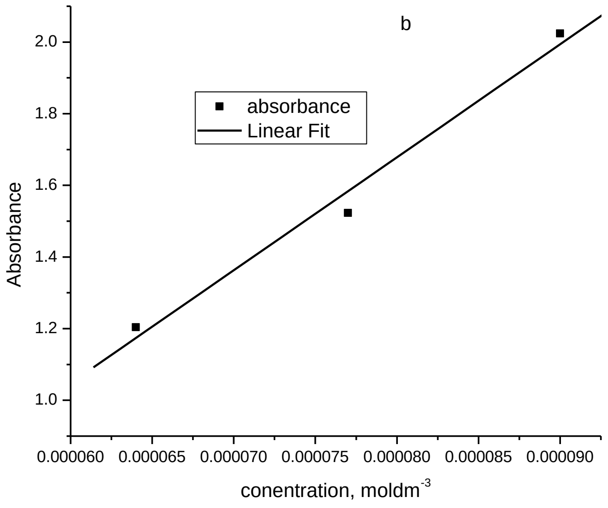


Figure 5: Concentration effect on the absorption spectra of 2,2`-bipyridine (a) and absorbance versus concentration (b) in acetonitrile.

In Figure 5, the UV-Vis absorption spectra of 2,2-bipyridine at three different concentrations in acetonitrile are presented. Two intense absorbance bands were observed at 236 nm and 281 nm. Band shape and the wavelength at the maximum absorbance for all the three different concentrations are similar. The peak ratio of the longer wavelength band to the shorter wavelength band is similar for all the three spectra, which shows that increment in absorbance of both bands is proportional. The plot of absorbance versus concentration (Figure 5b), presents the linear dependence of absorbance on concentrations which indicates that the effect of aggregation below the concentration 9.0 x10^{-5} moldm^{-3} on the absorption spectra is insignificant. From the plot, molar decadic absorption coefficient (ε) of 2,2'-bipyridine has been calculated using Beer`s law and found to be equal to (3154±404 m^{2}mol^{-1}). This value was used to prepare concentrations of 2,2'-bipyridine subsequently used in this project.

The parent molecule biphenyl shows an intense absorption at 246 nm with wavelength of maximum fluorescence occurring at 318 nm and the corresponding maximum excitation wavelength being 270 nm [27]. But, 2,2'-bipyridine shows two intense bands in all solvents used and this effect has been already ascribed as it is an indication that a twist along the central bond is small since it has been shown that both empirically and theoretically, for a large twist, only one band is usually observed [11].

3.1.2. Excitation Spectra of 2,2'-Bipyridine

The variations in fluorescence intensity as a function of the excitation wavelength for a fixed observation wavelength represents the excitation spectrum. For a single transition the band shape of absorption and excitation spectra are similar, provided that there is a single species in the ground state. In contrast, when several species are present, or when a sole species exists in different forms in the ground state (aggregates, complexes, rotamers, tautomeric forms, etc.), the excitation and absorption spectra are no longer superimposable [16]. Thus, any pronounced variation, in the band shape, indicates the existence of more than one transition, which may be attributed to the existence of multiple absorbing species. Since in the excitation spectrum, only the bands corresponding to the fluorescent species are observed, difference between the absorption and excitation spectra may result. Furthermore, in the absorption spectrum, the bands corresponding to the various species may not be resolved when the electronic structure of the excited species are closer; and also influence of the intermolecular and intramolecular interaction forces may not be resolved in absorbance spectrum as in excitation spectrum. Consequently, broadened band is observed.

As cited in the theoretical section, 2,2'-bipyridine is a compound that exists in cis/trans isomeric forms, thus difference between the two (absorption and excitation) is expected.

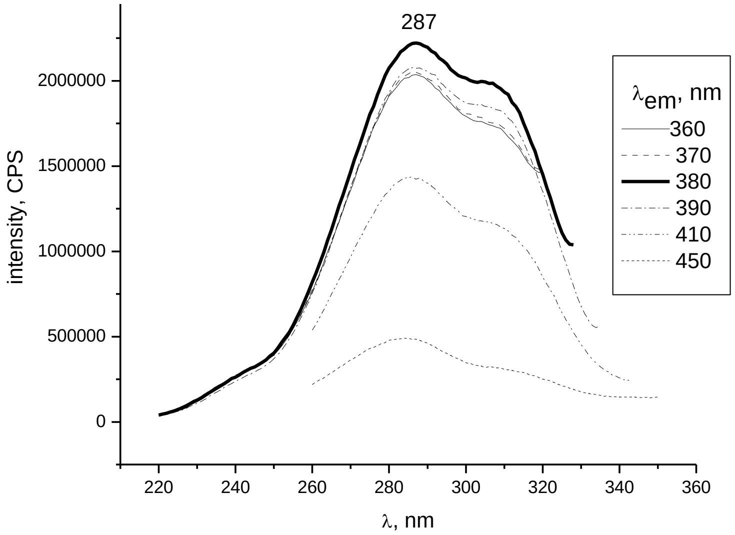


Figure 6: Uncorrected excitation spectra of 2,2'-bipyridine in acetonitrile.

Figure 6 presents the excitation spectra of 2,2'-bipyridine for varying emission wavelengths from 360-450 nm. Upon increasing the emission wavelength from 360 nm to 450 nm, intensity of the excitation spectra increases up to 380 nm and then starts to fall. This indicates that within the range of emission wavelengths stated, the population of emitting species are maximum at emission wavelength around 380 nm. The band shape above emission wavelengths 360 nm is relatively independent of the emission energy. Thus, only one excitation band observed indicates the presence of one species predominantly in the electronic ground state that emits in the range between 360 to 450 nm.

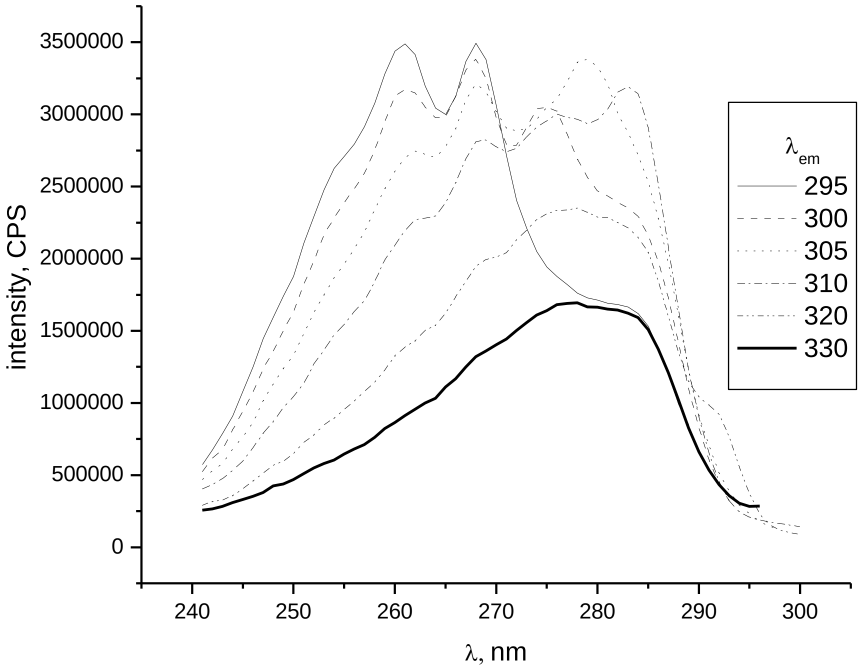


Figure 7: Uncorrected excitation spectra of 2,2'-bipyridine in p-dioxane

In 1, 4-dioxane, the excitation spectra showed two bands depending on the emission wavelength in the range λ_{em} 295 to 370 nm. On increasing the emission wavelength, the intensity of the lower energy band progressively increases while the intensity of the higher energy band decreases.

