FLUORESCENCE SPECTRAL STUDIES ON THE INTERACTION OF BIOTIN WITH 4-DICYANOMETHYLENE-2,6-DIMETHYL-4H-PYRAN(DDP) DYE IN AN AQUEOUS SOLUTION

Thesis submitted

in partial fulfilment of the requirements

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By

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DECLARATION

I Thirumalai.T, hereby declare that the Project Report entitled **"FLUORESCENCE SPECTRAL STUDIES ON THE INTERACTION OF BIOTIN WITH 4-DICYANOMETHYLENE-2,6-DIMETHYL-4H-PYRAN(DDP) DYE IN AN AQUEOUS SOLUTION"** done by us under the guidance of **Dr.S.GAYATHRI Ph.D.**, Assistant Professor Department of Chemistry, Sathyabama Institute of Science and Technology is submitted in partial fulfilment of the requirements for the award of Master of Science degree in Chemistry.

DATE: 08/05/2023

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T.THIRUMALAI

ABSTRACT

Photophysical studies of DDP dye with water soluble Biotin, were carried out in aqueous solution. Addition of biotin to DDP dye results in a fluorescence quenching accompanied with a blue shift in the emission maximum (6 nm). The fluorescence quenching is attributed to the promotion of the ICT process in between the donor moiety (Oxygen) and the acceptor moiety (C≡N) dye ring structure. The fluorescence quenching of DDP DYE is found to be dynamic in nature. The fluorescence decay of DDP DYE in the presence of BIOTIN shows tri-exponential decay, which implies that there exists a heterogeneous micro environment around the vicinity of the dye with variation in the fluorescence lifetime and their relative distribution. The interaction of DDP DYE with BIOTIN in water is found to be largely hydrophobic, but BIOTIN containing several hydrogen-bonding functional groups (biotin moieties and amino acids) also influences the excited state spectral properties of DDP DYE dye. BIOTIN serves as an excellent promoter of electron transfer (ET) through space between the donor and acceptor moiety of DDP DYE. The variation in the reduction and oxidation potential of DDP DYE on the addition of BIOTIN is elucidated from cyclic voltammetry studies and the existence of multi-environment of DDP DYE in BIOTIN is elucidated from time resolved fluorescence lifetime measurements.

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LIST OF SYMBOLS AND ABBREVIATIONS

ABBREVATIONS

SL.NO	ABBREVIATIONS	DEFINITION
1	AO	Atomic Orbital
2	CT	Charge transfer
3	DCM	Dicyanomethylene-2-methyl-6-p-dimethylaminostyryl- 4H-pyran
4	DDP	Dicyanomethylene-2,6-dimethyl-4H-pyran
5	FRET	Fluorescence resonance energy transfer
6	fs	Femtoseconds
7	HOMO	Highest occupied molecular orbital
8	ICT	Intramolecular Charge Transfer
9	LUMO	Lowest occupied molecular orbital
10	MEP	Molecular electrostatic potential
11	MO	Molecular orbital
12	ns	Nanoseconds
13	ps	Picoseconds
14	TAC	Time to Amplitude Convertor
15	TCSPC	Time Correlated Single Photon Counting
16	TICT	Twisted Intramolecular Charge Transfer
17	TRP	Tryptophan
18	TRY	Trypsin
19	TYR	Tyrosine
20	UV-Vis	Ultraviolet-Visible

SYMBOLS

SL.NO	SYMBOLS	DEFINITION
1	**χ**	Chi
2	**C**	Concentration
3	**Q**	Concentration of the Quencher
4	**I**	Fluorescence intensity of dye with hydrogen-bonding solutes
5	**Io**	Fluorescence intensity of free dye
6	***f***	Frequency
7	***υ***	Frequency in cm-1
8	**τ**	Lifetime
9	**mol L-1**	Number of moles per litre
10	**π**	Pi
11	***B***	Relative amplitude
12	**K_{sv}**	Stern-Volmer constant
13	**nm**	Wavelength

CHAPTER 1

INTRODUCTION

Introduction to the subject of photochemistry in microheterogeneous system, discussion about its occurrence, application to different form of the microheterogeneous system and its photophysical process. The term “microheterogeneous” are logical relevance that “heterogeneous” environment at microscopic level. In heterogeneous system at the microscopic level with the presence of charged interfaces in hydrophilic and hydrophobic domains. There are two principle reason for micro heterogeneous system self- assemblies, i) photophysical and photochemical processes to probe the dynamic and static properties of these organized system and ii) excited-state, molecular processes under novel microenvironment. The solute distribution can be inhomogeneous through out the entire volume of solution/aggregate. There may be unique properties in hydrophobic and hydrophilic cavities/cages/pockets/pools/pores that can be requisition the solutes. charges interfaces where electrostatic effects can play a dominant role in influencing the solutes distribution and their reactions. For these systems we need optically transparent (non-turbid) solutions readily amenable to photochemical investigation by steady-state and pulsed photolysis methods. As the system become increasingly complex, no single technique can provide all the answer and even unambiguously resolve different processes. There numerous physical or chemical methods that we can utilize to supplement.

Many of the important photochemical and photophysical processes occur on extremely fast time scales. One reason for this is that many photochemical reactions are unimolecular i.e. they do not rely on chance collisions between reactants. The time scales involved thus depend on the very fast rearrangement of electrons and (sometimes) atoms. Reactions that occur on these timescales are difficult to analyse using steady- state absorption and fluorescence spectroscopy, as short-lived intermediates states only have a small contribution to the time integrated spectrum. The photochemistry and photophysics of molecules has been a topic of interest for many years. Designing of more efficient organic displays,[2,3,4] solar cells [5,6]and optical properties when the system absorbs (or emits) a photon. Study of the photo-

excitation and subsequent relaxation of molecules also gives fundamental insights into the mechanisms and variables that affect these processes.

1.2 Biotin significance and biological importance:

Biotin is commonly known as vitamin B.it is helps to convert all the carbohydrates to producing energy to the body. Vitamin H commonly known as Biotin.it also help to body metabolize fat, protein and used to nervous system function properly.Your body needs biotin to metabolize carbohydrates, fats, and amino acids, the building blocks of protein. Biotin is often recommended for strengthening hair and nails, and it's found in many cosmetic products for hair and skin. Like all B vitamins, it is a water soluble, meaning the body does not store it.

1.2.1 Hair and nail problems:

Very weak evidence suggests that biotin supplements may improve thin, splitting, or brittle toe and fingernails, as well as hair. Biotin, combined with zinc and topical clobetasol propionate, has also been used to combat alopecia areata in both children and adults.

1.2.2 Diabetes:

Preliminary research indicates that a combination of biotin and chromium might improve blood sugar control in some people with type 2 diabetes, but biotin alone doesn't seem to have the same effect. More research is needed to determine whether biotin has any benefit.

1.2.3 Biological importance:

Role of Biotin in fatty acid biosynthesis the cytosolic pathway (extra-mitochondrial pathway, De Novo synthesis of fatty acids) is a major pathway for synthesis of fatty acids. Synthesis of fatty acid from Acetyl-CoA takes place outside mitochondria. Acetyl-CoA forms Citrate which comes out of the mitochondria can cleave to give Acetyl-CoA (as such cannot come out of the mitochondria). Tissue involved in cytosolic fatty acid biosynthesis: Liver, adipose tissue, mammary gland, brain, kidney.

1.3 BIOTIN STRUCTURE PROPERTIES:

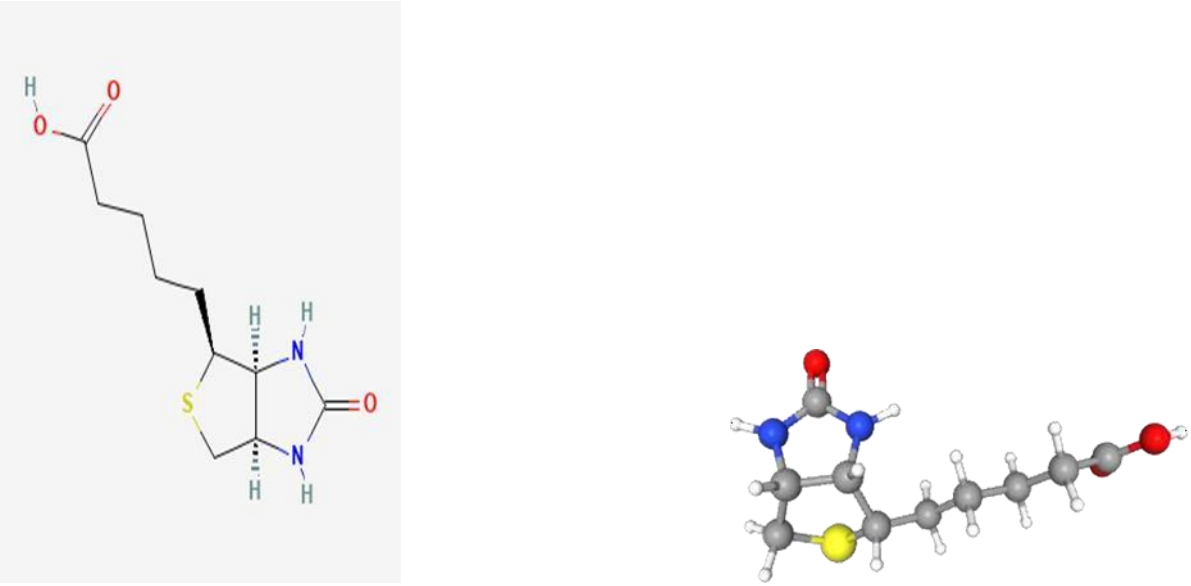

Fig 1.2 Structure of Biotin

Hexahydro-2-oxo-1H-thieno(3,4-d)imidazole-4-pentanoic acid is the chemical formula for biotin. Every live cell contains trace amounts of growth factor. It is found in abundance in the liver, kidney, pancreas, yeast, and milk, mostly bound to proteins or polypeptides. Cancerous tissue has a greater biotin level than normal tissue. Biotin is an enzyme cofactor found in trace levels in all living cells. Biotin is sometimes referred to as vitamin H, vitamin B7, and coenzyme R. It is found in abundance in the liver, kidney, pancreas, yeast, and milk, mostly bound to proteins or polypeptides. Biotin is regarded as an essential nutrient. Our biotin requirements are met in part by diet, endogenous reutilization of biotin, and maybe by capturing biotin produced by gut.

The biotin moiety is covalently bound to the epsilon amino group of a Lysine residue in each of these carboxylases in a domain 60-80 amino acids long. Evidence is emerging that biotin participates in processes other than classical carboxylation reactions.

Roles for biotin in cell signaling and chromatin structure are consistent with the notion that biotin has a unique significance in cell biology.

