

PHOTOPHYSICAL STUDIES OF DDP DYE WITH MALTOSE IN AQUEOUS SOLUTION

**Submitted in partial fulfillment of the requirements for the award of
Bachelor of science in chemistry**

by

YOGALAKSHMI.S (Reg.No.40030043)



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



SATHYABAMA

INSTITUTE OF SCIENCE AND TECHNOLOGY

[DEEMED TO BE UNIVERSITY]

Accredited "A" Grade by NAAC | 12B Status by UGC | Approved by AICTE

www.sathyabama.ac.in

DEPARTMENT OF CHEMISTRY

BONAFIDE CERTIFICATE

This is to certify that this Project Report is the bonafide work of **YOGA LAKSHMI.S (40030043)** who carried out the project entitled "**PHOTOPHYSICAL STUDIES OF DDP DYE WITH MALTOSE IN AQUEOUS SOLUTION**" under my Supervision from December 2022 to April 2023.

S. Gayathri
Guide

Dr.S. GAYATHRI Ph.D.,

Assistant Professor

Department of chemistry

J. Venkatesh
08/05/23
HOD CHEMISTRY
DEPARTMENT OF CHEMISTRY
SATHYABAMA
INSTITUTE OF SCIENCE AND TECHNOLOGY
(DEEMED TO BE UNIVERSITY)
Jeedapalar Nagar, Rajiv Gandhi Salai
Chennai - 600 119

Submitted for viva Examination held on 08/05/2023



Internal Examiner



External Examiner

DECLARATION

YOGA LAKSHMI.S (Reg.no. 40030043), here by declare that the project Report entitled **"PHOTOPHYSICAL STUDIES OF DDP DYE WITH MALTOSE 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

PLACE: CHENNAI

Yoga Lakshmi.S
SIGNATURE OF THE CANDIDATES

ACKNOWLEDGMENT

I am pleased to acknowledge by sincere thanks to Board of Management of **SATHYABAMA INSTITUTE OF SCIENCE AND TECHNOLOGY** for their kind encouragement in doing this project and for completing it successfully ,I am grateful to them.

I would to express my sincere and deep sense of gratitude to my project guide Dr.S.GAYATHRI for his valuable guidance,suggestions and constant encouragement which paved way for the successful completion of my project.

I wish to express my thanks to all teaching and Non-teaching staff members of the Department of chemistry who were helpful in many ways for the completion of the project.

YOGA LAKSHMI.S

CONTENTS

CHAPTER NO.	TITLE	PAGE NO.
1.	ABSTRACT	
2.	INTRODUCTION 1.1 STRUCTURE OF MALTOSE 1.2 STRUCTURE OF DDP	
3.	AIM AND SCOPE 2.1 AIM 2.2 SCOPE 2.3 PREPARATION OF SOLUTION	
4.	OBJECTIVE AND RESEARCH 3.1 PHOTOPHYSICAL STUDIES	
5.	RESULT AND DISCUSSION UV ABSORPTION STUDIES 4.1 DDP DYE 4.2 SUGAR ALONE 4.3 DDP DYE WITH SUGAR ALONE	
6.	EMISSION STUDIES 5.1 DDP DYE 5.2 SUGAR ALONE 5.3 DDP DYE WITH SUGAR ALONE	
7.	CONCLUSION	
8.	REFERENCE	

CHAPTER-1

Abstract

Photo physical techniques were employed to hydrogen-bonding self assemblies forming solutes maltose interaction with 4-dicyanomethylene 2, 6-dimethyl-4H-pyran (DDP) dye in aqueous medium. water soluble were investigated by Addition of Maltose 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 Maltose signifies the presence of heterogeneous micro environment of DDP dye surrounded by varying proportion of the solute and water molecules around the dye moiety. Maltose influence the excited state characteristics of DDP dye resulting in the formation and promotion of different distinguishable micro environment. The hydrophobicity of Maltose derivatives along with the hydrogen-bonding properties of maltose-water and maltose-Maltose largely influence the photo physical nature of DDP dye is emphasized.

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

CHAPTER-2

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, maltose interaction with fluorophores^[6-7] has been carried out in both aqueous and non-aqueous solvents, and the influence of maltose-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 maltose and alkyl substituted maltose molecular assemblies.

Studies involving the role of hydrogen-bonding interaction and the hydrophobic interaction involving maltose on organized micelles^[7], reversed micelles^[5,6], vesicles, mono layers, polymers and with cyclodextrins^[4] have been well established. In spite of reports available on the properties of maltose and alkyl maltose derivatives, there is no concrete evidence about the mechanism and the nature of interaction involving maltose and alkyl maltose interaction with intrinsic and extrinsic fluorophores. Interestingly, the influence of maltose 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 maltose with bio molecules and with that of the hydrophobic moieties, the mechanism and the nature of the interaction existing between maltose 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.