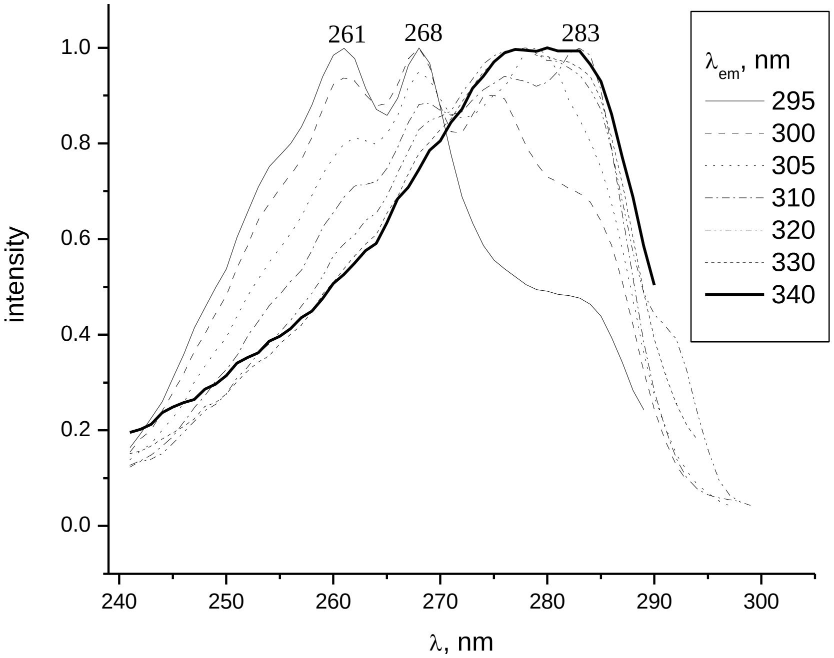


Figure 8: Peak normalized uncorrected excitation spectra of 2,2’-bipyridine in p-dioxane solution

A distinct isosbestic point (a common point between two components of absorption, i.e., a point of equilibrium) observed at 271 nm, is a clear indication of an equilibrium condition of population density between the ground absorption states.

In polar solvents, p-dioxane, excitation spectra was found to vary with emission wavelength compared to its respective absorption spectra. These two different excitation spectra for varying emission wavelength observed in p-dioxane indicate that the origins of the two fluorescence excitation bands are different. Generally, for pure dye molecules in solution, the absorption and fluorescence excitation spectra are usually identical and can be used interchangeably. However, the presence of two fluorescent ground state conformers or two structural isomers proportionally or the generation of the two conformational or two structural isomers in proportional amount in the excited states show emission wavelength dependent excitation spectra [18, 28].

3.1.3. Emission Spectra of 2,2’-Bipyridine

A series of emission spectra of 2,2`-bipyridine were obtained at different excitation wavelengths in solvents acetonitrile, p-dioxane and tetrahydrofuran.

Generally, if the solute molecule becomes more polar in the excited state, there will be greater electrostatic stabilization of the excited states, relative to the ground state by interaction with the polar solvent. The greater the polarity of the solvent, the lower will be the energy of the Frank-Condon (FC) excited state. Solvent effects on the absorption spectra of 2,2'-bipyridine was found to be small. However, fluorescence spectra were found to depend greatly on the polarity of the solvents used. Since fluorescence usually takes place from equilibrated excited states, excited electrons from higher energy levels usually relax to the lowest excited states, S_1' via internal conversion or vibrational relaxation. Compared to the time required for electrons to transit from ground state to one of the higher energy excited electronic states, solvent relaxation takes longer time. After excitation has taken place, solvents re-orient themselves around the excited molecule leading to decrease in the energy of the excited state.

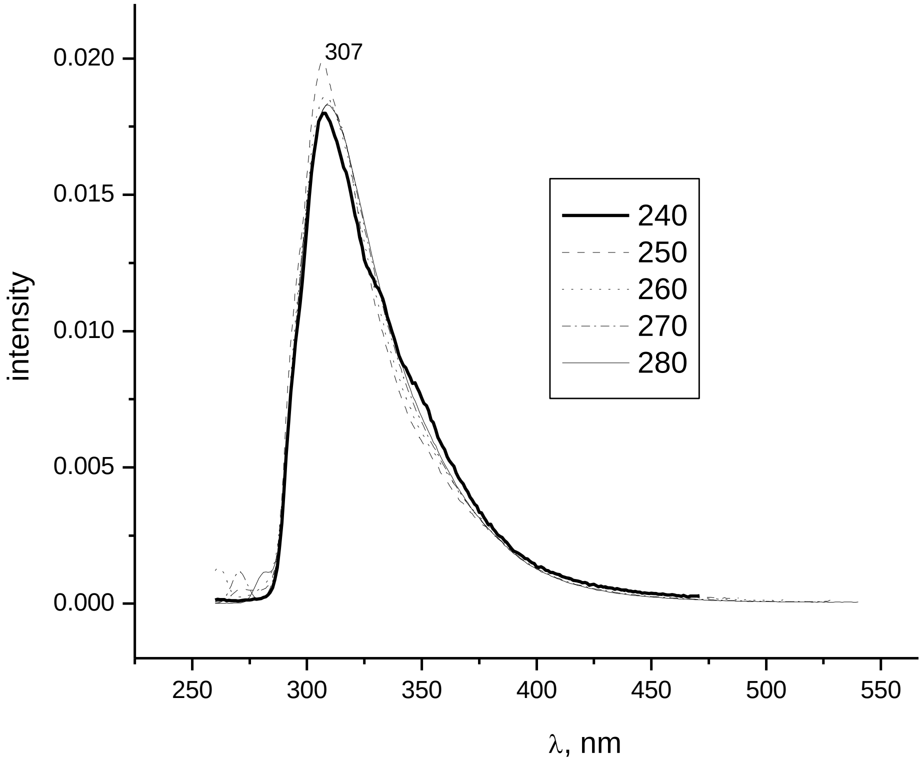


Figure 9: Area normalized emission spectra of 2,2'-bipyridine in tetrahydrofuran at different excitation wavelength.

Emission spectra of 2,2'-bipyridine in tetrahydrofuran shows one intense band at 307 nm for excitation wavelengths between 240 to 280 nm. However, two emission bands were observed for excitation wavelengths 225-240 nm and 250-305 nm respectively in acetonitrile and p-dioxane (Figures 11 and 12). This shows that the nature of solvent determines the nature of the species that emits within the ranges stated. Therefore one can conclude that, in tetrahydrofuran, one absorbing species predominates in the ground state.