1.3.1 PROPERTIES:

Chemical and physical properties. The biotin molecule is activated by polarization of the O and N-1′ atoms of the ureido nucleus. • This leads to increased nucleophilicity at N-1′, which promotes the formation of a covalent bond between the electrophilic carbonyl phosphate formed from bicarbonate and ATP, and allows biotin to serve as a transport agent for CO2. The Vitamins. Fundamental Aspects in Nutrition and Health. Biotin is covalently bound to its enzymes by an amide bond to the ε-amino group of a lysine residue and C-2 of the thiophane nucleus.

1.4 EXITED STATE PROPERTIES:

Fluorescence is a member of the ubiquitous luminescence family of processes in which susceptible molecules emit light from electronically excited states created by either a physical (for example, absorption of light), mechanical (friction), or chemical mechanism. Fluorescence is the property of some atoms and molecules to absorb light at a particular wavelength and to subsequently emit light of longer wavelength after a brief interval, termed the fluorescence lifetime. The process is governed by three important events, all of which occur on times scales that are separated by several orders of magnitude which is clearly explain by the jablonski enery diagram. Excitation of a susceptible molecule by an incoming photon happens in femtoseconds (10-15 seconds), while vibrational relaxation of excited state electrons to the lowest energy level is much slower and can be measured in picoseconds (10- 12 seconds). The final process, emission of a longer wavelength photon and return of the molecule to the ground state, occurs in the relatively long time period of nanoseconds (10E-9 seconds). The electronic state of a molecule determines the distribution of negative charge and the overall molecular geometry. For any particular molecule, several different electronic states exist (illustrated as S(0), S(1), and S(2) in Figure 1.5, depending on the total electron energy and the symmetry of various electron spin states.

Each electronic state is further subdivided into a number of vibrational and rotational energy levels Although the entire molecular fluorescence lifetime, from excitation to emission, is measured in only billionths of a second, the phenomenon is a stunning manifestation of the interaction between light and matter that forms the basis for the

expansive fields of steady state and time-resolved fluorescence spectroscopy and microscopy.

Because of the tremendously sensitive emission profiles, spatial resolution, and high specificity of fluorescence investigations, the technique is rapidly becoming an important tool in genetics and cell biology.

The wavelength of maximum absorption (red line in the center) represents the most probable internuclear separation in the ground state to an allowed vibrational level in the excited state.

Harmonic potentials associated with the vibrational modes available in each state. The quantised vibrational levels and their probability distributions (coloured red) are also shown.

The probability distributions for each vibronic state are given by the square of the wave function at the allowed levels of a harmonic oscillator. Vibronic transitions (between vibrational levels of two electronic states) occur with high probability when there is a large overlap between the wave function of the occupied vibrational level of the initial electronic state and that of the final electronic state [1].

As a consequence, photoexcitation from the excited-state often produces an electronic state in a vibrational level higher than the lowest available mode ($v' > 0$). The state produced by such transitions is known as a Franck-Condon state**.**

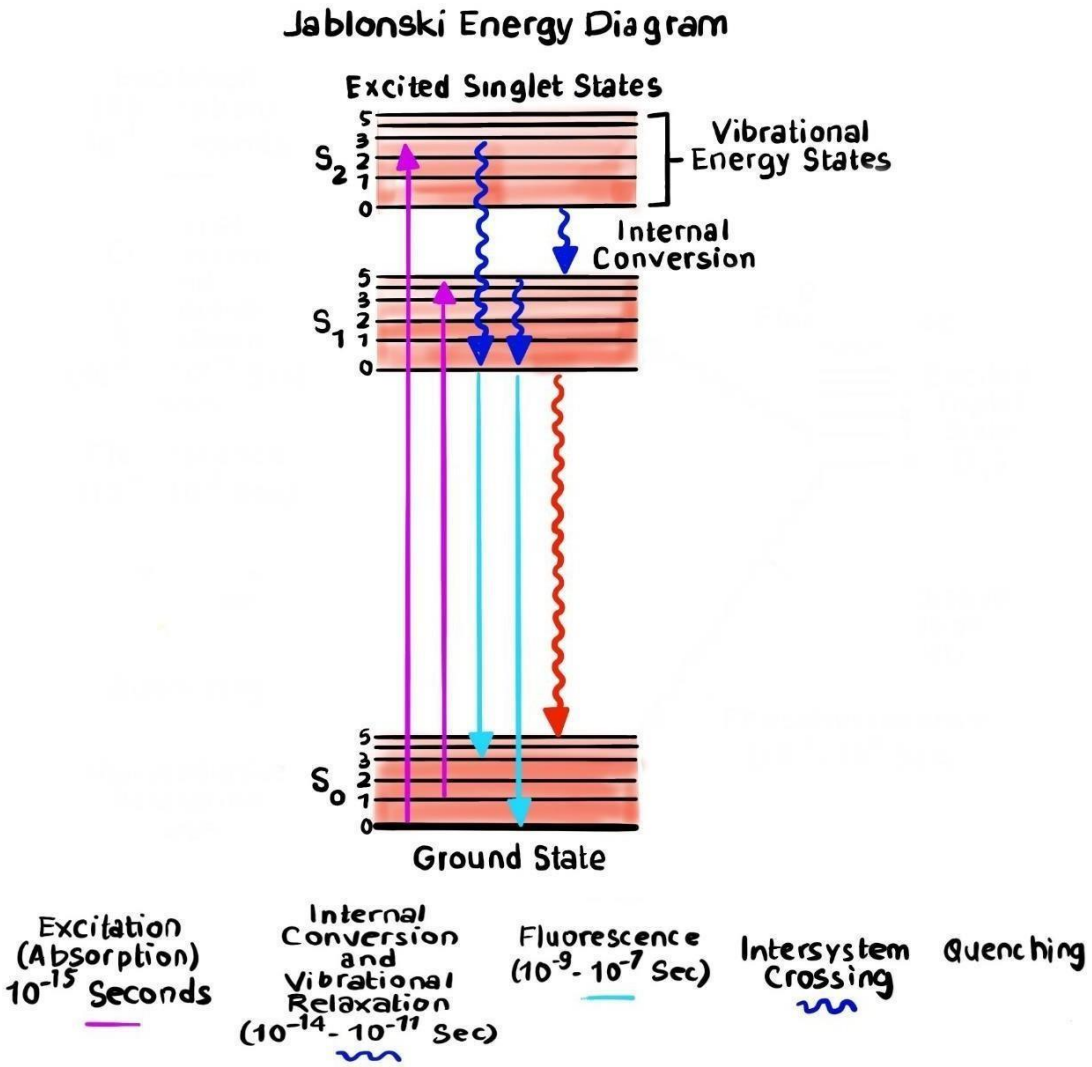

Fig 1.4(A) Jablonski Diagram

Scanning through the absorption spectrum of a fluorophore while recording the emission intensity at a single wavelength (usually the wavelength of maximum emission intensity) will generate the excitation spectrum. Likewise, exciting the fluorophore at a single wavelength (again, preferably the wavelength of maximum absorption) while scanning through the emission wavelengths will reveal the emission spectral profile. The excitation and emission spectra may be considered as probability distribution functions that a photon of given quantum energy will be absorbed and ultimately enable the fluorophore to emit a second photon in the form of fluorescence radiation. According to Franck Condon Principle: The wavelength of maximum absorption (red line in the center) represents the most probable internuclear separation in the ground state to an allowed vibrational level in the excited state. harmonic potentials associated with the vibrational modes available in each state.

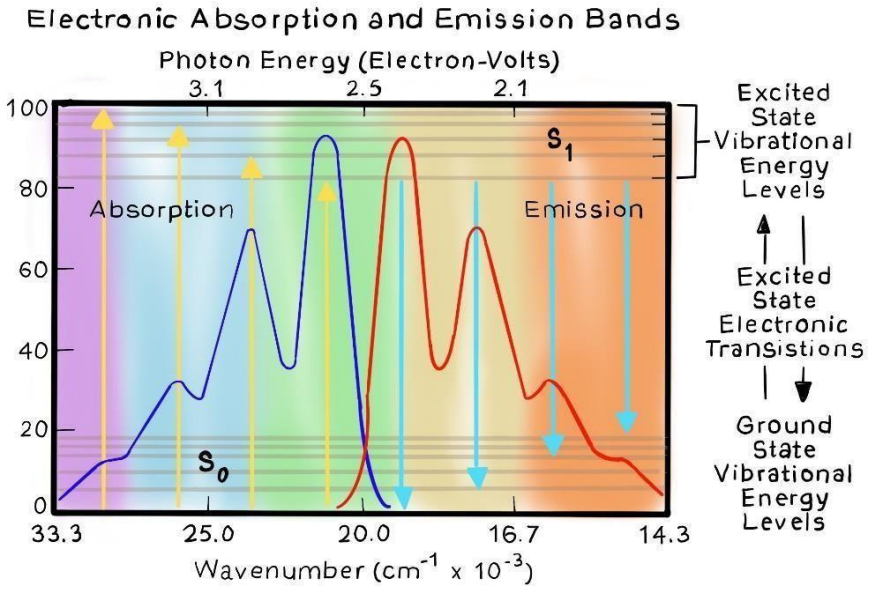

Fig 1.4(B) Electronic Absorption and Emission Bands

1.5 SOLVATION:

Solvation refers to the reorientation of solvent molecules to accommodate a changed charge or spatial distribution of a solute following photo-excitation. When there is a large difference in the dipole moments of the ground and excited states μ = μe − μg , this solvent reorganization can significantly lower the energy of the excited-states. The dipole-dipole interactions between vitamin and solute stabilize polar excited states, lowering their energy and therefore red-shifting the fluorescence band. Due to the large number of solvent molecules and degrees of freedom of solvent configuration, the potential energy of a molecule versus solvent coordinate can be treated as a classical continuum rather than a quantized system. As with the Franck-Condon principle for vibronic transitions, electronic transitions occur essentially instantaneously with respect to solvent orientation.

Thus the solvent is in a non-equilibrium configuration immediately following excitation and must reorganize into the lowest energy excited-state configuration. Relaxation of the vibronic state of the molecule again produces a non-equilibrium solvation configuration as the ground electronic state has a smaller dipole moment. Because of this, in a time-resolved experiment one would expect to see a shift in the energy of the

excited-state transitions (such as fluorescence and excited state absorption - to be discussed in chapter 3) to the red within ones to tens of picoseconds. Conversely, ground-state dynamics would be expected to shift to the blue as the solvent reorganizes, thereby lowering the energy of the molecular ground-state-solvent system.