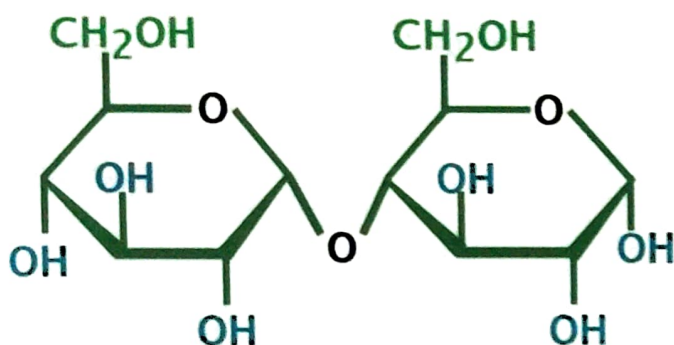
It is advantageous to use the intrinsic fluorophores for these investigations in order to avoid complicated labeling with an extrinsic dye. The fluorescence spectral properties of protein have been largely confined to the tryptophan amino acid. Tryptophan can be preferentially excited above 295 nm in 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 MALTOSE

Hydrogen Bonding in the Maltose Complex. The 2m| Fo| – D|Fc| X-ray map for the maltose complex at 1.1 Å resolution shows that the entire maltose molecule is wellordered, consistent with earlier studie^[10-15] In the corresponding nuclear density map for the maltose complex, the gamaltose moiety is very well-defined, but some parts of the glucose moiety are less clear due to a combination of higher mobility and cancellation efffects from the aliphatic hydrogen atoms on maltose, which is not

deuterated. Nevertheless, the hydrogen-bonding network between the inward-facing hydroxyl groups of maltose 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 maltose 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 maltose to the protein apparently leads to minor conformational strain within maltose^[13-15].

Maltose



The advantage of Maltose 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, **maltose** is non-corrosive, non-toxic, does not produce any adverse effect to the environment and have excellent solubility in water which makes Maltose an excellent host molecule to study the photo physical properties of a water soluble fluorescent probe. Further, Maltose is non-corrosive, non-toxic and does not produce any adverse effect to the environment^[12,13].

Maltose is readily soluble in water and possesses several advantages over other solutes. Maltose disrupts and reorients the water structure through hydrogen-bonding interactions. Even though the properties of water and Maltose in aqueous solutions have been extensively studied, there exists a large variation in their unusual behaviour related to the physical and chemical properties of maltose in solvents especially in aqueous solutions. Aqueous Maltose solution has maltose- maltose, maltose-water and

water-water hydrogen-bonding interactions. The structures of various hydrogen-bonded Maltose-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 Maltose among chemists and biologists. The peculiar behaviour of Maltose 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 Maltose and its derivatives in aqueous solutions reveal that there exists strong hydrogen-bonding interactions^[14,15]. In our study wherein the concentration of Maltose is varied and that of the fluorescent probe is fixed.

APPLICATIONS

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

USES OF MALTOSE

- Maltose 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, maltose 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 dyes and its derivatives 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 inter molecular charge transfer (ICT)-based dye.

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

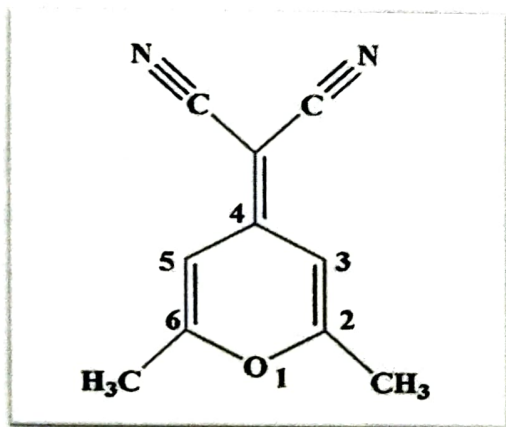
DDP is generally referred as a derivative of DCM type dyes which belongs to the class of red luminescence compound [7-9]. DDP dye have a strong acceptor group dicyanomethylene $\text{C}(\text{CN})_2$ in 4th position and a donor moieties in 2nd and 6th positions (Scheme1). Pyranilidene derivatives used 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 Maltose and alkyl Maltose 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 Maltose and alkyl Maltose derivatives is probed by fluorescence spectral technique. The hydrogen-bonding interaction of Maltose-solvent

and its influence on the fluorescence emission and lifetime of DDP dye is compared with other alkyl Maltose 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 Maltose 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 serves 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(maltose) with DDP dye are largely governed by the solute and the solute-solvent interactions. Like Glucose, Maltose 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-3

AIM AND SCOPE

2.1 AIM

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

2.2 SCOPE

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

MATERIAL AND METHOD

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

2.3PREPARATION OF THE SOLUTION

- ❖ To 10ml solution container, 2ml of (0.4M) maltose 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

3.1 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 from an excited state to a lower energy state.)