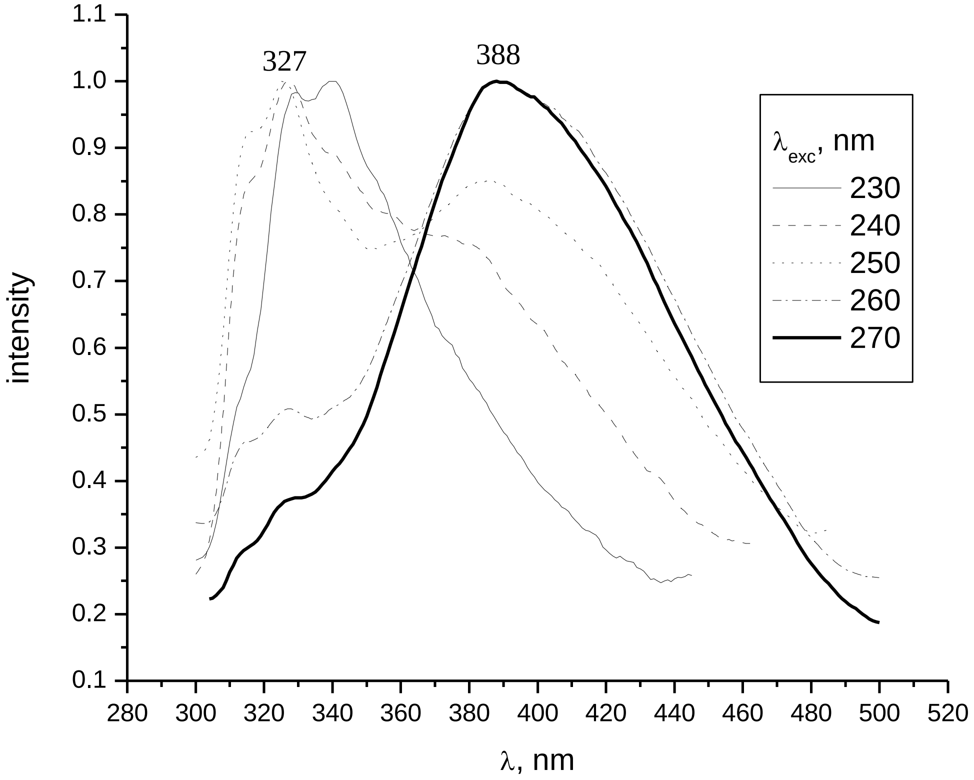


Figure 10: Peak normalized emission spectra of 2,2'-bipyridine in acetonitrile.

Figure 10 presents the emission band of 2,2'-bipyridine in a solution of a polar solvent, acetonitrile, up on excitation at different wavelengths. The spectra show dependence on the excitation energy up on changing the excitation wavelength from 225 nm through 285 nm. From the figure, it can be observed that intensity and position of the two bands seem to depend significantly on the excitation energy in the ranges used. As the excitation wavelength increases the intensity of the longer wavelength band increases at the expense of the shorter wavelength emission band. For excitation wavelengths above 250 nm, only the longer wavelength band was observed.

This change is an indicative of the absence of the mother-daughter relationship between the two emission bands and thus the observed excitation wavelength dependence of the emission spectrum can be attributed to different species in the electronic ground state present in the solution. Furthermore, their relative intensities dependence on the excitation wavelength violates the Kasha Rule stating that the same emission spectrum is generally observed irrespective of the excitation wavelength; i.e. a single excitation band gives rise to a single emission spectrum. And also, normalized emission spectra in the wavelength range 230 nm-270 nm gives an isoemissive point centered at 367 nm (Figure 10), indicating two emitting states originating from two equilibrium structures of a molecule in the ground state.

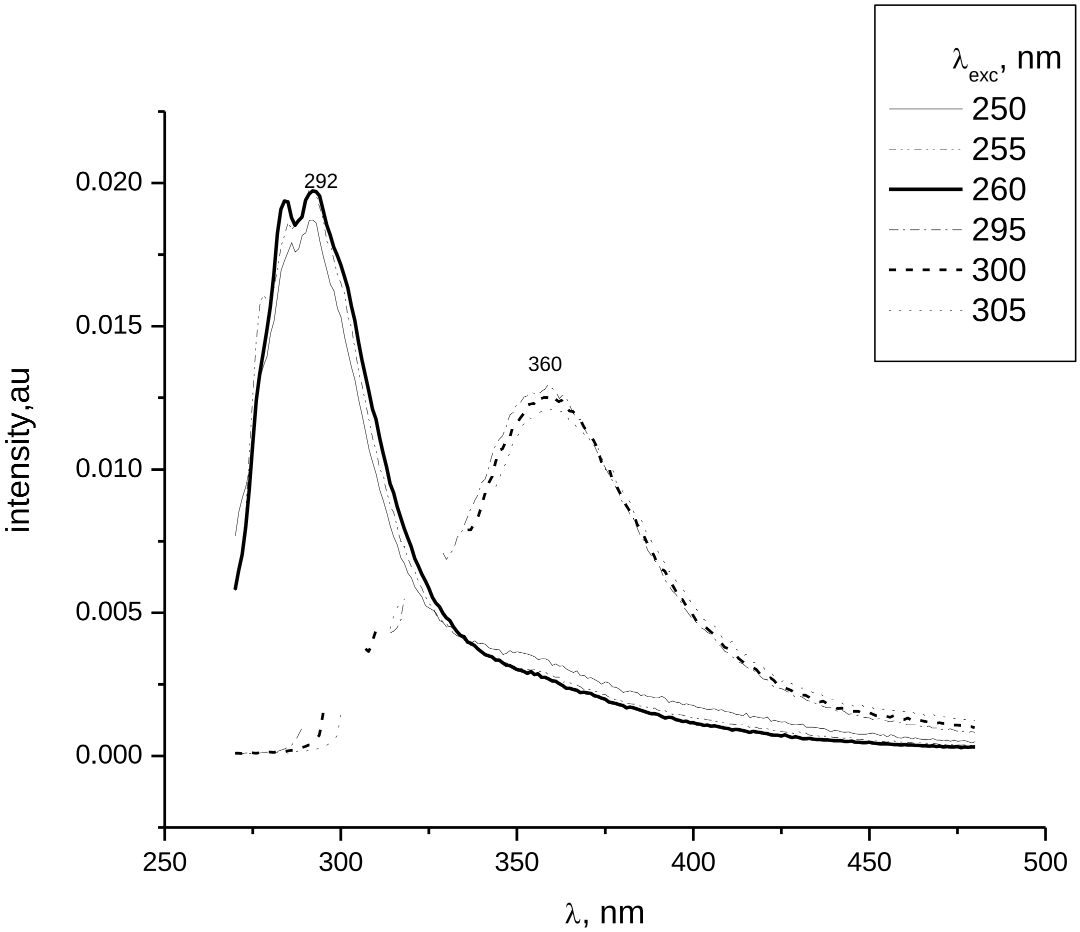


Figure 11: Area normalized emission spectra of 2,2’-bipyridine in p-dioxane

In p-dioxane, shown in Figure 11, a clear separation between the two emission bands is observed upon increasing the excitation wavelength in the ranges 250 nm to 305 nm. For the excitation wavelength below 260, the band at 292 nm is observed as the dominant spectra whereas above

λ_{exc} 260 nm the intensity of the band at 292 decreases while the band at 360 nm increases and above λ_{exc} 295 nm only the band at 360 is observed.

For the reason stated above (in acetonitrile), two different ground state species that fluoresce are responsible for the observed properties. Wide energy separation observed between the two bands suggests that the longer wavelength emitting species is well stabilized in excited state relative to the shorter wavelength emitting one. Compared to spectra observed for p-dioxane, red shift in both bands is observed in acetonitrile. Wide separation in the emission band maxima compared to excitation band maxima indicates that the solvent re-orientation takes place.