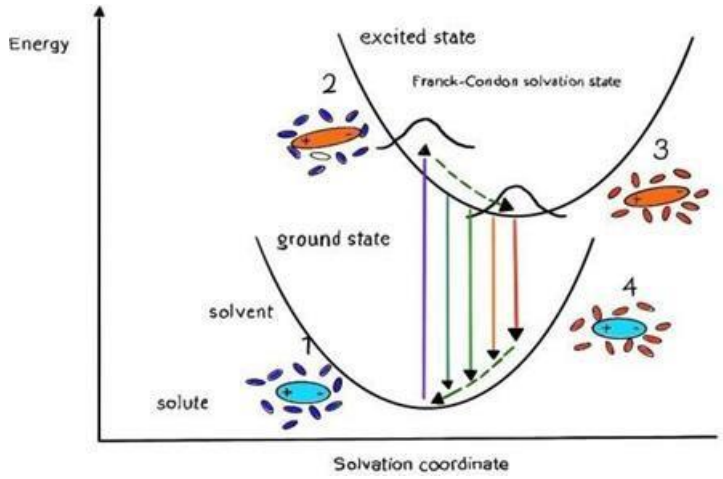

Fig 1.5 .Relaxation vibronic state of molecule

1.6 SYNTHESIS OF DDP DYE:

For synthesis of 4- dicyano methylene- 2,6-dimethyl-4H- pyran (DCM intermediate), a mixture of malononitrile (1 mmol), 2,6-dimethyl-4H-pyran-4-one (1 mmol) and acetic anhydride (0.5 ml) was refluxed for 1.5 h. The unreacted acetic acid was aspirated off and the residue was washed with 50 ml. of boiling water and collected to give soft brown material.

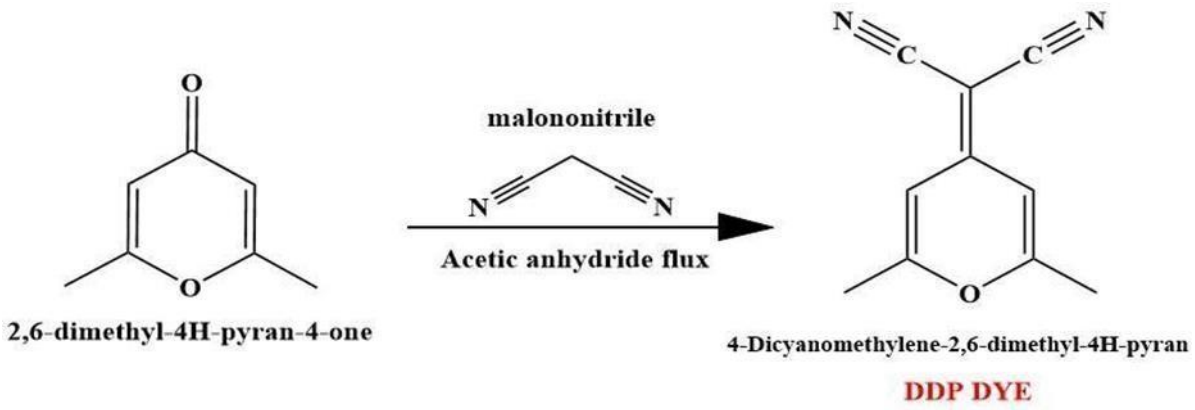

Fig 1.7 Synthesis of DDP Dye

CHAPTER 2

LITERATURE SURVEY

2.1 Webster and W. McGolgin. Arylidene dye lasers, US patent 3,852,683, 1974:

DCM is a fluorescent dye developed by the Eastman-Kodak company, in the mid-1970s BY F. Webster and W. McGolgin. to extend the range of dye lasers into the red spectrum. Its spectral properties include a broad fluorescence band, long excited-state lifetime, and large solvent-dependent Stokes shift. DCM has been widely studied for its excited-state dynamics and molecular configuration. It is a donor-π-bridge-acceptor molecule, containing an electron-donating dimethylamino group and an electron-accepting dicyano group. Photoinduced intramolecular charge transfer is expected to occur in such systems, involving the migration of the excited free electron from the donor to the acceptor before relaxing back to the ground state. Research on DCM has focused on its energy, excited-state dynamics, and potential applications in sensing and imaging.

2.2. Absorption of DCM Dye in Ethanol: Experimental and Time DependentDensity Functional Study:

Seyed Hassan Nabavia, (2018) has studied Absorption of DCM Dye in Ethanol: Experimental and Time Dependent Density Functional Study in which he describes the synthesis of the DCM intermediate and DCM dye using a combination of malonitirile, 2,6-dimethyl-4H-pyran-4-one, (N, N-Dimethyl) benzaldehyde, toluene, piperidine, and acetic acid. The intermediate was obtained through refluxing, washing, and recrystallization, while the dye was produced by refluxing the intermediate under argon gas for 20 hours, followed by column chromatography for purification. The hydrogen NMR spectrum of the final DCM product is available in supporting information. A literature survey may explore the properties and uses of DCM dyes, the synthesis of pyran derivatives, and the potential applications of the intermediate in organic synthesis.

2.3. Fluorescence umpolung enables light-up sensing of N-acetyltransferasesand nerve agents:

Chenxu Yan, (2021) discusses the recent advances in the development of Intramolecular Charge Transfer (ICT) based fluorophores along with tailoring their emission properties for high-fidelity bioimaging by investigating the use of high-performance donor-π-acceptor (D-π-A) fluorescent dyes, such as dicyanomethylene-4H-pyran (DCM), quinoline-malononitrile (QM), and so on, for biosensing and bioimaging applications. These fluorophores are known for their high sensitivity to electron disturbance and large Stokes shifts, making them promising platforms for developing fluorescent dyes and probes. However, the interaction of the donor receptor with electron-withdrawing targets (EWTs) can suppress the ICT pathway and quench the fluorescence, limiting their applicability. To address this issue, a molecular design strategy has been proposed wherein an indazole building block is inserted into the D-π-A motif to regulate intramolecular rotational driving energy. This strategy leads to a fluorescence umpolung effect, completely overturning the EWT-induced quenching mode into the light-up mode. The straightforward insertion of an indazole building block into the D-π-A fluorophores can substantially increase intramolecular rotation, resulting in a rotation-induced dark state, whereas the incorporation of EWTs can efficiently suppress rotation and enhance fluorescence. This strategy expands our understanding of ICT mechanisms and enables the design of ICT probes for light-up sensing of EWTs, such as N-acetyltransferases and nerve agents.

2.4. Photophysical and Electrochemical studies of 4-dicyanomethylene 2,6-dimethyl- 4H-pyran (DDP) dye with amides in water:

Somasundaram Gayathri, (2018) discussed that the DDP dye, also known as Dicyanomethylene-2,6-dimethyl-4H-pyran, which is an intermediate in the preparation of DCM dye was synthesized and studied the photophysical and electrochemical properties of DDP dye with formamide and alkyl-substituted amides in water were investigated. The addition of amides results in an isosbestic point and fluorescence enhancement of the DDP dye. The position of emission maxima of DDP dye shifted towards the blue and red regions with the addition of ACM and DMF, respectively. The fluorescence lifetime and relative amplitude of DDP dye vary significantly by the addition of amides in an aqueous solution, which is influenced by the amide water

hydrogen-bonding network and hydrophobic influences of the alkyl-substituted amides. The interaction between dye and amide is predominantly through hydrogen bonding. EIS results showed that there are at least three different microenvironments that support the existence of different fluorescence lifetimes of DDP dye. The fluorescence spectral technique is an efficient tool to elucidate the nature of the interaction of water-soluble probes with hydrogen-bonding solutes. Overall, the study provides insights into the photophysical and electrochemical properties of DDP dye and its interactions with different amides in aqueous solution.

2.5. Spectro electrochemical Investigation of 4-dicyanomethylene 2,6-dimethyl-4H-pyran (DDP) dye with Guanidine hydrochloride (GuHCl) in water:

Somasundaram Gayathri, (2018) investigates the interaction between guanidine hydrochloride (GuHCl) and 4-dicyanomethylene-2,6-dimethyl-4H-pyran (DDP) dye through photophysical and electrochemical techniques. The results indicate that the interaction between DDP dye and GuHCl is primarily through hydrogen bonding, although electrostatic interactions also play a role in the aqueous phase. The addition of amides to the aqueous solution affects the ground state and excited state properties of DDP dye, as evidenced by fluorescence enhancement and shift in the emission maximum. Hydrogen bonding and hydrophobicity of the amides are the main factors influencing this photophysical nature. The formation of a dye- water-amide hydrogen-bonding network along with dye-water and amide-water hydrogen-bonding assemblies is responsible for this effect.

2.6. Photophysical and Electrochemical Impedance Spectral studies on the interaction of 4-dicyanomethylene 2, 6-dimethyl- 4H-pyran (DDP) dye with urea derivatives in aqueous solution:

Somasundaram Gayathri, and Rajaraman Vasanthi, (2018) performed studied the interaction between urea derivatives and DDP dye using photophysical and electrochemical techniques. The addition of urea derivatives to DDP dye resulted in fluorescence enhancement and a shift in emission wavelength. Fluorescence lifetime studies showed the coexistence of three different components, indicating a heterogeneous microenvironment around the dye. The urea derivatives influenced the excited state characteristics of the dye, resulting in the formation of different microenvironments. Electrochemical impedance spectral studies validated the

existence of multiple environments attributed to urea-water structural behaviour. The urea derivatives' hydrophobicity and hydrogen-bonding properties influenced the dye's photophysical and electrochemical nature.

2.7. Rapid assay for avidin and biotin based on fluorescence quenching:

Biotin was covalently tagged with a BODIPY dye which can undergo an efficient distance-dependent fluorescence selfquenching. Multivalent binding of avidin with the BODIPY-labeled biotin (B581/591-biotin, either in aqueous buffer, or anchored on the surfaces of lipid vesicles or lipid bilayers coated on glass beads) induces aggregation of the BODIPY dye (up to four dyes for each avidin) to result in a decrease in fluorescence intensity due to fluorescence self-quenching. The system can be used to perform a rapid, direct assay for avidin and competitive assay for biotin with high sensitivity (<50 pM for avidin and <0.2 nM for biotin) and selectivity. The assay method is generally applicable for detection of all the species involved in a multivalent binding interaction.

2.8. Biotin-4-Fluorescein Based Fluorescence Quenching Assay for Determination of Biotin Binding Capacity of Streptavidin Conjugated Quantum Dots:

The valency of quantum dot nanoparticles conjugated with biomolecules is closely related to their performance in cell tagging, tracking, and imaging experiments. Commercially available streptavidin conjugates (SAv QDs) are the most commonly used tool for preparing QD-biomolecule conjugates. The fluorescence quenching of biotin-4-fluorscein (B4F) provides a straightforward assay to quantify the number of biotin binding sites per SAv QD. The utility of this method was demonstrated by quantitatively characterizing the biotin binding capacity of commercially available amphiphilic poly (acrylic acid) Qdot ITK SAv conjugates and poly (ethylene glycol) modified Qdot PEG SAv conjugates with emission wavelengths of 525, 545, 565, 585, 605, 625, 655, 705, and 800 nm.

CHAPTER 3

AIM AND SCOPE

AIM:

The main aim is to study the interaction between DDP dye (Host) and Biotin (Guest) molecule and to find the hydrogen bonding of Biotin in aqueous solution. With the help of UV-Visible spectroscopy, Emission spectroscopy, Fluorescence life time spectroscopy I studied the hydrogen bonding of Biotin in the aqueous solution.

