# PHOTOPHYSICAL STUDIES OF DDP DYE WITH LACTOSE IN AQUEOUS SOLUTION

**Submitted in partial fulfillment of the requirements for the award of**

**Bachelor of science in chemistry**

**by**


**MARAGATHAMBIGAI.P (Reg.No.40030021)**

**DEPARTMENT OF CHEMISTRY**

**SCHOOL OF SCIENCE AND HUMANITIES**

**SATHYABAMA INSTITUTE OF SCIENCE AND TECHNOLOGY**

**(DEEMED TO BE UNIVERSITY)**

**Accredited with Grade "A" by NAAC JEPPIAAR NAGAR,**

**RAJIV GANDHI SALAI,**

**CHENNAI-600 119**

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## BONAFIDE CERTIFICATE

This is to certified that this Project Report is the bonafide work of **MARAGATHAMBIGAI.P (40030021)** who carried out the project entitled **"PHOTOPHYSICAL STUDIES OF DDP DYE WITH LACTOSE IN AQUEOUS SOLUTION "** under my Supervision from December 2022 to April 2023.

S. Gayathri
Guide

**Dr.S.GAYATHRI Ph.D.,**

Associate Professor

Department of chemistry

J. Kanikkai Raj 08/05/
Head of the department
HEAD OF THE DEPARTMENT
DEPARTMENT OF CHEMISTRY
SATHYABAMA
INSTITUTE OF SCIENCE AND TECHNOLOGY
(DEEMED TO BE UNIVERSITY)
Jeppiaar Nagar, Rajiv Gandhi Salai
Chennai – 600 119

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Submitted for viva Examination held on 08 . 05 . 2023

**Internal Examiner**

**External Examier**

## DECLARATION

MARAGATHAMBIGAI.P (Reg.no. 40030021),here by declare that the project Report entitled **"PHOTOPHYSICAL STUDIES OF DDP DYE WITH LACTOSE IN AQUEOUS SOLUTION"** done by us under the guidance of Dr.S.GAYATHRI Ph.D., Associate Professor, Department of chemistry,SATHYABAMA INSTITUTE OF SCIENCE AND TECHNOLOGY is submitted in partial fulfillment of the requirement for the award of Bachelor of Science degree in chemistry.

DATE: 08. 05. 2023

P. Maragathambigai

PLACE: CHENNAI

SIGNATURE OF THE CANDIDATES

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**MARAGATHAMBIGAI. P**

## Abstract

Photo physical techniques were employed to hydrogen-bonding self assemblies forming solutes **l**actose **i**nteraction with 4-dicyanomethylene 2, 6-dimethyl-4H-pyran (DDP) dye in aqueous medium. water soluble were investigated by Addition of lactose derivatives to DDP dye which is an Intramolecular Charge Transfer (ICT) based fluorescent probe, results in a fluorescence enhancement accompanied with a shift towards the red region. The coexistence of DDP dye with lactose signifies the presence of heterogeneous micro environment of DDP dye surrounded by varying proportion of the solute and water molecules around the dye moiety. lactose influence the excited state characteristics of DDP dye resulting in the formation and promotion of different distinguishable micro environment. The hydrophobicity of lactose derivatives along with the hydrogen-bonding properties of lactose-water and lactose-lactose largely influence the photo physical nature of DDP dye is emphasized.

**Keywords:** DDP dye; lactose derivatives; Hydrogen-bonding; fluorescence emission.

# CONTENTS

# CHAPTER-1

# INTRODUCTION

## Photo physics of probes in self assemblies and molecular aggregates

The concept of photo physics and photo chemistry in micro heterogeneous systems are of utmost importance in the field of chemistry and biology[1-9].Photo physical studies serve as a vital link in establishing and elucidating the structure and properties of the probe molecules in the micro heterogeneous environment in their ground and excited states[1-3].Most of the biophysical and biochemical studies of the micro heterogeneous systems involve the changes in the microscopic level and are widely applicable to elucidate the structure of a large protein molecule or a polymer backbone.

Organized self-assemblies act as an excellent medium for studying the photo physical properties of fluorophores[1-5].The presence of hydrophilic and hydrophobic groups in micelles results in a large variation in the photo physical properties of the probes is a well known phenomenon[1]. Micelles form self assemblies in aqueous solution and act as an ideal medium to probe the photo physical properties. Likewise, lactose interaction with fluorophores[6-7] has been carried out in both aqueous and non-aqueous solvents, and the influence of lactose-solvent hydrogen-bonding properties on the photo physical properties of the probe results in variation in the spectral properties of the fluorescent probe. This provides an excellent approach to study in depth involving the interaction of extrinsic and intrinsic fluorophores in lactose and alkyl substituted lactose molecular assemblies.