The wavelength dependency observed in acetonitrile and p-dioxane, resulted in dual emission. Dual emission generally, can have its origin from (a) concentration dependent aggregation of molecules in the ground or excited state, (b) two modes of electronic transitions, (c) the presence of two different compounds in the ground or excited state and (d) the presence of two different structures of the same molecule.

There are experimental and theoretical evidences for the fluorescence originating from association of 2,2’-bipyridine which appears at longer wavelength [5, 29]. In our case, measurements of the absorption and fluorescence spectra were carried out at very low concentrations in such a way that the absorbance of the sample for fluorescence analysis was adjusted to be always lower than 0.1, so that formation of molecular aggregate is less probable. Hence emission from aggregate cannot be the origin of the dual emission observed in 2,2’-bipyridine. Option (b) and (c) cannot also be considered to be the origins of the dual emission observed in our case because had the dual emission been from two modes of transitions, (b) or from the presence of two different compounds in the ground or excited state, (c), then there would not have been excitation wavelength dependence. However, in our case a significant excitation wavelength dependence of the two emission spectra is observed. Hence, the most probable origin for the two emission bands observed for 2,2’-bipyridine is the presence of two different structures of the same molecule.

Similarly, one can easily identify whether equilibrium between ground state electronic structures or equilibrium between excited state electronic structures are responsible for dual fluorescence by studying the dependence of emission spectra on excitation wavelength. If only single excitation wavelength is responsible for the observed emission bands, that is, if emission spectrum is independent of excitation wavelength, excited state equilibrium is responsible for the observed bands [16, 30]. However, if the emission spectrum shows dependence on excitation wavelength, equilibrium between ground state electronic structures is responsible for the observed bands. Therefore, the presence of two ground state species, most probably cis/trans conformers, are responsible species for the observed wavelength dependency of the excitation and emission spectra.

Since excitation spectrum depends on emission wavelength and vice versa, it is possible to identify which excitation band corresponds to the low or higher energy emission band. In acetonitrile, the 3D spectra of 2,2'-bipyridine gives two contour centers (Figure 12). The excitation at λ_{Exc} = 257 nm gives the emission band at λ_{Em} = 327 nm, and the second excitation band at λ_{Exc} = 286 nm corresponds to the emission band at λ_{Em} = 388 nm (Figures 6 and 10).

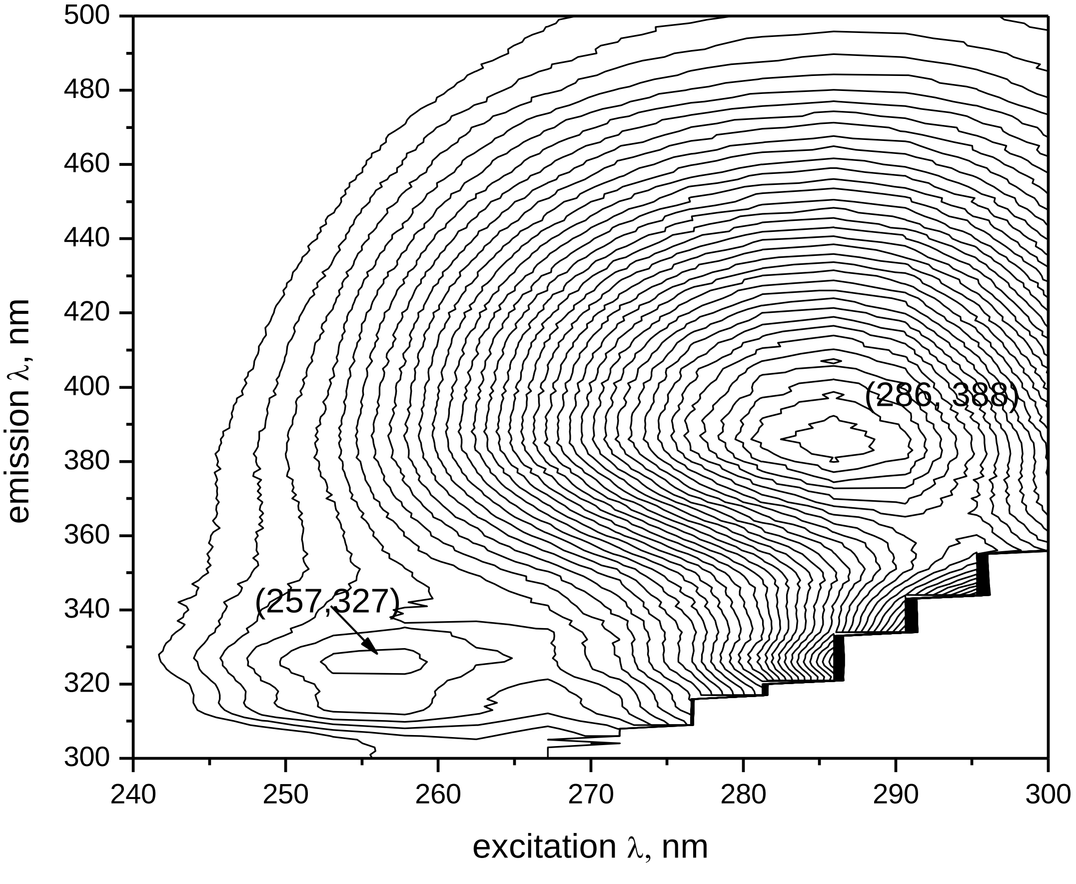


Figure 12: Contour plot of 2,2'-bipyridine in acetonitrile solution.

Presence of two contour centers supports explanations given for the existence of two species that absorbs and emits at their own distinct wavelength. From observations, dual emission is observed in the relatively more polar solvents acetonitrile and p-dioxane whereas only single emission is observed in an intermediate polar tetrahydrofuran.

3.1.4. Effect of Metal Ions on the Spectra of 2,2'-Bipyridine

2,2'-bipyridine is a bidentate ligand and in the metal chelate compounds, a perfectly planar cis conformer is maintained [7, 9, 10]. Titration of 2,2'-bipyridine solution in acetonitrile with a less concentrated zinc solution ($2.3x10^{-5}$ $moldm^{-3}$) showed a gradual increase in the absorbance of the metal chelate compound and a decrease in the absorbance of the metal free ligand indicating that the more stable trans conformer is being converted to cis metal chelated form. The spectra observed in Figure 13 in acetonitrile are in complete agreement with the report in aqueous solution [10].

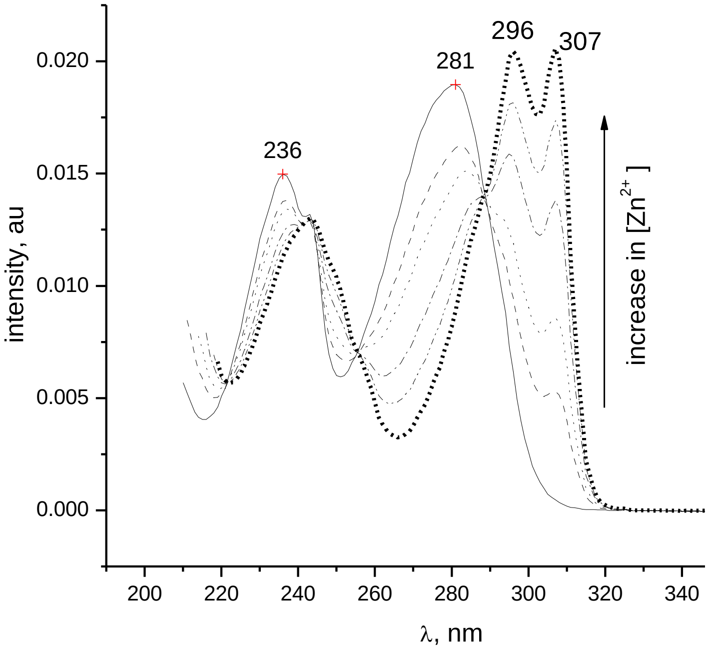


Figure 13: Area normalized absorption spectra of 2,2'-bipyridine in acetonitrile at different Zn^{2+} ion concentrations added drop wise, $[Zn^{2+}] = 2.3x10^{-5}$ $moldm^{-3}$