SCOPE:

The photophysical and photochemical process of fluorophore molecules in hydrogen-bonding self-assemblies forming supramolecular systems is an intensive and interesting field of research. The process happening in aqueous medium is more source reliable and extensively useful in the field of biology and medicine. The nature of interaction between the probe with molecules or compounds that form supramolecular self-assemblies and molecular aggregates in aqueous solution provides a basis for studying the ground and excited state properties of the probe. The photophysical properties of the fluorescent probes in general are influenced by polarity of the solvent, refractive index, dielectric constant, temperature and viscosity of the medium. Apart from these influences, the solvent assisted hydrogen-bonding interactions induced by the solutes, the presence of hydrophobic moieties;charged species influences and governs the photophysical properties of these probes. The variation in the quantum yield, decrease or increase in the fluorescence intensity, fluorescence lifetimes and shift in the emission maxima of the fluorophore in the presence of host molecules provides a better knowledge in determining the exact location of the probe and its orientation in a microheterogeneous environment.

The case study of ICT based DDP dye interaction with hydrogen-bonding solutes forms the basis of the other results in the present study. DDP dye possess several advantages over the conventional DCM type dye (Red dye) based on its better solubility in water and ICT nature. The photophysical studies and the fluorescence spectral properties (fluorescence emission, lifetime and ansisotropy) of DDP dye was monitored and explored extensively with Biotin(vitamin) in aqueous solution.. The mechanism and the nature of interaction involving these hydrogen-bonding solutes with DDP dye are characterized by the change in the fluorescence intensity and the fluorescence lifetime, which is correlated to the combined effect of hydrogen-bonding and hydrophobic nature of these solutes. The orientation of DDP dye is not confined to homogeneous environment and this was elucidated from fluorescence spectral techniques. The existence of dye in different microenvironments was established from the multi exponential lifetime characteristics of DDP dye in the presence of various solutes.

CHAPTER 4

MATERIAL AND METHODOLOGY

4.1 MATERIALS AND METHODS:

The preparation of DDP DYE dyes were carried out as reported in the literature . DDP DYE stock solution was freshly prepared in triple distilled water. BIOTIN, laboratory grade sample of CAS No 9000-01-05 was purchased from Merck. 2% (w/v) solution of BIOTIN was prepared by adding 2 g of spray dried powder to 100 mL of triply distilled water. Sulfuric acid (GR grade with assay 98.2%) was purchased from Qualigens India Ltd. Acid catalyzed hydrolysis was carried out by the addition of 50 mL of 0.5 N sulfuric acid to 50 mL of BIOTIN solution (1:1) ratio with constant stirring in order to prepare a clear homogeneous bulk solution. The temperature was maintained at 68-70 °C.

4.2 EXPERIMENTAL METHODS:

Absorption spectra were recorded using an Agilent 8453 diode array spectrophotometer. Fluorescence measurements and steady state anisotropy measurements were performed in Fluoromax-4P spectrometer (Horiba Jobin Yvon). The emission spectrum was recorded by carrying out a corrected spectrum following the literature reported and the instrument voltage and slits were kept constant during the course of recording the fluorescence intensity. Time-resolved fluorescence decays were obtained by the time correlated single-photon counting (TCSPC) method. A diode pumped Millenia V CW laser (Spectra Physics) was used to pump the Ti– sapphire rod in a Tsunami picosecond mode-locked laser system (Spectra Physics). The 750 nm (85 MHz) beam from the Ti–sapphire laser was passed through a pulse picker (Spectra Physics, GWU 23PS) to generate 4 MHz pulses. The second harmonic output was generated by a flexible harmonic generator (Spectra Physics, GWU 23PS). A vertically polarized 377 nm laser was used to excite the sample. The fluorescence of DDP DYE dye was monitored at magic angle (54.7o). This was counted by a MCP-PMT apparatus (Hamamatsu R3809U) after being passed through the monochromatorand was proceeded through a constant fraction discriminator (CFD), a time-to- amplitude converter (TAC) and a multichannel analyzer (MCA). The instrument response function for this system is ~50 ps. The obtained fluorescence decays were analyzed by using IBH software (DAS-6) which is based on reconvolution technique

using iterative nonlinear least squares method. The reduction potential and oxidation potential of DDP DYE (10 mL of 1.5×10-4 M) in the absence and presence of BIOTIN and their respective diffusion coefficients were evaluated by electrochemical experiments.The diffusion coefficients of both fluorophore and quencher were determined experimentally as per the literature reported.

4.2.1 UV-VIS SPECTROSCOPY:

Ultraviolet–visible (UV/Vis) spectroscopy is based on the absorption of the electromagnetic radiation in UV/Vis region, with the wavelength ranges of 200–400 nm, called 'ultraviolet spectroscopy,' and 400–800 nm, called 'visible spectroscopy.

The Principle of UV-Visible Spectroscopy is based on the absorption of ultraviolet light or visible light by chemical compounds, which results in the production of distinct spectra. Spectroscopy is based on the interaction between light and matter. When the matter absorbs the light, it undergoes excitation and de-excitation, resulting in the production of a spectrum.

When matter absorbs ultraviolet radiation, the electrons present in it undergo excitation. This causes them to jump from a ground state (an energy state with a relatively small amount of energy associated with it) to an excited state (an energy state with a relatively large amount of energy associated with it). It is important to note that the difference in the energies of the ground state and the excited state of the electron is always equal to the amount of ultraviolet radiation or visible radiation absorbed by it.

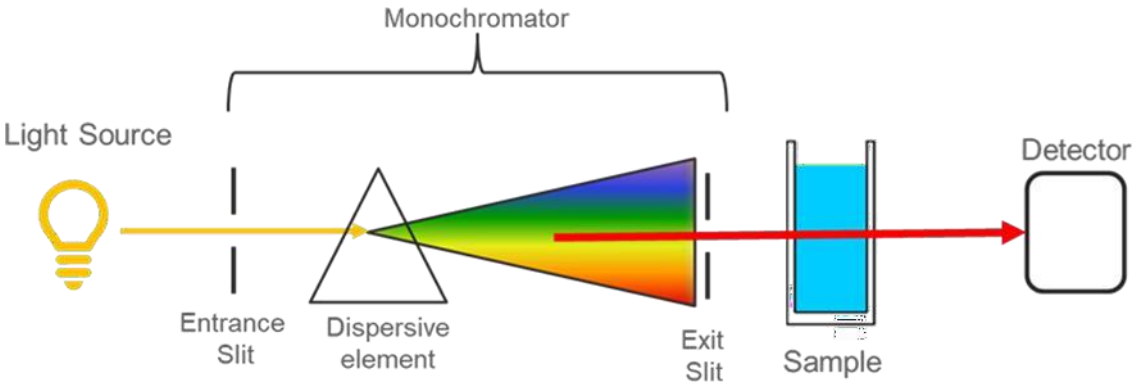

Fig 4.1 Uv-Visible spectrometer

4.2.2 FLUORESCENCE EMISSION SPECTROSCOPY:

Emission spectroscopy is a spectroscopic technique which examines the wavelengths of photons emitted by atoms or molecules during their transition from an excited state to a lower energy state.

An emission spectrum consists of all the radiations emitted by atoms or molecules, whereas in an absorption spectrum portions of a continuous spectrum (light containing all wavelengths) are missing because they have been absorbed by the medium through which the light has passed; the missing wavelength.

Whenever electromagnetic radiations interact with atoms and molecules of matter, the electrons in these atoms may absorb energy and jump to a higher energy state, losing their stability.

To regain their stability, they need to move from the higher energy state

to the previous lower energy state. To accomplish this job, these atoms and molecules emit radiations in various regions of the electromagnetic spectrum.

This spectrum of radiation emitted by electrons in the excited atoms or molecules is known as the emission spectrum.

It can be defined as the emission spectrum of a chemical element or chemical compound is the spectrum of frequencies of electromagnetic radiation emitted due to an atom or molecule transitioning from a high energy state to a lower energy state.

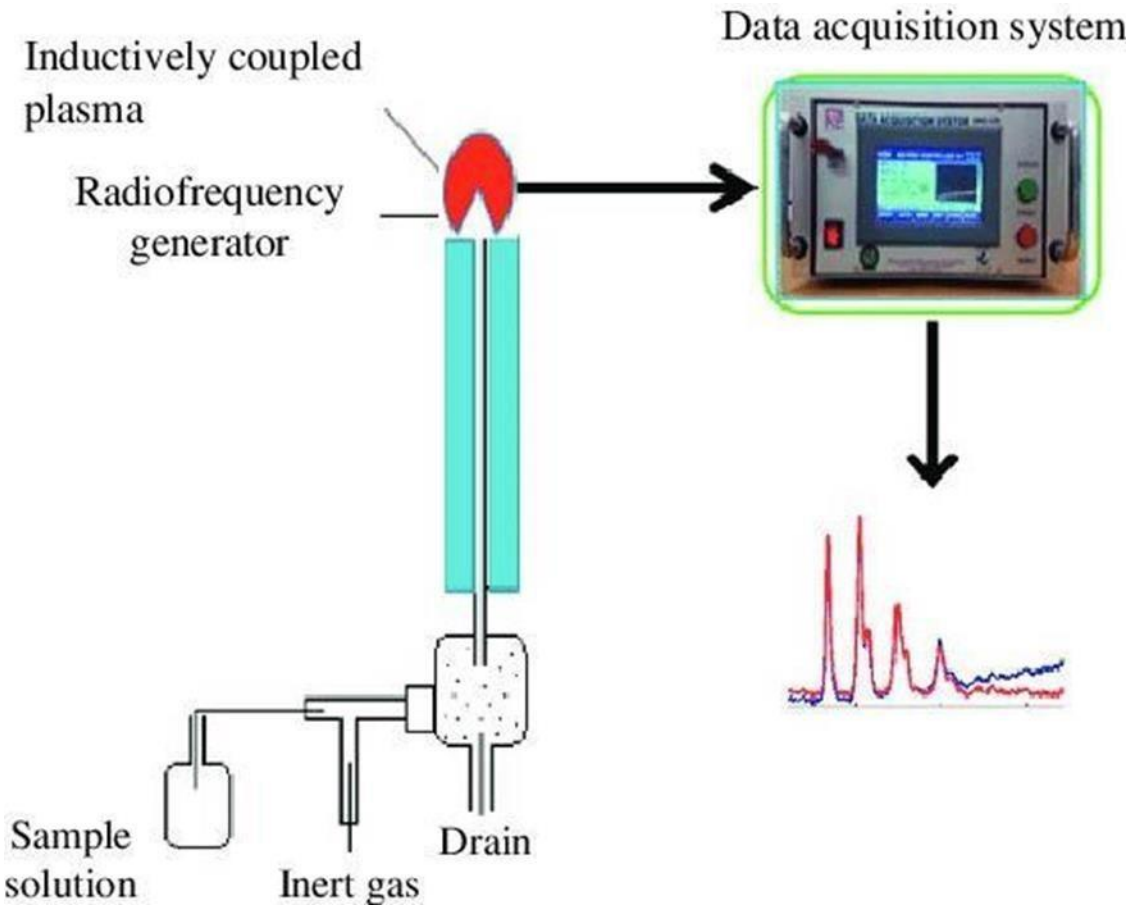

Fig 4.2 FL Spectrometer

4.2.3.TIME-CORRELATED SINGLE PHOTON COUNTING -TCSPC:

Fluorescence lifetime (FLT) is the time a fluorophore spends in the excited state before emitting a photon and returning to the ground state. FLT can vary from picoseconds to hundreds of nanoseconds depending on the fluorophore.