Studies involving the role of hydrogen-bonding interaction and the hydrophobic interaction involving lactose on organized micelles[4], reversed micelles[4], vesicles, mono layers, polymers and with cyclodextrins[4] have been well established. In spite of reports available on the properties of lactose and alkyl lactose derivatives, there is no concrete evidence about the mechanism and the nature of interaction involving lactose and alkyl lactose interaction with intrinsic and extrinsic fluorophores. Interestingly, the influence of lactose concentration resulting in a large variation in the fluorescence spectral properties of these probes is of utmost importance in the field of spectroscopy. Even though, there are numerous reports based on the interaction of lactose with bio molecules and with that of the hydrophobic moieties, the mechanism and the nature of the interaction existing between lactose and bio molecules is an area of unanswered domain.

**Fluorescence spectral techniques.**

Photo physical and photochemical properties of probe or drug are monitored by fluorescence spectroscopic techniques, which is so far the most efficient and much reliable tool for the chemists and biologists in particular[1,10].

Fluorescence spectroscopy is the technique involved in establishing the location of a fluorescent probe in micro heterogeneous environment like protein and peptide aggregates. The photo physical properties of the fluorescent probe are attributed to the micro and macro environment of the medium. Since most of the reactions occur in the time scale ranging from few picoseconds to microseconds in the solution phase, fluorescence spectroscopic techniques are widely used in establishing the properties of the fluorescent probes. Further, a low concentration (in μM) of the probe is only required to establish the spectral properties.

Fluorescence spectral techniques possess several advantages compared to other conventional methods. The nature of the probe environment is reflected by fluorescent spectroscopy. The parameters investigated are excitation and emission spectral shapes, maxima, vibrational fine structure, fluorescence lifetime, quantum yield and degree of depolarization of emission. In the micro heterogeneous medium, changes in the nature of the micro environment experienced by the probe on transfer from the aqueous or organic medium to the host are effectively reflected in the emission properties of the fluorescent probe in. Further, the solvent molecules contribute to the variation in the emission spectrum due to the polarity nature of the solvent and the change in the dipole moment. A change in the fluorescent quantum yield of the fluorescent amino acids like tryptophan, tyrosine and phenylalanine on protonation or deprotonation also influences the spectral properties of the fluorophore[10].

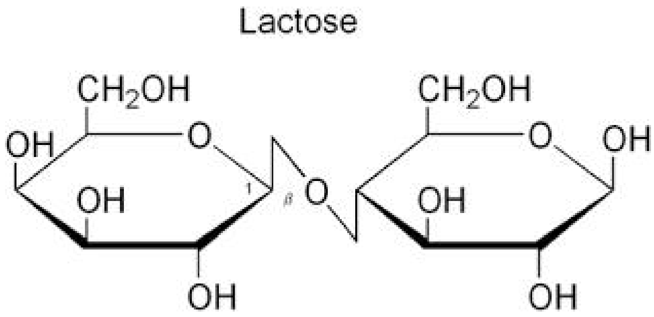
Fluorescence arguably constitutes the most widely used experimental approach in the field of protein folding due to several advantages. Fluorescence signals are exquisitely sensitive to the immediate environment of the probe or protein, change in temperature, viscosity, polarity changes, very high signal to noise ratio and particularly useful for in depth thermodynamic studies. In addition to all these advantages the fluorescence lifetime of the probes are in the nanosecond and picosecond time scale[1,6] which could be carried out in a picosecond or femtosecond lifetime domain instrument, which seems to be the limiting factor.

Fluorophores commonly used to probe structural, functional and dimensional information of a bio-molecule, or solutes situated in a highly heterogeneous environment are divided into two major classes[9]; intrinsic and extrinsic. Intrinsic fluorophores are those, which occur naturally in biological macromolecules, and in proteins.The dominant intrinsic fluorophore is the indole group of the amino acids that are fluorescent in nature.

It is advantageous to use the intrinsic fluorophores for these investigations in order to avoid complicated labeling with an extrinsic dye.The fluorescence spectral properties of protein have been largely confined to the tryptophan amino acid.Tryptophan can be preferentially excited above 295 nm in proteins and the indole moiety is sensitive to the local environment.Tryptophan displays a substantial spectral shift, which corresponds either to the native or denatured form and the fluorescence spectra depends on the degree of exposure of the tryptophanyl side chains to the polar, aqueous solvent and upon its proximity to the self quenching groups[1,5,9]. Tyrosine and phenylalanine are not sensitive to the micro environment as compared to that of tryptophan and does not provide valuable information regarding the dynamics of protein folding.

## 1.1 STRUCTURE OF LACTOSE

Hydrogen Bonding in the Lactose Complex. The 2m| Fo| − D|Fc| X-ray map for the lactose complex at 1.1 Å resolution shows that the entire lactose molecule is wellordered, consistent with earlier studie [10-15]In the corresponding nuclear density map for the lactose complex, the galactose moiety is very well-defifined, but some parts of the glucose moiety are less clear due to a combination of higher mobility and cancellation effects from the aliphatic hydrogen atoms on lactose, which is not deuterated. Nevertheless, the hydrogen-bonding network between the inward-facing hydroxyl groups of lactose and the protein is very clear, and the protonation states of interacting residues are unambiguously defined.The directionalities of the three key H-bonding groups on the inside of the lactose molecule can now be described In the following discussion, −OH will be used to refer to the hydroxyl groups and −O when referring specifically to the oxygen atoms.Interestingly, binding of lactose to the protein apparently leads to minor conformational strain within lactose[13-15].