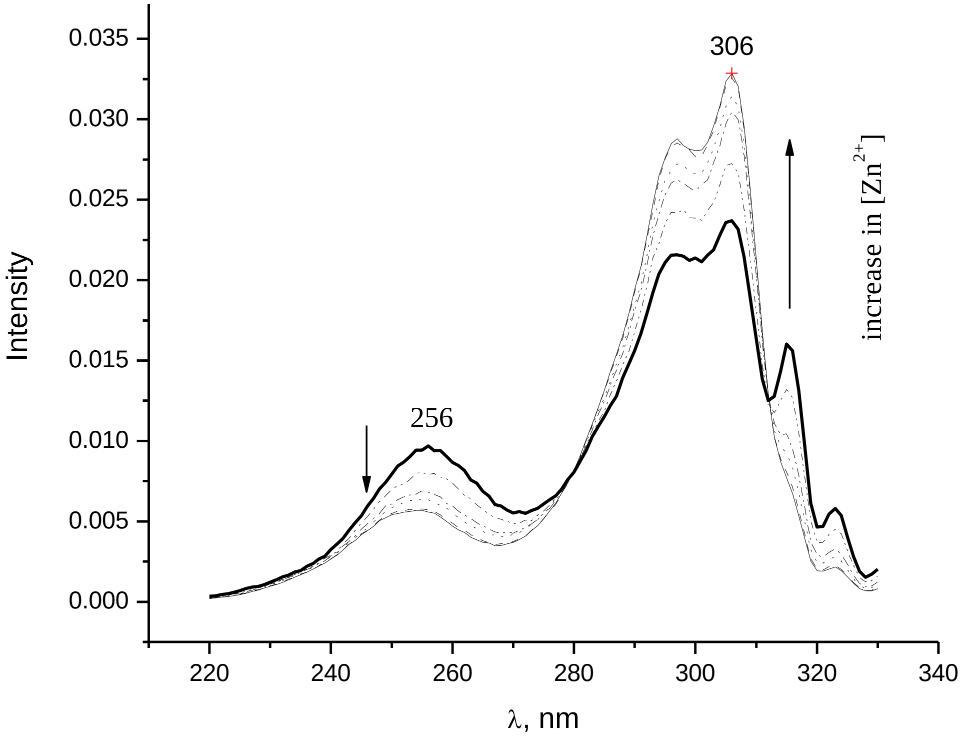


Figure 14: Effect of metal ions on the excitation spectra of 2,2'-bipyridine in acetonitrile, λ_{Em} = 360 nm. (Area normalized uncorrected excitation spectra).

Excitation spectra of metal chelated ligand showed peak maxima at 306 nm and 256 nm with an isosbestic point at 280 nm. Increasing Zn^{2+} ion concentration increases the intensity of the band at 306 nm whereas the band at 256 nm decreases. Compared to the absorbance spectra (Figure 13), relatively similar band shape and absorption maxima were observed. The intensity changes with Zn^{2+} ion concentration observed for both bands indicates the change of the metal-free 2,2'-bipyridine (band at 256 nm) to the metal-chelated complex (band at 306 nm). The presence of isosbestic point verifies existence of two absorbing species in equilibrium at the ground electronic state. The presence of the band at 256 nm confirms the band observed in contour plot (Figure 12) and therefore, it is from the metal free ligand. Since the presence or absence of metal ions in solutions of 2,2'-bipyridine switches between the cis/trans conformations; and metal-chelated 2,2'-bipyridine is of cis form [7,9,10], the band at 256 nm can be assigned to be originated from the metal free trans structure of 2,2'-bipyridine.

Generally, aromatic molecules complexed with closed shell diamagnetic metal ions such as zinc, cadmium and gallium fluorescence strongly, with fluorescence spectra similar to the chromospheres. The lowest excited states of metal complexes of Zn^{2+} with ligand are well known to possess ligand-localized states [10].

3.1.5. Effect of pH on the spectra of 2,2'-bipyridine

As a result of the acido–basic properties in the ground and excited states, absorption and fluorescence spectra are pH-dependent [16]. The effect of pH on the absorbance of 2,2'-bipyridine was presented in Figure 15 showing a shift in the absorbance bands from 236 and 281 nm to 243 and 303 nm for the shorter and longer wavelength respectively. Stepwise decreases in pH of the 2,2'-bipyridine solution in acetonitrile increases the intensity of the 303 nm band and decreases the intensity of the 281 nm band with an isosbestic point at 289 nm. The effect observed for both metal ion and pH on the absorption spectra are in agreement with the work in aqueous solution [10].

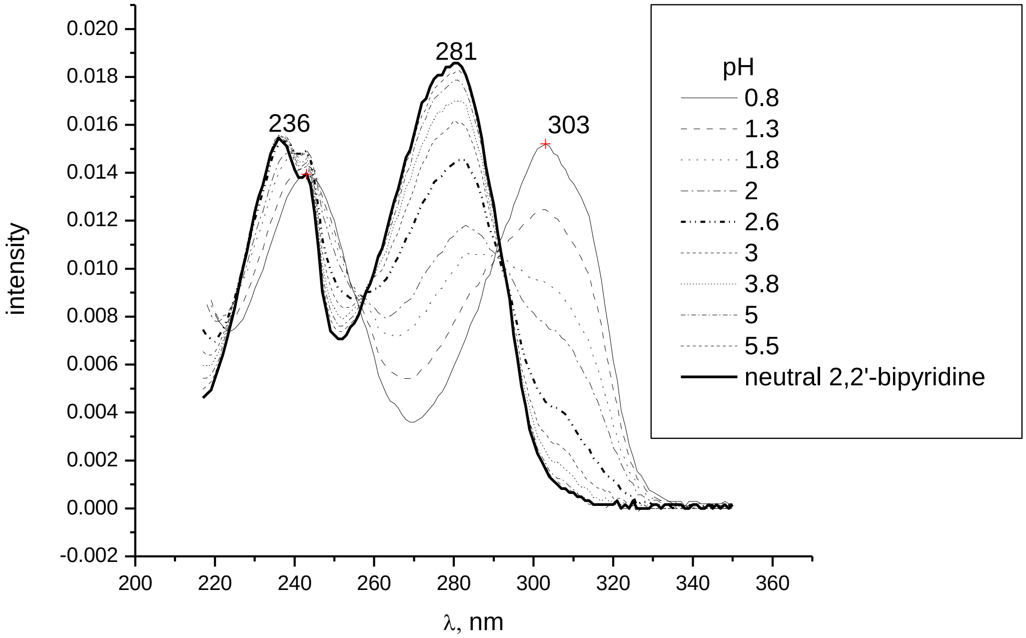


Figure 15: Area normalized absorption spectra of 2,2'-bipyridine at different pH in acetonitrile.