It is affected by external factors, such as temperature, polarity, and the presence of fluorescence quenchers. Fluorescence lifetime is sensitive to internal factors that are dependent on fluorophore structure.

into the energy relaxation and dynamics of the species under study, such as energy transfer between molecular states, molecular rotation, and dynamic quenching.

Fluorescence is a type of luminescence caused by photons exciting a molecule, raising it to an electronic excited state. It's brought about by absorption of photons in the singlet ground state promoted to a singlet-excited state.

Three important factors influencing the intensity of fluorescence emission were theoretical analyzed, including the absorption ability of excitation photons, fluorescence quantum yield, and fluorescence saturation & fluorescence quenching.

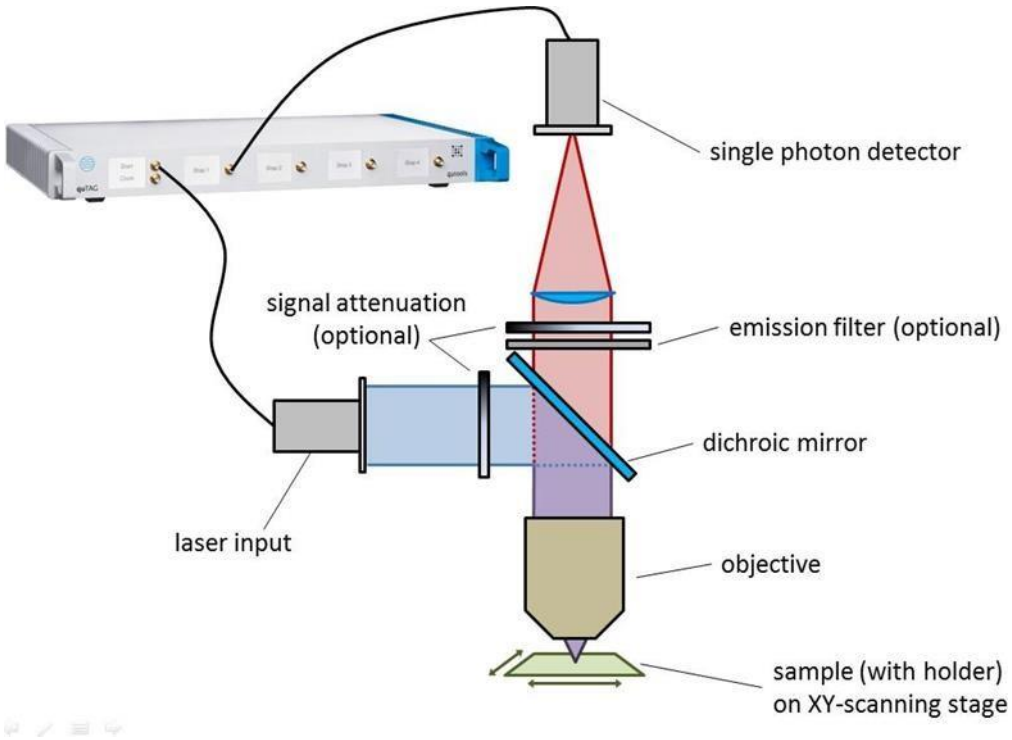

Fig 4.3TIME-CORRELATED SINGLE PHOTON COUNTING -TCSPC

CHAPTER 5

RESULTS AND DISCUSSION

5.1 ABSORPTION SPECTRAL STUDIES:

The absorption spectrum of DDP DYE in water exhibits a maximum at ~378 nm and this band is assigned to the charge transfer from the ring nitrogen to the carbonyl oxygen center by local excited (LE) state. The absorption spectrum of DDP DYE in the absence of BIOTIN in water. No significant change in the dye absorption maximum results on the addition of BIOTIN to DDP DYE.

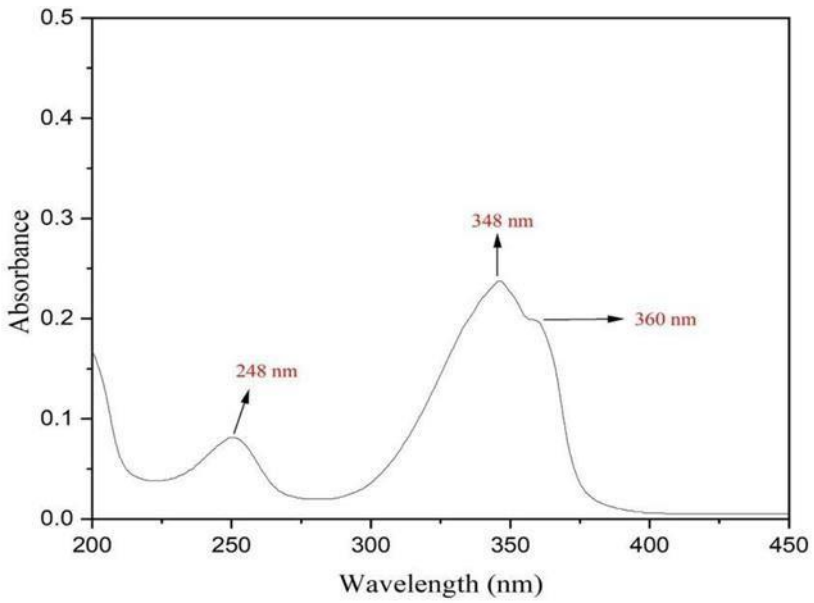

Fig 5.1 Absorption spectra of DCM dye (8 x10^{-5} M) in water.

The increase in the absorbance ~270 nm is attributed to the strong absorbance of BIOTIN. However the intramolecular charge transfer (ICT) absorption maximum of DDP DYE is not influenced by the addition of BIOTIN such that no characteristic interaction could be ascertained at the ground state energy level. This is due to the close spacing of the energy levels and no concrete mechanism could be established at this level.

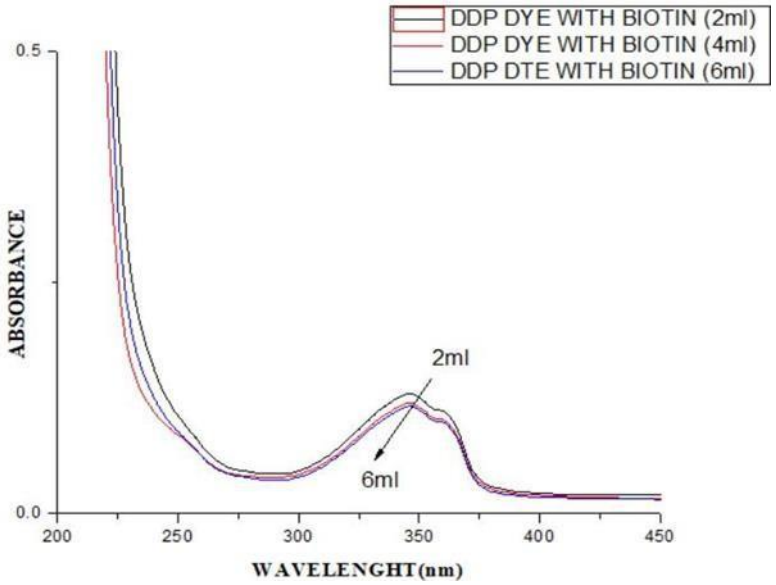

Fig 5.1.2 Absorption spectra of DDP dye (8 x 10-5 M) with varying concentrations of Biotin in water. The concentration of Trypsin: 1) 2.0 M, 2) 4.0 M, 3) 6.0 M

5.2 FLUORESCENCE SPECTRAL

The fluorescence spectrum of DDP DYE exhibits a broad peak at ~440 nm in water when excited at the longest wavelength absorption maximum. The fluorescence ~440 nm is assigned to the LE state emission. Addition of BIOTIN to DDP DYE results in a quenching of fluorescence intensity without the appearance of new emission band even at high concentration of BIOTIN (2.2865×10-5 M).This observation eliminates the formation of exciplex as the intermediate and addition of BIOTIN results in a gradual decrease in the fluorescence intensity. The fluorescence maximum shifts towards the blue region (~8 nm) with decrease in the fluorescence intensity as increase in the concentration of BIOTIN indicates that the polarity of the medium decreases. It was observed that addition of 1.428×10-5 M of BIOTIN results in 85% quenching of the DDP DYE fluorescence. The extent of fluorescence quenching of DDP DYE on the addition of BIOTIN. Interestingly, the extent of fluorescence quenching after the addition of 1.428×10-5 M of BIOTIN to DDP DYE remains the same with no further significant decrease in the fluorescence intensity. The fluorescence quenching of DDP DYE by BIOTIN is attributed to the promotion of ICT through space. Moreover, the addition of BIOTIN does not suppress the ICT process as observed in the interaction of DDP DYE dyes with solutes like urea derivatives,

bovine serum albumin, guanidine hydrochloride and amides. The addition of these solutes results in fluorescence Quenching and has been reported. BIOTIN promotes the electron transfer process through space resulting in promotion of ICT process. The schematic representation of the most probable orientation

chemical changes in surrounding ground state molecules. A variety of processes can result in quenching which includes excited state reactions, energy transfer, electron transfer, complex formation and collisional quenching.

Bimolecular quenching rate constants for the electron transfer process were determined by using the Stern-Volmer (S-V) relation.

where F0 and F are the fluorescence intensity of DDP DYE in the absence and presence of BIOTIN, respectively. Ks-v is the Stern-Volmer constant, kq is the bimolecular quenching rate constant, 0 is the fluorescence lifetime of DDP DYE in the absence of quencher. The S-V plot exhibits nebiotintive deviations from the linearity at higher concentration of BIOTIN. The representative S-V plot for the DDP DYE- BIOTIN system.The downward curvature may be due to DDP DYE located in distinct environment of BIOTIN with differently accessible to quencher. Generally, Stern-Volmer plot will exhibit downward curvature if the multi-existence of fluorophores, one of which is accessible to quenchers and the other being inaccessible. Similar to present results that downward curvature in Stern-Volmer plot caused by DDP DYE located in distinct environment of BIOTIN, previously many literatures have reported on determining the fraction of protein fluorescence accessible to quenchers.