The advantage of lactose to mankind is vital in biological and environmental studies because of its involvement as a waste product in our daily life and helps in sustained smooth functioning of the metabolic activities**.** Further, **l**actose is non-corrosive, non-toxic, does not produce any adverse effect to the environment and have excellent solubility in water which makes lactose an excellent host molecule to study the photo physical properties of a water soluble fluorescent probe. Further, lactose is non-corrosive, non-toxic and does not produce any adverse effect to the environment[12,13]. lactose is readily soluble in water and possesses several advantages over other solutes. lactose disrupts and reorients the water structure through hydrogen-bonding interactions. Even though the properties of water and lactose in aqueous solutions have been extensively studied, there exists a large variation in their unusual behaviour related to the physical and chemical properties of lactose in solvents especially in aqueous solutions. Aqueous lactose solution has lactose- lactose, lactose-water and water-water hydrogen-bonding interactions. The structures of various hydrogen-bonded lactose-water complexes with the existence of hydrogen-bonded networks [13,14] have been confirmed by experimental and molecular simulation methods [14,15]. Although there have been many conflicting reports and considerable debate about the role of lactose among chemists and biologists. The peculiar behaviour of lactose in aqueous solutions and its varying hydrogen-bonding properties exhibited in water is still an area of considerable challenge for the chemists particularly in the field of photo physics of fluorescent probes in water[9,15].The high permittivity and viscosity of lactose and its derivatives in aqueous solutions reveal that there exists strong hydrogen-bonding interactions[14-15]. In our study wherein the concentration of lactose is varied and that of the fluorescent probe is fixed.

## APPLICATIONS

- Lactose hydrolysis in fluid milk products
- Control of lactose crystallization in concentrated dairy Products
- Formulated pet food applications
- Production of oligosaccharides and expolysaccharides.

## USES OF LACTOSE

- Lactose is used as a nutrient in preparing modified milk and is also added to infants' food to match the composition of human milk.
- It helps the body to absorb calcium and a variety of other minerals, like magnesium, copper, and zinc.
- Because of its physical and functional properties, lactose is added as an ingredient in pharmaceutical products like an excipient and diluent for tablets and capsules.

## 1.2 DDP dye

### 4-[DICYANOMETHYLENE]-2,6[DIMETHYL]-4H-PYRAN

DDP dye and its derivates are highly efficient laser dye. It is hydrophobic.

2-cyano groups withdraw strongly the electron density from the methyl substituent.

DDP dye is classified as inter molecular charge transfer (ICT)-based dye.

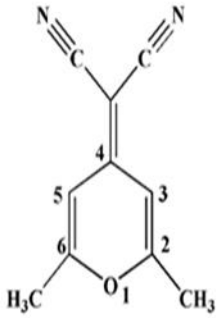
The structure of DDP dye has a strong acceptor group dicyano-methylene-$C(CN)_2$ in the fourth position and a donor (methyl) substituted in second and sixth position.

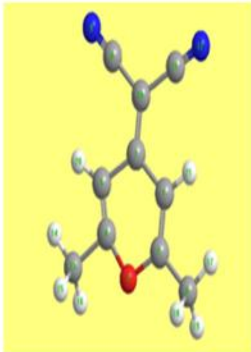
DDP is generally referred as a derivative of DCM type dyes which belongs to the class of red luminescence compound[7-9].DDP dye have a strong acceptor group dicyanomethyleneC$(CN)_2$ in 4th position and a donor moieties in 2nd and 6th positions (Scheme1). Pyranylidene derivatives used so far are mostly concerned with optoelectronic properties such that they are deposited on the OLED hole transport layer or used as dopants in a polymer matrix [9]. Most of the photo physical studies were carried out with DCM dye only due to the presence of Twisted Intramolecular Charge Transfer (TICT) behavior [3-14]. The striking difference is that DDP dye does not exhibit TICT behaviour. Further the quantum yield and the photo physical properties of these dyes are governed by the number of donor and acceptor moieties [13]. In that case to get best electroluminescence material with high quantum yield and better solubility in water, DDP dye was used as the ideal host material to explore the spectrochemical and electrochemical properties in the presence of hydrogen-bonding solutes like lactose and alkyl Lactose derivatives.

Interestingly, DDP dye is soluble in water compared to that of DCM dye and possesses significant advantage over DCM dye in the field of photo physical, photochemical and photobiological studies in the presence of a water soluble solute in aqueous solution. Further, DDP dye are widely used in quantum electronics and optoelectronics as active media of tunable lasers, emitting layers of organic light diodes [11-14] and in nonlinear optics as the starting material. A recent report on the interaction of amides with DDP dye reveals that the absorption, emission and fluorescence lifetime properties are influenced by the nature of the solutes, concentration of the solute, hydrogen-bonding and hydrophobic interactions [12]. The variation in the ground and excited state properties of DDP dye in the presence of lactose and alkyl lactose derivatives is probed by fluorescence spectral technique. The hydrogen-bonding interaction of lactose-solvent and its influence on the fluorescence emission and lifetime of DDP dye is compared with other alkyl lactose derivatives.