In absorption spectra, the similarity between the acidified 2,2'-bipyridine solution and the metal-chelated spectra indicates that the monocation forms predominantly exists in solution has a cis structure [7, 9, 10]. The presence of the isosbestic point and change in intensity of the bands with pH also shows the interconversion of cis/trans structures present in equilibrium at around isosbestic point.

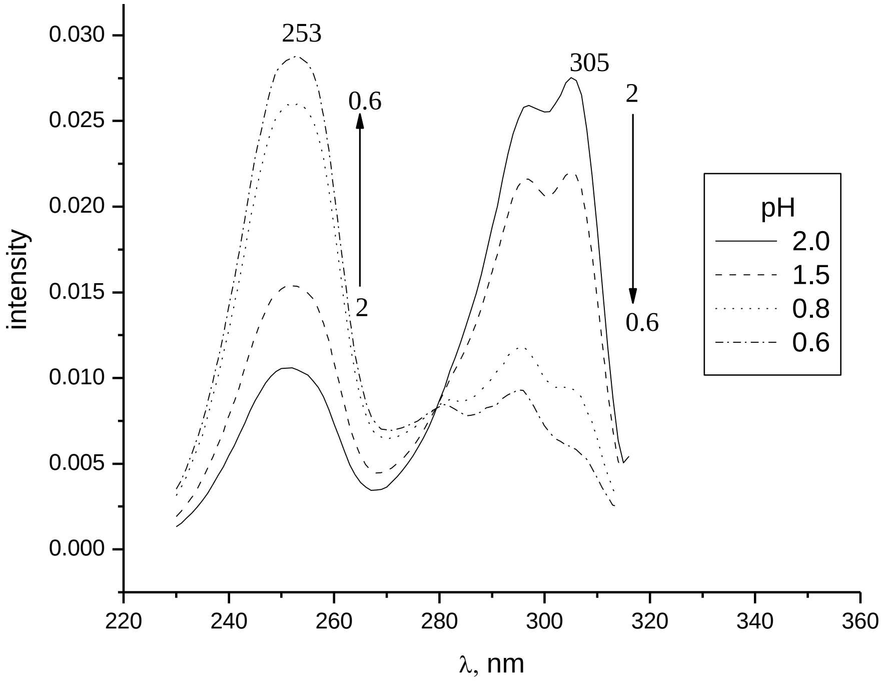


Figure 16: Effect of pH on the excitation spectra of 2,2'-bipyridine in acetonitrile at λ_{em}=325 nm. (Area normalized uncorrected excitation spectra).

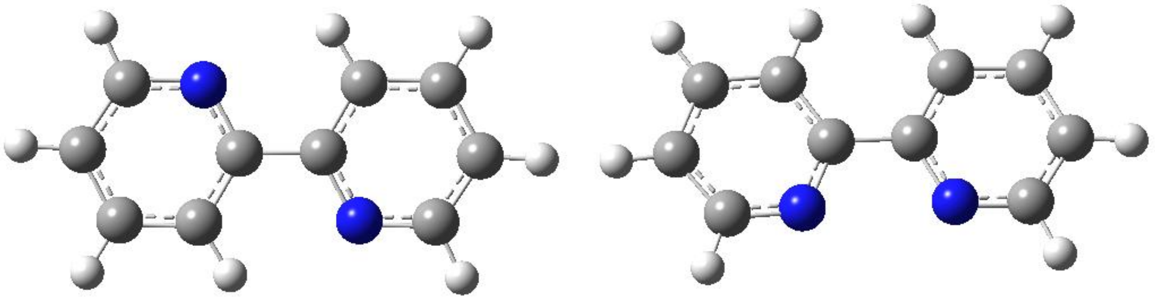
The excitation spectra of the acidified 2,2'-bipyridine solution of pH between 0.6 to 2 show two bands at 253 and 305 nm in acetonitrile. On decreasing the pH of the solution, the band at 253 nm increases in intensity while the band at 305 nm decreases. Closer similarity is observed between the band shapes of the excitation spectra given in Figure 14 and excitation spectra of acidified 2,2'-bipyridine solution (Figure 16) with an isobestic point appeared at 280 nm in both figures. Compared to the spectra depicted in Figure 14, the excitation band at 253 nm observed for acidified solution corresponds to the band at 256 nm and 305 nm corresponds to 306 nm.

Since biprotonated 2,2'-bipyridine was reported to exist in strongly acidic solution at low pH [10, 11], and from viewpoint of charge distribution, a complete planar trans form is most favorable form of the di-cation. Hence, decrease in the intensity of the longer wavelength band (305 nm) and increase in the shorter wavelength band (253 nm) observed with decrease in pH can be accounted as due to the change in the monoprotonated cis conformation to the biprotonated trans conformation. This observation is in agreement with the assignment of the structure of the species responsible for the contour center at (257, 327) nm shown in Figure 12 to trans form of 2,2’-bipyridine. In line with the previous reports, the monoprotonated 2,2’-bipyridine is of cis structure that has relatively similar band shape and absorption wavelength maxima to the metal-chelated complex (Figures 15 and 18). Therefore, the mono-protonated 2,2’-bipyridine that forms in acidic solution has a cis structure with the excitation spectra maxima at 305 nm.

3.2. Computational Investigation of Molecular Properties of 2,2’-Bipyridine

Optimization

Both isomeric structures of 2,2’-bipyridine (IA and IB) are optimized using the DFT/B3LYP/6-31G method. The resulting structures were planar with dipole moment 3.5 D for IB and 0.00D for IA.

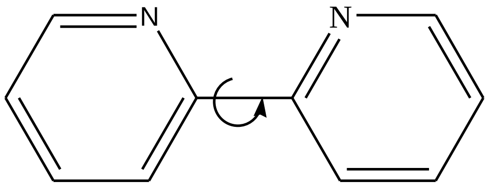


IA IB

Scheme 2: Optimized structure of 2,2’-bipyridine IA and IB

Potential Energy Curves

Structures related by bond rotations usually interconvertible at room temperature. As it was already described 2,2’-bipyridine is among the molecule that is expected to rotate about the bond joining the two rings (C2-C’2) exhibiting a different conformational behavior to that of biphenyl [31]. The presence of the two N atoms in the ortho/ ortho’ position relative to the bond joining the rings substantially affects the geometry of this molecule. Energy should be supplied to twist a bond into a specific conformation known as barrier to rotation or torsional energy, which is defined as relative energy difference between highest energy conformer and lowest energy conformer.



Scheme 3: Rotation of the pyridyl ring about the C-C' bond.

For 2,2'-bipyridine potential energies were calculated for every 10^o rotations of the one of pyridine ring as shown in scheme 3 and the results obtained are summarized in Figure 17 and Table 2.