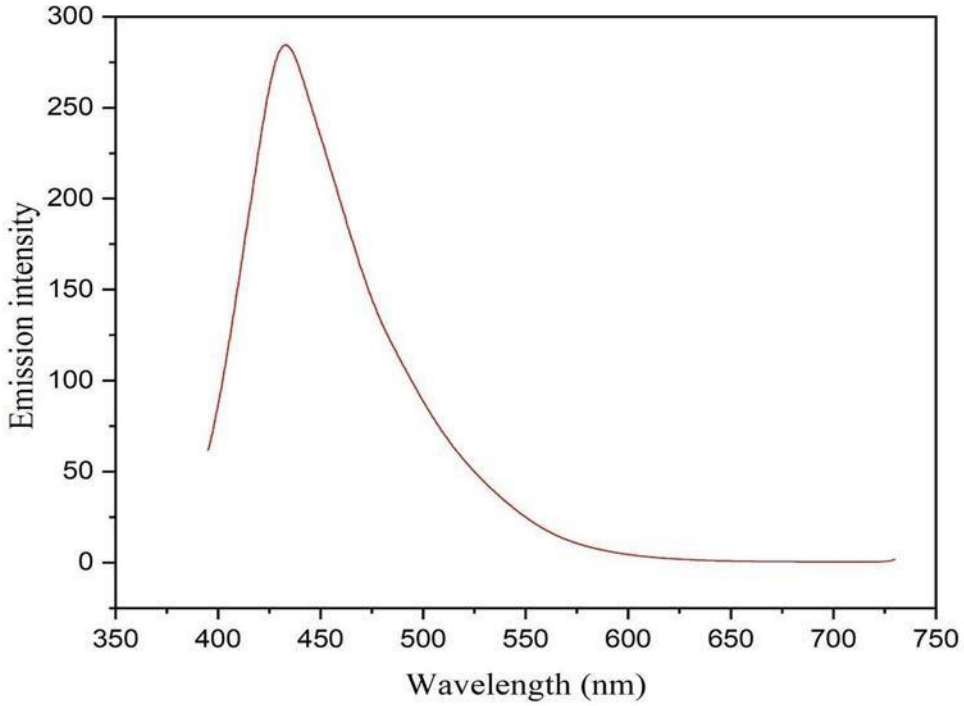

Fig 5.2.1. Emission spectra of DDP dye (8 x 10^{-5} M) in Water.

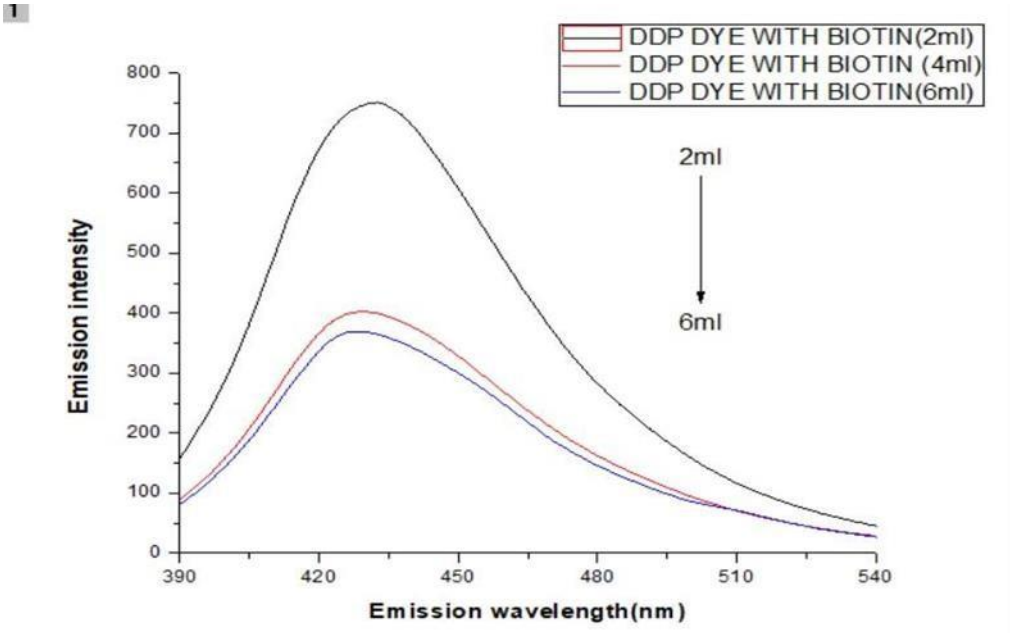

Fig 5.2.2.Emission spectra of DDP dye (8 x 10-5 M) with varying concentrations of Biotin in water. The concentration of Trypsin: 1) 2.0 M, 2) 4.0 M, 3) 6.0 M

The Stern-Volmer plot deviates from linearity and the quenching process was analyzed by a modified Stern-Volmer equation by Lehrer,

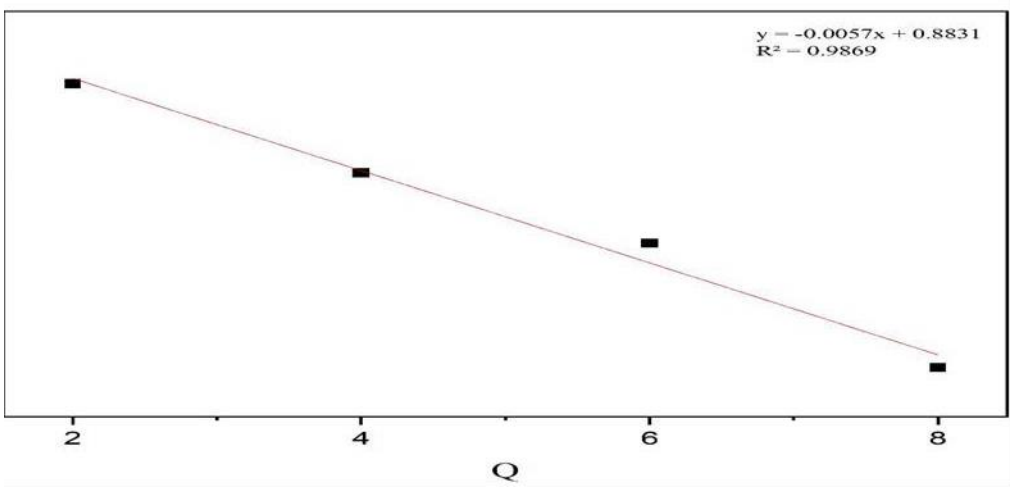

Fig 5.2.3 Extent of fluorescence quenching of DDP dye on the addition of Biotin.

5.3.TIME-RESOLVED FLUORESCENCE STUDIES:

Where fa is the fraction of initial fluorescence that is accessible to quencher, Ka is the Stern-Volmer quenching constant of the accessible fraction and ΔF is the F0-F. versus 1/[BIOTIN] exhibits a straight line, which confirms the 1:1 stoichiometry of DDP DYE with BIOTIN (Inset of Figure 4). The association constant was determined by dividing the intercept by the slope of the straight line. The log K was estimated to be 5.75 which indicate that the DDP DYE dye is strongly associated within BIOTIN. The dye probably resides mostly in the hydrophobic region compared to that of hydrophilic or aqueous region

The fluorescence lifetime of DDP DYE exhibits a single exponential behavior in aqueous solution. The fluorescence lifetime of 500 ps is attributed to the ICT process through space between the methoxy group and the acridinedione ring. On the addition of BIOTIN to DDP DYE the fluorescence lifetime decay of DDP DYE exhibits a tri-exponential behavior. The fluorescence decay of DDP DYE is shown in Figure 5. Addition of 3.23×10-6 M BIOTIN to DDP DYE results in the fluorescence lifetime of

213 ps (86%), 1.65 ns (5.23%) and 4.27 ns (8.77%). The 213 ps component is attributed to the free dye component present in the aqueous solution and this was evident from the relative amplitude of DDP DYE dye. Further, increase in the concentration of BIOTIN results in gradual decrease in the fluorescence lifetime of the free dye component, such that at very high concentration of BIOTIN (2.29×10-5 M), the relative population of free DDP DYE is 36 % only. Addition of BIOTIN results in the formation of a heterogeneous micro environment in aqueous phase wherein the dye molecule prefers to reside in the close vicinity of comparatively hydrophobic environment which is in accordance to the fluorescence emission and anisotropy studies.

Fluorescence lifetime of a fluorophore serves as a sensitive parameter for exploring the local environment around a fluorophore and it is sensitive to the excited state reactions. The binding of fluorophore to the host molecules like proteins (aggrebiotintes), micelles and or biotinized self-assemblies, the solvent relaxation around the fluorophore, and change in the microenvironment results in a marked variation in the fluorescence lifetime of the probe in aqueous medium.

BIOTIN consists of subiotinr amino acids and other hydrophobic moieties such that promotion of different microenvironment is facilitated in the aqueous phase. DDP DYE is hydrophobic in nature and prefers to orient towards the less hydrophilic environment. BIOTIN easily diffuses into the DDP DYE resulting in large decrease in the fluorescence lifetime of DDP DYE (Table 1). Based on the fluorescence lifetime results, it can be assumed that the relatively long-lived components (2 and 3) correspond to the DDP DYE located in the different environment of BIOTIN.

several subiotinr and amino acid moieties. Presence of these moieties creates several hydrophilic and hydrophobic phases and the medium tends to be more . In our previous work, we have measured the fluorescence lifetime of same DDP DYE with addition of different concentration of BSA and reported that the DDP DYE reside in hydrophilic and hydrophobic pockets of BSA with lifetimes of ~2 and 6.5 ns respectively [6]. In the present work, the observed fluorescence lifetimes (2 and 3) of DDP DYE are closely similar to the previously reported values of DDP DYE with BSA]6]. This indicates that the DDP DYE is located both in hydrophilic and hydrophobic phases of BIOTIN which is further supported by the higher binding constant value.

Interestingly, the fluorescence lifetime components of 2 and 3 almost remain the same with increase in concentration of BIOTIN, but their corresponding relative amplitude increases gradually (Table 1). This reflects that DDP DYE dye preferentially resides near the close proximity of subiotins that are present in the aqueous solution obtained by the hydrolysis of BIOTIN. When the ratio of dye: BIOTIN is above 1: 0.5, there is not much change in the relative amplitude of the fluorescence lifetime components 2 and 3. The decrease in the fluorescence lifetime of free dye is attributed to the electron transfer from BIOTIN to DDP DYE dye and definitely not due to hydrogen-bonding interaction. Moreover, the ICT process through space between the methoxy group and the acridinedione ring of DDP DYE is enhanced by the presence of BIOTIN and this phenomenon is in agreement from the decrease in the fluorescence lifetime of free dye from 500 ps to 50 ps.