In our present study, we focus on the variation in the photo physical properties of an ICT based dye, 4- Dicyanomethylene-2,6-dimethyl-4H-pyran (DDP) in the presence of lactose Further, a complete mechanism of the origin of fluorescence emission and lifetime of DDP dye in the presence of water soluble non fluorescent solutes are very limited in the literature to the best of our DDP dyes and its derivates are highly efficient laser dyes. It is hydrophobic.2-cyano groups withdraw strongly the electron density from the methyl substituent.

- DDP dye is classified as intermolecular charge transfer (ICT)-based dye.
- The structure of DDP dye has a strong acceptor group di cyano-methylene-$C(CN)_2$ in the fourth position and a donor (methyl) substituted in second and sixth position.



- DDP dye has been widely employed as a fluorescent material.
- DDP dye is basic in nature.
- Further the electroluminescent and photo physical properties are significantly enhanced by varying structure of the electron donating groups.
- Main point of DDP dye is attributed to its solubility in water and it also servers a better light emitter organic material.
- It also possesses the property of not crystallizing easily when coated upon polymeric surfaces.Which portrays DDP dye as a useful probe molecule to study the electroluminescent properties in an aqueous solution.
- The spectroscopic nature of many surface-bound fluorescent probes are found to be highly sensitive to the local micro environment formed by the layer around the close vicinity. Considering the high sensitivity of fluorescence and the salient structural information that it can provide,photo physical techniques are predominantly confined to the bulk region where in the structure of the solvent and solvent induced properties govern the absorption and emission phenomena.
- Spectroscopic outcomes-provides better knowledge for understanding the nature of the interaction.
- Photo physical studies of a well-known water-soluble fluorescent solute like sugars(lactose) with DDP dye are largely governed by the solute and the solute-solvent interactions.Like glucose, lactose are classified as a water-soluble hydrogen bonding.

### 1.2.1 PREPARATION OF DDP DYE

The mixture of 2,6-dimethyl-4-pyrone (4: 1.25g, 10.06 mol) and malononitrile(0.783g, 11.85mmol) in 5mL of acetic anhydride was stirred and heated to 140 degree for 1 hour under nitrogen atmosphere. The acetic acid was aspirated off, and the residue was washed with 10ml of boiling water and collected by filtration to give brown material.

# 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 Dependent Density 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-acetyltransferases and 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.

# CHAPTER-3

# AIM AND SCOPE

## 3.1 AIM

The Aim of the project is to study the photo physical studies of DDP dye with lactose with aqueous solution.

## 3.2 SCOPE

- Sugar solution(LACTOSE) in different concentration.
- Hydrogen-bonding interaction of Guest-Host molecule.
- organized self-assemblies in water
- Photo physical properties of DDP – lactose Fluorophore charge transfer (CT) state

## MATERIAL AND METHOD

- Making up dye.
- Making up the solution with the following concentration.

## 3.3 PREPARATION OF THE SOLUTION

- To 10ml solution container, 2ml of (0.4M) lactose solution with 1ml of DDP dye and add water to the remaining part of solution.

This process is classified under the physisorption because of weak forces, reversible after with atmosphere by filtration, and forms multilayer.

# CHAPTER-4

## 4.1 OBJECTIVE THIS RESEARCH WORK

- Fluorescence spectroscopy is the technique involved in establishing the location of a fluorescent probe in microheterogeneous environment like protein and peptide aggregates. The photo physical properties of the fluorescent probe are attributed to the micro and macro- environment of the medium. Since most of the reactions occur in the time scale ranging from few picoseconds to microseconds in the solution phase, fluorescence spectroscopic techniques are widely used in establishing the properties of the fluorescent probes. Further, a low concentration (in µM) of the probe is only required to establish the spectral properties.
- Photo physical studies were subjected to in depth analysis of hydrogen-bonding
- The mechanism and the nature of interaction involving these hydrogen-bonding solutes with ICT dye are characterized by the changes in the microenvironment.
- Hydrogen-bonding interaction of Guest-Host molecule.
- The photo physical properties of fluorescent labels define the particular applications in with they are most beneficial, considering the sensitivity, efficiency, and operative lifetime of the detection process.
- The term photo physics refers to a number of physical processes induced by absorption of light that do not lead to overall changes in the chemical identity of the molecule.
- Examples include vision and photosynthesis.
- The properties mainly include fluorescence emission, absorption band shift, photodynamic process, and photothermal conversion of dye assemblies.