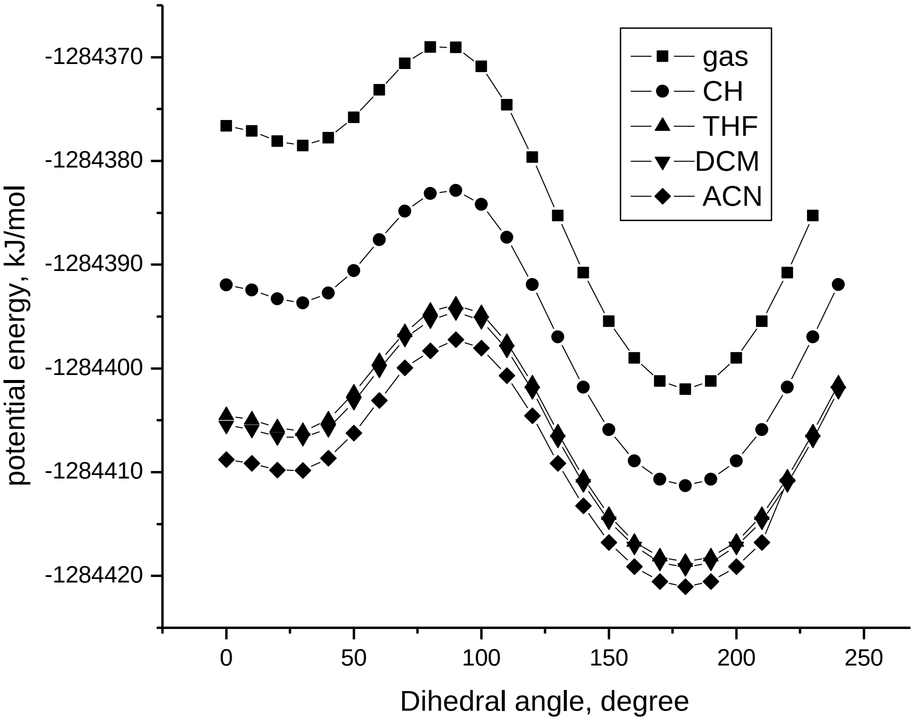


Figure 17: Rotational angle about NCC'N' versus ground state potential energy for every10^o rotation of the pyridyl ring using DFT/B3LYP/STO-3G in vacuum and solvents

Table 2: Ground state energy of the local minima (cis) and absolute minima (trans) of 2,2'-bipyridine in different solvents in every 10^o rotation of the bipyridyl ring using DFT/B3LYP with STO-3G basis set and CPCM solvent model.

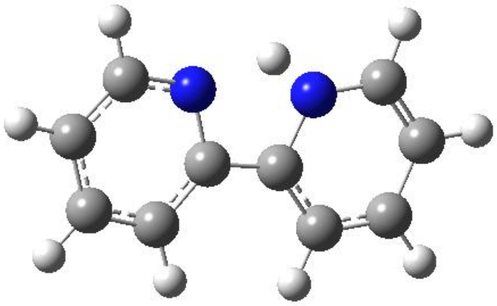
Solvent	Trans (kJmol^{-1}) $\Delta E_{gas\text{-}solvent}$	Cis (kJmol^{-1}) $\Delta E_{gas\text{-}solvent}$	Rotational barrier (kJmol^{-1})	$\Delta E_{cis\text{-}trans}$ (kJmol^{-1})
Vacuo			32.96	23.46
Cyclohexane	9.01	15.50	28.47	17.63
Tetrahydrofuran	17.01	27.50	24.82	12.63
Dichloromethane	17.01	28.50	24.63	12.51
Acetonitrile	19.05	31.35	23.81	11.20

As can be seen in the potential energy curves (Figure 17), maximum energy was reached when twisting angle about the NCC'N' reaches 90^o, perpendicular configuration of the pyridyl rings to each other. There are two energy minima: cis-like structure with 30^o twisted out of plane as local minima and the planar trans structure as absolute minima. For ground state 2,2'-bipyridine in vacuo, interconversion of cis/trans requires additional energy that overcome the barrier energy of 32.96 kJ/mol; and the trans isomer (absolute minima) is more stabilized compared to the 30^o cis-like structure (local minima) by about 23.46 kJ/mol. As the polarity of the solvent increases, the stability of both conformers increases.

The extent of stabilization of the cis-like conformer is greater than the trans conformer in all solvents used indicating that cis shows more solvent dependent which is in consistent with the expected result and with earlier report by Jaime and Font [31].

The energy barriers for both conformers, 9.47 and 32.96 kJmol^{-1} for cis like and trans conformer respectively, are greater than the available room temperature thermal energy, 3RT/2 =.3.77 KJmol^{-1}.This indicates that room temperature thermal energy is not enough to exchange one conformer in to the other. Hence, the equilibrium structure of the molecule in vacuum can exist independently and contribution of the two conformers will appear as a distinct ground state species. In polar solvents like acetonitrile, the energy of activation to interchange one another is increased to 12.6 KJmol^{-1} for cis like structure and decreased to 23.8 KJmol^{-1} for trans with overall decrease in energy between cis/trans conformers to 11.20 kJmol^{-1}. This change indicates that in polar solvents, in addition to the overall stability of the two conformers, the stability of the cis-like structure is enhanced.

The existence of cis and trans structures of 2,2’-bipyridine distinctively in ground state, as the calculated value indicates, supports the suggested justifications given for the causes of the wavelength dependency of emission and excitation spectra observed experimentally.



Scheme 4: Optimized structure of the mono-protonated 2,2’-bipyridine using DFT/B3LYP /STO-3G basis set and CPCM solvent model in acetonitrile.

Optimized monoprotonated 2,2’-bipyridine was found to possess cis structure in acetonitrile which is in agreement with the experimental result [10]. The potential energy curve of the monoprotonated 2,2’-bipyridine given in Figure 18 shows two energy minima for 0 and 180^{o} ` and energy maxima in the range between 80 to100$^{0}$ on rotational angle about N-C-C`-N.

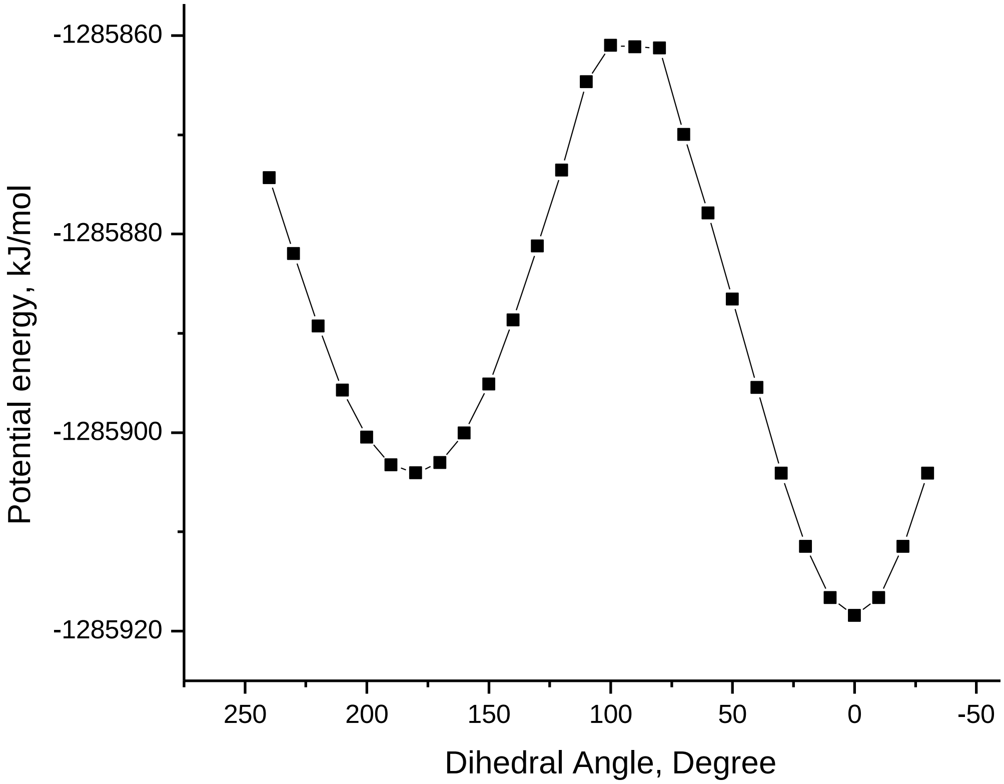


Figure 18: Rotational angle versus ground state potential energy of mono protonated 2,2’-bipyridine using DFT/B3LYP/STO-3G and CPCM solvent model in acetonitrile.