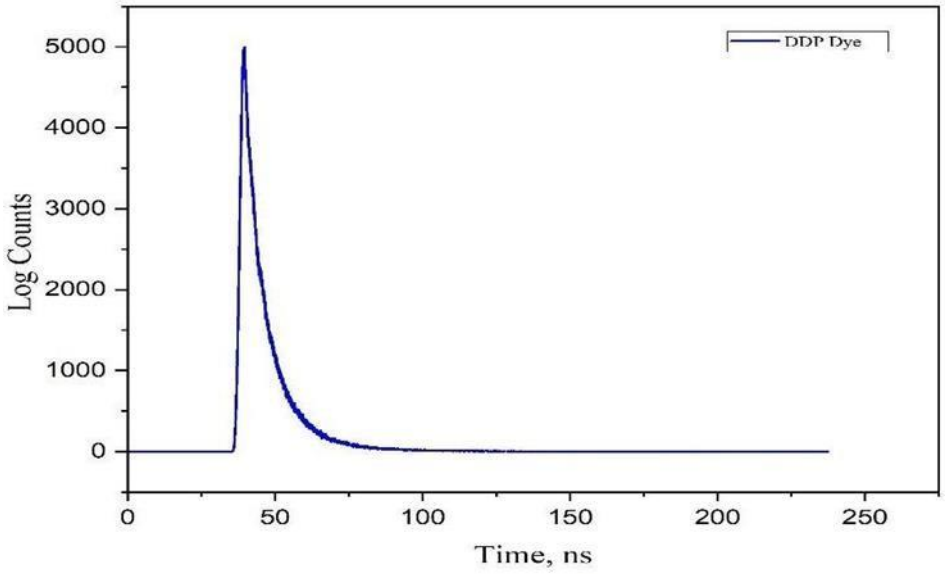

Fig 5.3.1.Fluorescence decay of DDP dye

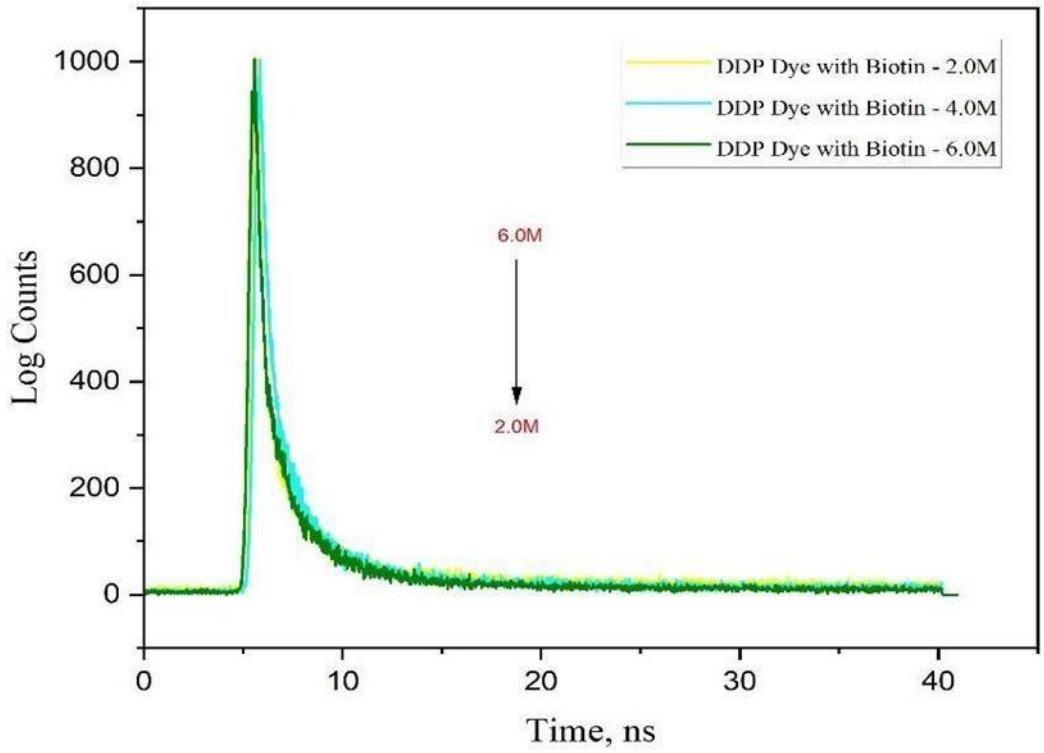

Fig 5.3.2. Fluorescence decay of DDP dye (8 x 10-5 M) as a function of TRYPSIN in water. λex370 nm and λem 436 nm. (A) DDP dye alone; (B) i) DDP dye + Trypsin 2.0M, ii) DDP dye+ Trypsin 4.0M, iii) DDP dye + Trypsin 6.0 M.

The promotion of electron transfer results in a 10 fold decrease in the LE state emission as observed form steady state spectral studies (Figure 2). The fluorescence lifetime of free unbound dye is ~500 ps and this decreases by 10 fold on the addition of BIOTIN.

Moreover the water molecule is situated to the close vicinity of the carbonyl oxygen and hydrogen-bonding exits between the carbonyl oxygen and water molecule as shown in Scheme 3. This is to ascertain that the distance between the donor and acceptor moieties in DDP DYE does not change in the presence of solvent molecule. Further the electron transfer is well promoted by the presence of BIOTIN resulting in an effective ICT through space. The promotion of electron transfer results in a 10 fold decrease in the LE state emission as observed form steady state spectral studies (Figure 2). The fluorescence lifetime of free unbound dye is ~500 ps and this decreases by 10 fold on the addition of BIOTIN.

5.4 BIOTIN DDP-DYE HYDROGEN BONDING IN THE PRESENCE OF WATER MOLECULES:

The Role of Hydrogen Bonding in the Interaction of Biotin and DDP Dye in Aqueous Solutions in Different Phases:

In the shell phase,Biotin and DDP dye can form a well-organised hydrogen-bonding network with water molecules. The water molecules in the shell phase can surround the dye, and the hydrogen atoms of the water molecules can form hydrogen bonds with the electronegative atoms in the DDP dye and trypsin.

In this phase, the microenvironment of the dye is mainly influenced by the surrounding water molecules.In the intermediate phase, the dye is surrounded by fewer Biotin molecules in the aqueous solution. In this phase, hydrogen bonds between the dye and Biotin may form via Biotin residues.

The dye's microenvironment in this phase is influenced by both the surrounding water molecules and the Biotin residues.In the bulk phase, the dye is surrounded by more Biotin molecules in the aqueous solution.

The formation of hydrogen bonds between the Biotin molecules can influence the hydrogen bonding between the dye and Biotin during this phase.

The dye's microenvironment in this phase is primarily influenced by the surrounding Biotin molecules, but the presence of water molecules can also contribute to the hydrogen bonding interactions between the dye and Biotin.

The promotion of electron transfer results in a 10 fold decrease in the LE state emission as observed form steady state spectral studies (Figure 2). The fluorescence lifetime of free unbound dye is ~500 ps and this decreases by 10 fold on the addition of BIOTIN.

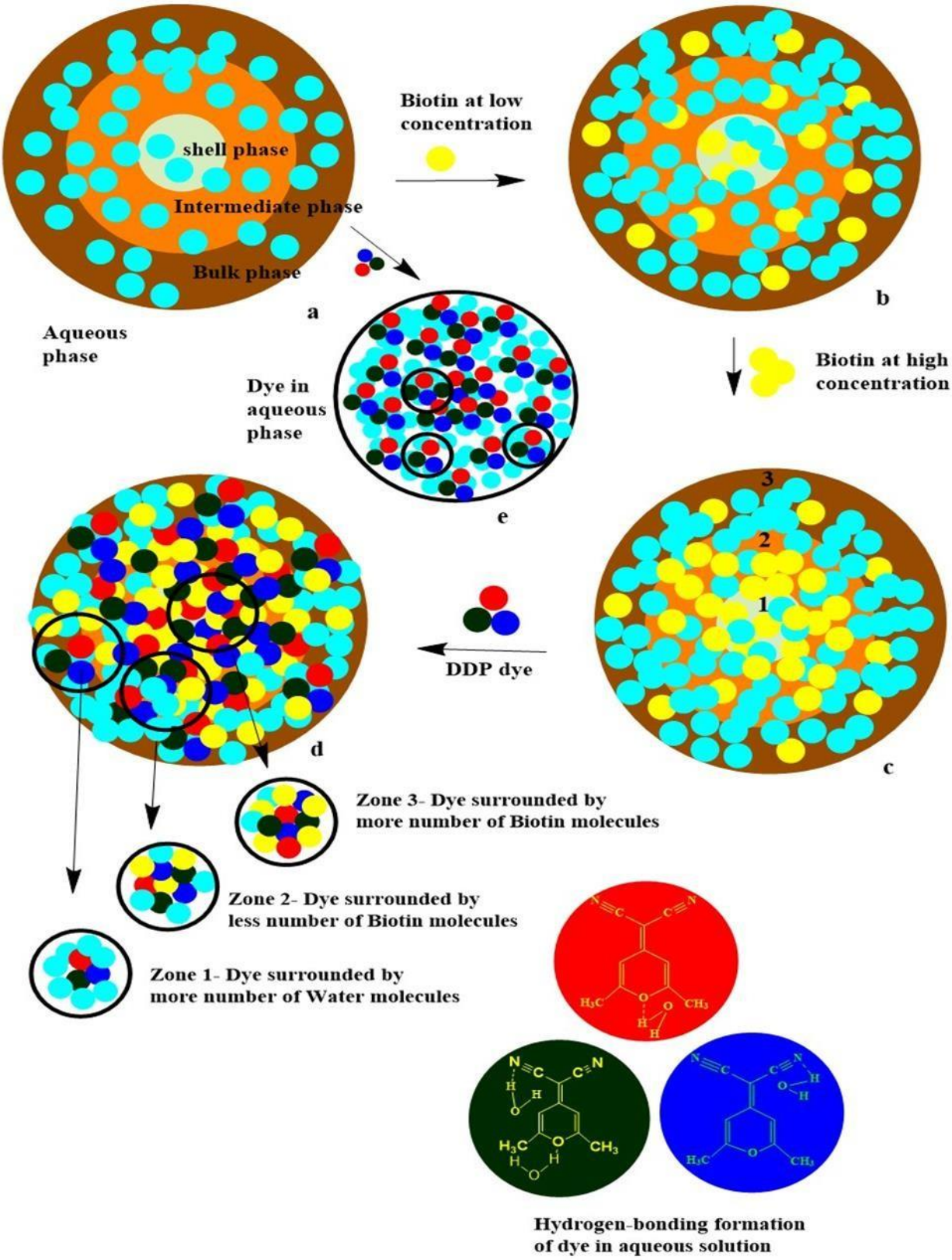

Fig.5.4. Hydrogen-bonding interaction Biotin with DDP dye in water

CHAPTER 6

SUMMARY AND CONCLUSION

The main aim is to study the interaction between DDP dye (Host) and Biotin (Guest) molecule and to find the hydrogen bonding of Biotin in aqueous solution. With the help of UV-Visible spectroscopy, Emission spectroscopy, Fluorescence life time spectroscopy I studied the hydrogen bonding of Biotin in the aqueous solution. As we increased the concentration there is a decrease in absorbance and fluorescence quenching taking place.