✓ **UV ABSORPTION** (The relative measure of the amount of light by a water sample compared with the amount of light absorbed by a pure water sample**)**

✓ **EMISSION STUDIES (**spectroscopic technique which examines the wavelengths of photons emitted by atoms or molecules during their transition form an excited state to a lower energy state.**)**

# CHAPTER- 5

## UV ABSORPTION STUDIES DYE ALONE

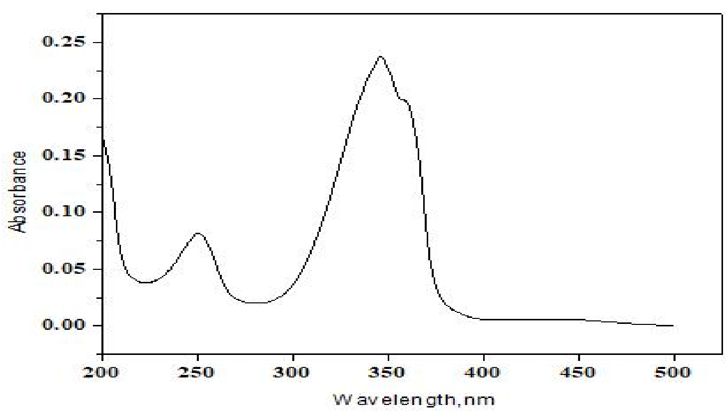
UV-Vis spectrophotometers are used in almost every laboratory in the world due to their measurement versatility. This guide is a snapshot of the main spectrophotometer uses, with links to application notes by measurement technique and industry type.

For many substances that undergo structural or conformational transitions, their UV-Vis absorbance also changes. UV visible spectroscopy can therefore be used to study conformational changes in molecules.

UV-Vis calculates the colour of a liquid or solid by measuring the visible light transmitted through a solution or reflected from a surface. Colour is often considered a quality indicator and UV-Vis can colour match or measure a change of colour in solution.

DDP dye exhibits two characteristic absorption peaks at 248 and 348 nm accompanied with a shoulder around 360 nm, wherein the peaks at 248 and 348 nm are attributed to the π- π* transition and charge transfer (CT) band [19].The electron density redistribution clearly suggests π → π* transition character, except the small fraction of σ → π* arising from the donor methyl groups for DDP dye.

### 5.1 ABSORPTION GRAPH FOR DDP DYE



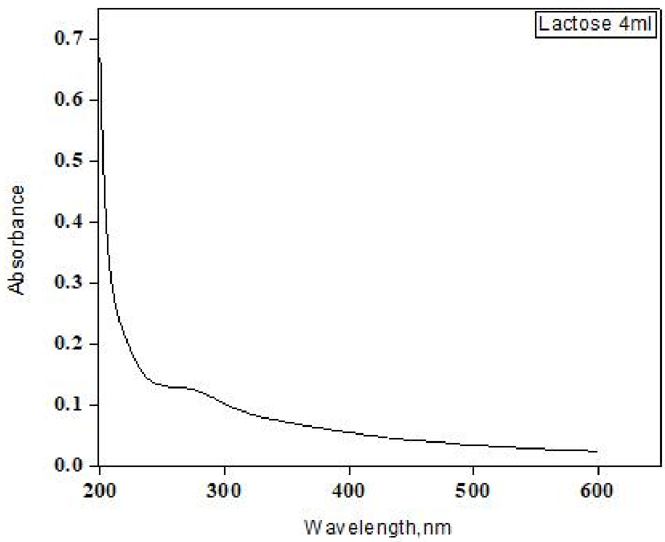

Transitions could not be categorized to have a pure CT character. DCM-styryl type dye exhibits many absorption peaks in the spectral range of 400-500 nm. Interestingly, no absorption peak above 400 nm resulted for DDP dye and this authenticates that the absorption spectral pattern is entirely different from DCM type dye that exhibits a TICT behavioural pattern

In the field of spectroscopy, the frequency or wavelength of a given sample which exhibits the maximum or the highest spectral value of absorption.

The higher the value, the more of a particular wavelength is absorbed.

In this graph we will see that absorption peak is at 430nm.

**5.2ABSORPTION GRAPH ONLY FOR SUGAR SOLUTION**




This graph shows us the wavelength of light that different photosynthetic pigments absorb.

A peak of distribution is a bump or high point in graph.

When we observe the absorption graph only with the sugar solution(lactose) it does not show any peaks or high point in it.