The potential energy curve for monoprotonated 2,2’-bipyridine exhibits one local minima for trans isomer and absolute minima for the cis structure for rotation angle between -50 to 250^{o} dihedral angle. Very high rotational energy barrier for monoprotonated species indicates that the probability of interconversion of the cis/trans conformers seems low.

Table 3: Ground state energy of the local minima (trans) and absolute minima (cis) of monoprotonated 2,2’-bipyridine in acetonitrile for every10^{o} rotation of the bipyridyl ring using DFT/B3LYP with STO-3G basis set and CPCM solvent model.

Rotational barrier (kJmol^{-1})	$\Delta E_{cis\text{-}trans}$ (kJmol^{-1})
57.44	14.38

For biprotonated 2,2’-bipyridine, even higher barrier energy but very low energy difference between the cis and trans structure was found with trans conformer being at the absolute minima.

Thus, comparison of the present experimental and computational studies of 2,2’-bipyridine indicates that the gradual changes observed in the band shape and position by varying pH, Zn^{2+} ion and excitation or emission wavelength can be attributed to the presence of cis/trans isomeric structures.

4. SUMMARY

Absorption spectra show small shift in solvents of different polarity indicating small difference between the dipole moments of the Franc-Condon (FC) excited state and the ground state and suggests a small degree of charge transfer in the FC excited state. Band shape of excitation spectra in p-dioxane show variation with emission wavelength. Upon increasing emission wavelength in p-dioxane, the intensity of the higher energy band diminishes and new band at the lower energy edge appears signifying presence of two ground state species emitting at different wavelength.

Two distinct emission spectra were observed upon progressive increase in excitation wavelengths in both acetonitrile and p-dioxane solvents producing more red shifted spectra in acetonitrile. The presence of two emitting species was supported by contour plot showing the correspondence between emission and excitation spectra. The analysis made show that the spectral changes related to wavelength dependency is due to the presence of two ground state equilibrium species (cis/trans isomers) that absorbs and emits photon of specific energy.

Stepwise addition of metal ions, Zn^{2+}, made possible the assignment of the shorter wavelength emission band as originated from trans and the longer wavelength band to cis conformer. pH effects on the spectra of 2,2’-bipyridine were similar to that of the metal ions for moderate pH values; and at very low pH, change in excitation spectra was observed which was supposed to be due to the formation of the diprotonated 2,2’-bipyridine.

Computational results predict the presence of two low energy conformers of 2,2’-bipyridine to be the cis-like (relatively less stable) structure and trans (more stable) structure. In contrast, cis structure is more stable in acidic media than the trans structure. The higher barrier energy compared to room temperature thermal energy, for both cis and trans structures, shows their existence distinctively at room temperature and the probability of interconversion of cis/trans is low. This result supports the experimental result that assigns the wavelength dependency to the presence of cis/trans structures in ground state.

Therefore, the origin of the spectral changes observed are due to the presence of ground state cis/trans conformers and their population changes in the presence of metal ions and pH of the solution. Progressive change in excitation wavelength, metal ion concentration and pH shows change in the fluorescence excitation spectra.

5. REFERENCES

[1] K. Rohatgi-Mukherjee, *Fundamentals of Photochemistry*, Wiley Eastern Limited, New Delhi, 1986

[2] A. Almenningen, O. bastiansen, S. Gundersen and S. Samdal *Acta Chem. Scand*. 1989, 43, 932

[3] R. Thummel, F. Lefoulon, R. Mahadevan *J. Org. Chem*. 1985, 50, 3824

[4] F. Sasse *Org. Synth., Coll.* 1973, 5,102

[5] E. Castellucci, L. Angeloni, G. Marconi, E.venuti and I. Baraldi *J.phys. Chem.* 1990, 94, 1740.

[6] A. Sarkar, S. Chakravorti *J. Luminesc*. 1995, 65, 163

[7] A. Ikemoto, K. Takemura, K. Seki, and M. Yagi *Spectrosc.Lett.*1996, 29, 1185

[8] M. Yagi, T. Kaneshima, M. torii, K. Matsu, and J. Higuchi *Chem. Phys.Lett.* 1992, 197, 457

[9] M. Yagi, H. Shirai, J. Ohta, and J. Higuchi *Chem. Phys.Lett.* 1989, 160, 13

[10] K. Nakamoto *J. phys. Chem.* 1960, 64, 1420

[11] S. Henry and Z. Hoffman *J. phys. Chem*. 1979, 83, 618

[12] G. Schulman, *Fluorescence and Phosphorescence Spectroscopy:International Series in Analytical Chemistry*,Vol. 59, Pergamon Press, New York, 1979.

[13] L. Merritt and D. Schroeder, Acta Cryst. 1956, 9, 801

[14] C. Boiteux, P. Michel, A. Marsura, J. Vains and F. López *J. Molecul Struct. THEOCHEM.* 2007, 811, 169

[15] C. Reichardit, *Solvent And Solvent Effect In Organic Chemistry*, 3rd Edition,Willey-Vch, Weinheim, 2003.

[16] B. Valeur, *Molecular Fluorescence*, Willey-VCH, Weinhein, 2000.

[17] W. Han, T Liu, F. Himo, A. Toutchkine, D. Bashford, K. Hahn, And L. Noodleman *Chemphyschem* 2003, 4, 1084

[18] M. Balon, C. Carmona, A. Munoz, *Chem. Phys*. 2004, 302

[19] M. Balon, A. Munoz, C. Carmona, *Chem. Phys*. 2003, 299, 67

[20] G. Kuzmin, *Pure And Appl. Chem*., 1993, 65, 1653

[21] R. Lakowicz, *Principles of Fluorescence Spectroscopy*, 2nd Edition, Kluwer Acadamic Plenum Press, New York, 1999.

[22] J. Konijnenberg, A. H. Huizer And C. A. G. O. Varma, J. Chem. Soc., Faraday Trans. 1989, 85, 1539

[23] S. Kapelle, W. Retting And R. Lapouyade, *Photochem. Photobiol. Sci.* 2002,1, 492

[24] S. Maheshwari, A. Chowdhury, N. Sathyamurthy, M. Panda And J. Chamdrasekhar, *J. Phys. Chem. A.* 1999, 103, 6257

[25] R. Ghanem, C. Carmona, P. Guardado, M. Munoz, M. Balon, *J. Photochem.Photobiol. A* 1999, 129, 57

[27] C. Young *Computational Chemistry: A Practical Guide for Applying Techniques to Real-World Problems*, John Wiley & Sons, Inc. New York, 2001.

[27] W. Bridges, J. Creaven and T. Williams *Biochem. J.* 1965, 96, 872

[28] C. Yao, H. B. Kraatz , R. Steer, *Photochem. Photobiol. Sci.* 2005, 4, 191

[29] A. Agresti, M. Bacci, E. Castellucci and P. Salvi *Chem. Phys. Lett.* 1982, 89, 324

[30] L. Dunford, J. Smith, E. Swinny and R. Markham, *Photochem. Photobiol. Sci.* 2003, 2, 611

[31] C. Jaime and J. Font *J. Org. Chem.* 1990, 55, 2637