REFERENCE:

1) Hankins D, Moskowitz Jand Stillinger F (1970) Water Molecule Interactions. J Chem Phys 53:4544–4554.

2) Costain CCand Dowling JM (1960) Microwave Spectrum and Molecular Structure of Formamide. J Chem Phys 32:158–165.

3) Petersen R (1960) Interactions in the binary liquid system N, NDimethylacetamide— water: viscosity and density. J Phys Chem 64:184–185

4) Smyth C P (1955), Dielectric Behavior and Structure, McGraw- Hill, New York,pp 53, 73.

5) Aquino AJA, Tunega D, Haberhauer G, Gerzabek MHand Lischka H (2002) Solvent Effects on Hydrogen BondsA Theoretical Study. J Phys Chem A 106:1862–1871.

6) Kumaran Rand Ramamurthy P (2014) J Lumin 148:277–284.

7) Johansson A, Kollman P, Rothenberg Sand McKelvey J (1974) Hydrogen bonding ability of the amide group. J Am Chem Soc 96:3794–3800.

8) Bard AJand Faulkener LR (1980) Electrochemical methods; fundamentals and applications; Department of Chemistry and Biochemistry; University of Texas at Austin:John wiley & sons, inc, New York.

9) Randles JEB (1948) A cathode ray polarograph. Part II.—The current-voltage curves. Trans. Faraday Soc. 44:327-338.

10) Hsieh B-Yand Chen Y (2007) Synthesis, Photophysics, and Electroluminescence of Copolyfluorenes Containing DCMDerivatives.Macromolecules. 40:8913-8923.

11) Carter MTand BardAJ (1987) Voltammetric studies of the interaction of tris(1,10-phenanthroline)cobalt(III) with DNA. J. Am. Chem. Soc. 109:7528– 7530.

12) Carter MT, Rodriguez MandBard AJ (1989) Voltammetric studies of the interaction of metal chelates with DNA. 2. Tris-chelated complexes of cobalt (III) and iron(II) with 1,10-phenanthroline and 2,2'-bipyridine. J. Am. Chem. Soc. 111:8901–8911.

13) Da-An Qin, Xiao-Qing Cai, Qian Miao, Zuo-Hui Wangand Mao-Lin Hu (2014) Cyclic Voltammetry and Molecular Docking Study of the Interactions of Two Derivatives of 5-fluorouracil with DNA. Int J ElectrochemSci 9:1608 – 1620.

14) Qu F, Li NQand Jiang YY (1998) Electrochemical studies of NiTMpyP and interaction with DNA. Talanta.45:787-93.

15) Stelian LUPU, U.P.B. Sci. (2011) Bull Series B 73:85.

16) Kumaran R and Ramamurthy P (2006)PET Suppression of Acridinedione Dyesby Urea Derivatives in Water and Methanol. J. Phys. Chem. B 110:23783- 23789.

17) WoodsL L(1958)Some Further Reactions of 2, 6-Dimethyl-4-pyrone.J. Am.

Chem. Soc. 80:1440– 1442.

18) Randles J E B (1948) A cathode ray polargraph. Part II – The current-voltagecurves. Trans. Faraday Soc. 44:327-338.

19) Ševčík A (1948) OscillographicPolaropgraphy with Periodical TriangularVoltage. Collect. Czech. Chem. Commun.349-377.

20) Nicholson R Sand ShainI(1964) Theory of Stationary ElectrodePolarography. Single Scan and Cyclic Methods Applied toReversible, Irreversible, and Kinetic Systems. Anal. Chem. 36:706-723.

21) Bard A J and Faulkner L A (2000) ElectrochemicalMethods: Fundamentals andApplications,2nd ed. Wiley-Interscience: New York Kissinger P and Heineman,

W. R. (1996) Laboratory Techniques in ElectroanalyticalChemistry, 2nd ed. Marcel Dekker, Inc: New York.

22) AgarwalP, Orazem ME and Garcia-RubioLH(1992) J. Electrochem. Soc, 139:1917.

23) Robert Cottis and Stephen Turgoose(1999)Electrochemical Impedance and Noise.NACE International, 1440 South Creek Drive, Houston, TX 77084, US,. Website: www.nace.org.

24) BarsoukovE and Macdonald JR(2005) Impedance Spectroscopy; Theory, Experiment and Applications, 2nded, Wiley Interscience Publications.

25) ScullyJR, Silverman DC and Kendig MW(1993) Electrochemical Impedance: Analysis and Interpretation, editors, ASTM,.

26) Atkins PW (1990) Physical Chemistry, Oxford University Press. 27)Oppenheim AV and Willsky AS(1983) Signals and Systems, Prentice-Hall. 28)Dobbelaar JAL(1990) The Use of Impedance Measurements in Corrosion

Research; The Corrosion Behaviour of Chromium and Iron Chromium Alloys,

Ph-D thesis TU- Delft.

29) Biotinspar Y, Johnson KL, McKenna JA, Bacic A, Schultz CJ (2001) The complex structure of arabinobiotinlactan-proteins and the journey towards understanding function. Plant Mol Biol 47: 161-176.

30) Williams PA, Phillips GO (2000) Gum Arabic, In Handbook of hydrocolloids, Woodhead Publishing Ltd, chapter (9), pp. 155-168.

31) Randall RC, Phillips GO, Williams PA (1989) Fractionation and characterisationof gum from Acacia Senebiotinl. Food Hydrocolloids, 3: 65-71.

32) Fenyo JC, Vandevelde MC. (1990). In gum and stabilizer for food industry, 5 (pp 17-23) IRL Press, Oxford.

33) Islam AM, Phillips GO, Sljivo A, Snowden MJ , Williams PA (1997) A review ofrecent developments on the regulatory, structural and functional aspects of gum Arabic. Food Hydrocolloids, 11: 493-505.

34) Idris OHM, Williams PA, Phillips GO (1988) Charecterisation of gum arabic fromAcacia Senebiotinl trees of different age and location using multidetection gel permeation chromatography. Food Hydrocolloids, 12: 379-388.

35) Goodrum LJ, Patel A, Leykam JF, Kieliszewski MJ (2000) Gum arabic glycoprotein contains glycomodules of both extensin and arabinobiotinlactan-glycoproteins. Phytochemistry, 54: 99-106.

36) Jurasek P, Kosik M, Phillips GO (1993) The classification of natural gums. Part

II. Characterisation of the gum arabic of commerce based on a chemometric study of amino-acid compositions . Food Hydrocolloids, 7: 157-174.

37) urasek P, Kosik M, Phillips GO (1993) The classification of natural gums. III.Acacia senebiotinl and related species (gum arabic). Food Hydrocolloids, 7: 255-280.

38) Snowden MJ, Phillips GO, Williams PA (1987) Functional characteristics of gumarabic. Food Hydrocolloids, 1: 291-300.

39) Randall RC, Phillips GO, Williams PA (1988) The role of the proteinaceouscomponent on the emulsifying properties of gum arabic.

40) Food Hydrocolloids, 2: 131-140.

41) Li X, Fang Y, Al-Assaf S, Phillips GO, Nishinari K, Zhang, H (2009) Rheologicalstudy of gum arabic solutions: Interpretation based on molecular self-association. Food Hydrocolloids, 23: 2394-2402.

42) Yadav MP, Ibiotinrtuburu JM, Yan Y, Nothnagel EU (2007) Chemical investibiotintion of the structural basis of the emulsifying activity of gum arabic. Food Hydrocolloids, 21: 297-308.

43) Li X, Fang Y, Zhang H, Nishinari K, Al-Assaf S, Phillips GO (2011) Rheologicalproperties of gum arabic solution: From Newtonianism to thixotropy. Food Hydrocolloids, 25: 293-298.

44) Nelson RE, Ander P (1972) Heterogeneity of gum arabic and its salts.

Carbohydrate Res 25: 81-98.

45) Park C, Lim KH, Kwon D, Yoon TH (2008) Biocompatible quantum dot 5 stabilized by gum Arabic. Bull Korean Chem Soc 29: 1277-1285.

46) Williams DN, Gold KA, Holoman TRP, Ehrman SH, Wilson OCJ (2006) Structure modification of magnetic nanoparticles using gum Arabic. J Nanopart Res 8: 749-753.

47) Zhang HY, Shan GB, Liu HZ, Xing JM (2007). Surface modification of γ-Al2O3 nano-particles with gum arabic and its applications in adsorption and biodesulfurization. Surf Coat Technol 201: 6917-6921

48) Bandyopadhyaya R, Nativ-Roth E, Regev, O, Yerushalmi-Rozen R (2002) Stabilization of individual carbon nanotubes in aqueous solutions. Nano Lett 2: 25-2

49) Kattumuri V, Katti K, Bhaskaran S, Boote EJ, Casteel SW, Fent GM, Robertson DJ, Chandrasekhar M, Kannan R, Katti KV (2007) Gum Arabic as a phytochemical construct for the stabilization of gold nanoparticles: in vivo pharmacokinetics and X- ray-contrast-imaging studies. Small, 3: 333-341.

50) Velikov KP, Zegers GE, Van Blaaderen A (2003) Synthesis and characterizationof large colloidal silver articles. Langmuir, 19: 1384-1389.

51) Mantell CL (1954) Technology of gum Arabic, In American Chemical Society, Division of Colloid Chemistry, Natural Plant Hydrocolloids, Washington, DC, 20- 32.

52) Mahendran T, Williams PA, Phillips GO, Al-Assaf S, Baldwin TC (2008) New insights into the structural characteristics of the arabinobiotinlactan-protein (AGP) fraction of gum Arabic. J Agric Food Chem 56: 9269-9276.

53) Glicksman M, Sand R (1973) Gum Arabic, L. Whistler (Eds.), Industrial Gums Academic press, New York, Vol 10, 197-263.

54) Fincher GB, Stone BA, Clarke AE (1983) Arabinobiotinlactan-protein: structure,biosynthesis and function.Annual Rev Plant Molecular Biol 34: 47-70.

55) Vendevelde MC, Fenyo JC (1985) Macromolecular distribution of Acacia Senebiotinl gum (Gum Arabic) by size exclusion chromatography. Carbohydrate Polymers, 5: 251-273.

56) Johnson W (2005) Final report of the safety assessment of Acacia catechu gum, Acacia concinna fruit extract, Acacia dealbata leaf extract, Acacia dealbata leaf wax, Acacia decurrens extract, Acacia farnesiana extract, Acacia farnesiana flower wax, Acacia farnesiana gum, Acacia senebiotinl extract, Acacia senebiotinl gum, and Acacia senebiotinl gum extract. Int J Toxicol 24: 75-118.

57) Chi X, Liu R, Zhang H (2011) Noncovalent interaction of Oxytetracycline with enzyme Trypsin. Biomacromolecules, 11: 2454-2459.

58) Zong W, Liu R, Sun F, Teng Y, Fang X, Chai J (2011) A new Strategy to identifyand Eliminate the inner filter effects by outer Filler technique. J Fluoresc 21: 1249- 1254