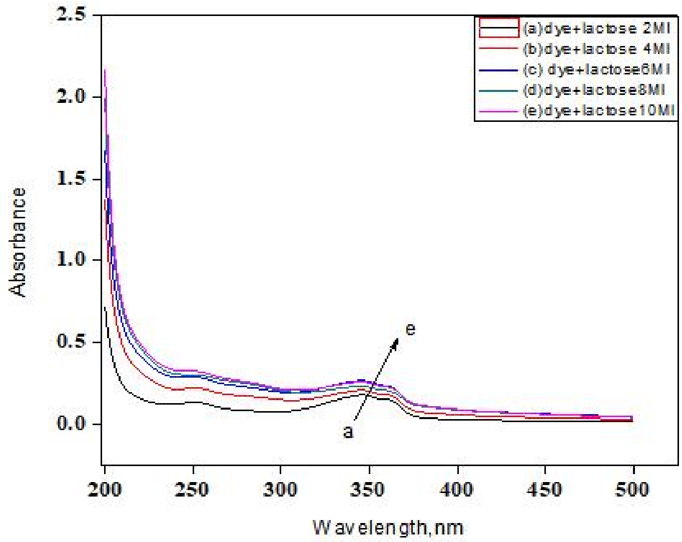
## 5.3 ABSORPTION GRAPH FOR LACTOSE SOLUTION WITH DDP DYE

DDP dye exhibits two characteristic absorption peaks at 248 and 348 nm accompanied with a shoulder around 360 nm as reported [9]. Addition of lactose results no significant change in the absorbance at the longest wavelength absorption maximum of DDP dye (figure 3).The absorbance at the ICT absorption maximum remains unaltered even in the presence of very high concentration of lactose. The increase in the absorbance around 280 ± 10 nm is attributed to the strong absorbance of lactose. Further, an isosbestic point in the absorption spectrum of DDP dye with lactose is correlated to the formation of a ground state complex. Our earlier studies of DDP dye with hydrogen-bonding assemblies of amides and lactose derivatives also resulted in an isosbestic point which signifies the presence of equilibrium between dye and lactose. From absorption spectral studies it is evident that lactose associates with dye molecules in the aqueous phase. In the present investigation, we account for the role of lactose that possesses hydrogen-bonding as well as hydrophobic moieties on the variation in the excited state nature of DDP dye. The existence of more than one environment of dye in equilibrium in the presence of lactose which associated to be the bound and free dye component.

As we have seen the absorption graph for DDP dye alone and sugar solution(lactose)alone.

The dye shows the peak in 430nm and the sugar solution alone doesn’t show any peak. The dye is characterized under the micro environment and Inter Charge Transfer.

Now absorption for our solution prepared with lactose and host dye graph as follows as given below.




From this graph we can say that the DDP dye enhances the sugar solution and shows the hydrogen bonding interaction between the DDP dye and guest lactose.

Here the peak show at 450nm shows the peak more than the DDP dye.

Green mark line shows the absorbance peak of 4ml of lactose with dye in aqueous medium.

Red mark line shows the absorbance peak of 6ml of lactose with dye in aqueous medium.

Brown mark line shows the absorbance peak of 8ml of lactose with dye in aqueous medium.

The graph above explains us that the DDP dye enhances the solution, the same way higher the volume of sugar solution with DDP dye shows the large hydrogen bonding interaction.

Hence the peak shown by the above given graph represents the absorbance.

# CHAPTER-6

## EMISSION STUDIES

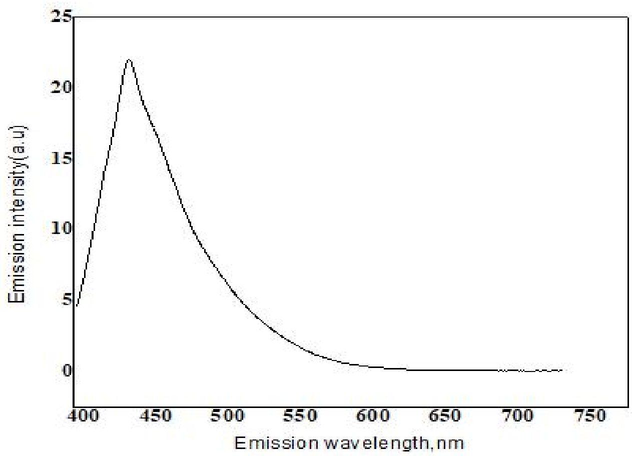
The emission spectrum of the chemical element or compound is the spectrum of the frequencies of electromagnetic radiation emitted due to an atom or molecule transitioning from a high energy state to lower energy state.

The process of elements releasing different photons of colour as their atoms return to their lower energy levels.

There are many ways in which atoms can be brought to an excited state. Interaction with the electromagnetic radiation is used in fluorescence spectroscopy.

The radiation emitted when the molecules decay back to the original energy state.

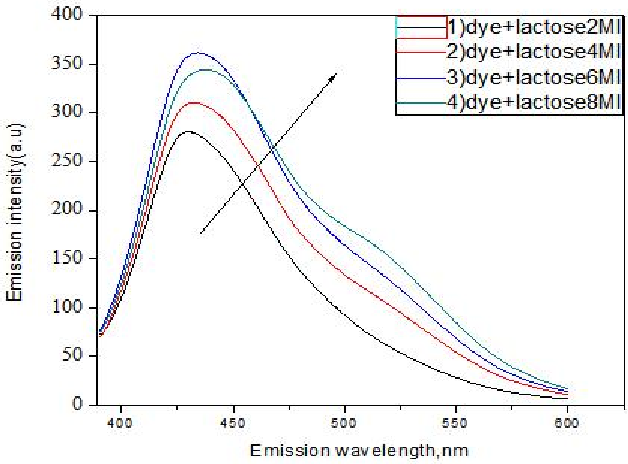
### 6.1 EMISSION GRAPH FOR ONLY DYE




In the graph, the Emission of a characteristic energy is shown as a peak on a graph. In this case, the height and width of the peak represent the line's intensity.

An emission spectrum consists of all the radiation emitted by atoms or molecules.

### 6.2 EMISSION GRAPH FOR DDP DYE WITH LACTOSE SOLUTION




When a molecule is photochemically promoted to an excited state, it does not stay there for long. The majority promotions are from $S_0$ to the $S_1$ state. Here this is also in the same state $S_1$.

Vibrational levels of $S_1$ are initially populated by excitation and decay for higher singlet states. Lowest vibrational level of $S_1$ is the important excited singlet state.

By releasing the energy in the form of light, a molecule in one state fall to a low vibrational state known as fluorescence which occurs within $10^{-9}$ seconds.

From this graph we can say that the DDP dye enhances the sugar solution and shows the hydrogen bonding interaction between the DDP dye and guest lactose.

Here the peak show at 450nm shows, the peak more than the DDP dye.

Red mark line shows the peak of 4ml of lactose with dye in aqueous medium.

Brown mark line shows the peak of 6ml of lactose with dye in aqueous medium.

Green mark line shows the peak of 8ml of lactose with dye in aqueous medium.

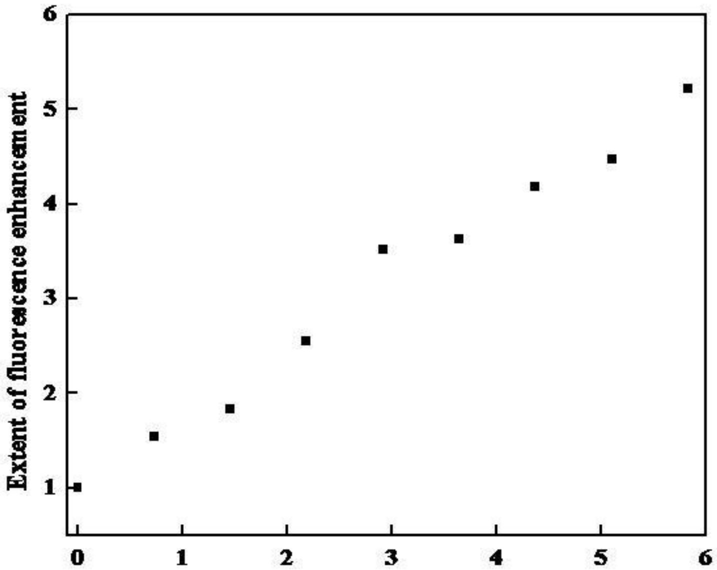
The graph above explains us that the DDP dye enhances the solution, the same way higher the volume of sugar solution with DDP dye shows the large hydrogen bonding interaction.

Hence the peak shown by the above given graph represents the Emission studies.

Quenching is a non-radiative process that involves the deactivation of an excited molecular entity by the external environmental effect a substituent. Therefore, an excited species can be quenched.

Addition of lactose to DDP dye results in a fluorescence enhancement . A broad emission maximum around 430 to 440 nm resulted on the addition of lactose as observed in the case of lactose, and a close observation on the emission spectra reveals the presence of two emission peaks of almost similar intensity separated by a few nm only. Interestingly, with a further increase in the concentration of lactose resulted in broad emission maxima at 435 ± nm with a larger fold of enhancement at the longest wavelength emission. The emission spectral studies of lactose with DDP dye reveals that the two emission peaks of DDP dye are partially separated and coalesce to form a single peak at higher concentration of lactose. The extent of fluorescence enhancement is accompanied with no considerable shift in the emission towards the red or blue region.

In general the excited state properties of intrinsic or extrinsic fluorophores are influenced by pH,viscosity, dielectric constant, dipole moment and refractive index of the medium.The shift in the emission maximum towards the red or blue region is generally attributed to increase in the dipole moment of the medium or through hydrogen-bonding interaction or change in the polarity of the medium. In our present study there is no significant shift in the emission maxima of the dye upon the addition of lactose. Interaction of Photoinduced Electron Transfer (PET) based acridinedione dyes with lactose resulted in a larger extent of fluorescence enhancement and shift in the emission maximum towards the blue region.The increase in the fluorescence intensity is attributed to the suppression of the PET process through space and the blue shift is attributed to the orientation of the dye predominantly in the hydrophobic pocket of lactose. lactose is a complex macro molecule which comprises several amino acids that contain polar and non-polar groups which induces hydrogen-bonding, hydrophobic interactions and electrostatic contributions. In our study the absence of any characteristic shift towards neither red nor blue region reveals that the dye is not oriented completely in the hydrophobic interior or hydrophilic exterior of lactose. The exact distribution of the dye could not be quantified from steady state measurements; rather DDP dye is distributed uniformly in an environment which is heterogeneous. This was established and elucidated based on the fluorescence lifetime decay studies only.




It is well known that lactose binds with drugs and fluorescent probes and the binding constant values are calculated from the fluorescence intensity measurements, which signifies the extent of probe–protein binding. The binding constant of lactose with fluorescent probes are determined using the modified form of the Benesi–Hildebrand equation which is so far the ideal tool in determining the nature of the interaction involving proteins[14]. Studies based on the interaction of probe/drug with lactose elucidate the importance of binding sites in lactose[13-14].The most preferred site for the binding of fluorescent probes in lactose is the hydrophobic exterior rather than the hydrophilic domain. The most probable location of the dye in lactose is ascertained by the binding constant value in comparison with other fluorescent probes. The binding site further provides valuable insight and information on the nature of the interaction and the stability of DDP dye with other ICT based dyes with lactose. Interaction of lactose with norharmane [18], AODIQ, p-(dimethylaminobenzamido-thiosemicarbazone), methylene blue [16] and nile blue [13], reveals a higher binding constant. The binding constant value, which is of the order of $10^4$ $M^{-1}$ reveals that the dye is located in the hydrophobic interior of the protein molecule. We have employed the modified Benesi–Hildebrand equation interaction of DDP with lactose as applied to other probe–protein systems.The binding constant of DDP dye with lactose is obtained from the plot of $(F_N–F_0)/(F_x–F_0)$ versus 1/[lactose]. The binding constant (K) calculated from the slope and the intercept is found to be $5.070 \times 10^{-3}$ $M^{-1}$ for DDP dye which is found to be similar to that of the ICT based probes interaction with lactose as proposed earlier. The binding constant value of

DDP dye with lactose confirms that the dye–protein interaction is comparatively hydrophobic in nature. This is further supported by time-resolved fluorescence decay measurements. The literature reports portray that the interaction of drugs with globular proteins also results in high binding constant value which is correlated to the binding of the drug in the hydrophobic interior of protein molecule.

If hydrophobic influences on the excited state properties of DDP dye would have been more predominant than hydrogen-bonding influences, a blue shift in the emission maxima would have resulted. On the contrary no blue shift in the emission results. The pattern of shift clearly reveals that apart from the hydrophilic and hydrophobic nature of lactose influence the emission spectra of DDP dye wherein the presence of microhetrogeneous population of the amino acids definitely governs the emissive nature of the dye. The fluorescence enhancement of DDP dye in the presence of lactose is larger in terms of fluorescence enhancement. The influence of lactose-solvent hydrogen-bonding properties on the photo physical behavior of fluorescent probe results either in an increase or decrease in the fluorescence emission and lifetime. This provides an excellent approach to study in depth regarding the interaction of water soluble fluorophores in the presence of large macromolecules.

## MECHANISM OF FLUORESCENCE ENHANCEMENT OF DDP DYE WITH LACTOSE

In general, the mechanism of fluorescence enhancement (FE) of fluorophore in the presence of lactose in aqueous or buffered solutions is attributed to two different mechanisms [16-20]. These mechanisms are postulated in regard to fluorescence enhancement of extrinsic probes involving globular proteins such that addition of lactose results in change in the absorption and emission spectral properties. A change in the microenvironment resulted by change in pH and viscosity by the addition of lactose influences the medium. One mechanism signifies that FE is due to the variation in the bulk viscosity and the polarity around the probe molecule. It was observed that the addition of protein result in the either decrease or increase in the polarity of the medium around the vicinity of the dye [16-17]. This mechanism signifies that the increase in the fluorescence intensity accompanied with a shift in the emission is attributed to the change in the microenvironment around the fluorophore resulting in the stabilization or destabilization of the charge transfer (CT) state.

Interaction of lactose an with fluorescent probes involving a change in the polarity around the fluorophore has been well documented in the literature involving probe–protein interaction[17]. Herein, DDP is an ICT based dye which exhibits only fluorescence enhancement and no characteristic shift is observed. On the contrary, another mechanism signifies an intermolecular energy transfer from the protein molecule to the probe resulting in a FE [15–19]. This mechanism was ruled out since there were no new emissive peaks arising from DDP dye on the addition of lactose. The emission intensity in the spectral range of 435 ± 10 was almost similar which authenticates that the local excited (FE) state emission is stabilized to a larger extent. This was confirmed from 3D emission contour spectral studies.

Apart from these mechanisms, FE is also correlated to the binding of the dye to the protein molecule resulting in the formation of a stable complex in the excited state [19] such that free dye and bound dye exists in solution. This mechanism was ruled out in our present study since there exists no free dye component in aqueous phase on the immediate addition of lactose. This was established based on the fluorescence lifetime amplitude distribution of the various lifetime components of DDP dye. Interestingly, the addition of lactose (hydrophilic and hydrophobic moieties) results in a complete change in the micro environment. The increase in the fluorescence intensity of DDP dye on the addition of lactose results in a red shift in the emission maximum and this is attributed to hydrogen-bonding interaction. This mechanism results in the stabilization or destabilization of the CT or FE state emission of the flourophore. The presence of lactose around the dye molecules compete with water molecules in forming hydrogen-bonding interaction such that the variation in the micro environment of dye is influenced by the concentration of lactose. The nature of the solute influences the excited state properties of DDP dye is illustrated from this present study.

# CHAPTER-7

## CONCLUSION

DDP dye interaction with a globular protein by various techniques like UV–Visible, fluorescence spectroscopy Studies were employed as a tool in establishing the most probable orientation of the dye in a micro heterogeneous environment. These techniques provide link in establishing the binding nature as well as preferred mode of interaction of DDP dye with lactose. The fluorescence enhancement reveals that the excited state properties are largely governed by the concentration of the protein.

# CHAPTER-8

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