CHAPTER-5

UV ABSORPTION STUDIES DYE ALONE

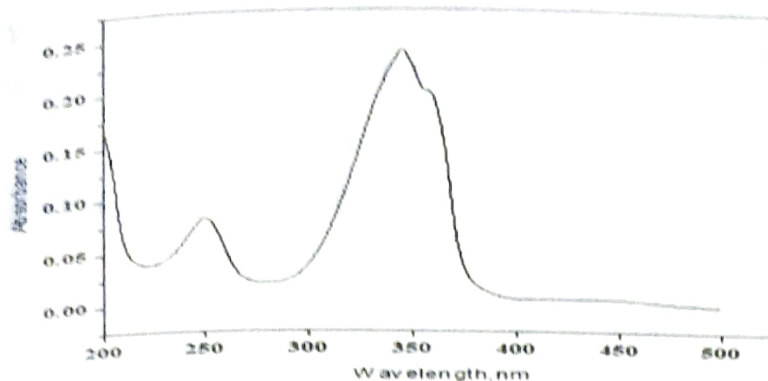
UV-Vis spectrophotometers are used in almost every laboratory in the world due to their measurement versatility. This guide is a snapshot of the main spectrophotometer uses, with links to application notes by measurement technique and industry type.

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 $\pi \rightarrow \pi^*$ transition character, except the small fraction of $\sigma \rightarrow \pi^*$ arising from the donor methyl groups for DDP dye.

4.1 ABSORPTION GRAPH FOR DDP DYE



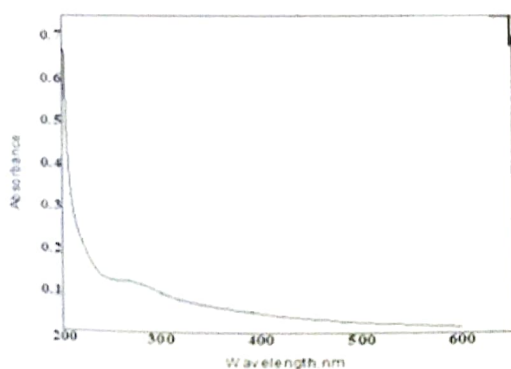
Transitions could not be categorized to have a pure CT character. DCM-styryl type dye exhibits many absorption peaks in the spectral range of 400-500 nm. Interestingly, no absorption peak above 400 nm resulted for DDP dye and this authenticates that the absorption spectral pattern is entirely different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

4.2 ABSORPTION 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(maltose) it does not show any peaks or high point in it.

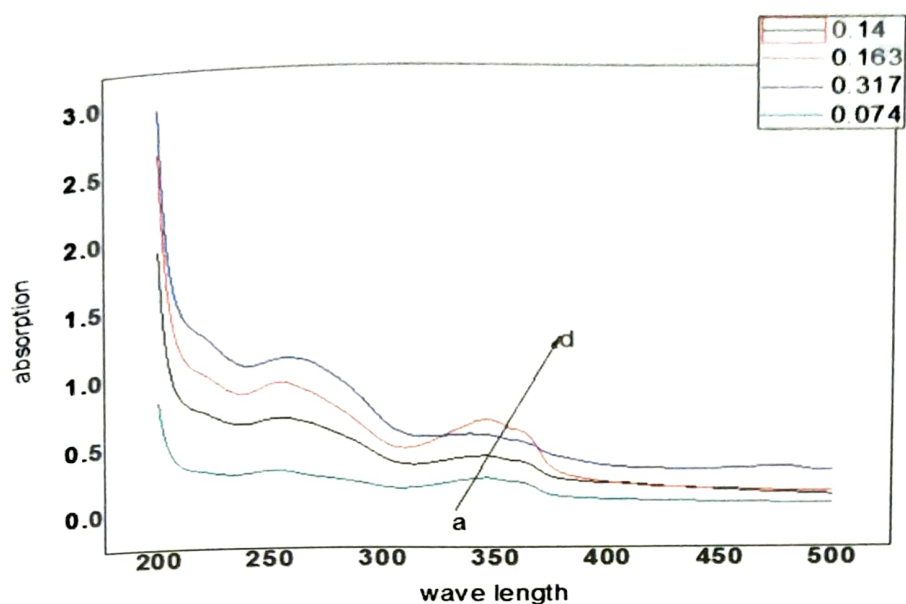
4.3 ABSORPTION GRAPH FOR MALTOSE SOLUTION WITH DDP DYE

DDP dye exhibits two characteristic absorption peaks at 248 and 348 nm accompanied with a shoulder around 360 nm as reported ^[9]. Addition of maltose 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 maltose. The increase in the absorbance around 280 ± 10 nm is attributed to the strong absorbance of maltose. Further, an isosbestic point in the absorption spectrum of DDP dye with maltose is correlated to the formation of a ground state complex. Our earlier studies of DDP dye with hydrogen-bonding assemblies of amides and maltose derivatives also resulted in an isosbestic point which signifies the presence of equilibrium between dye and maltose. From absorption spectral studies it is evident that maltose associates with dye molecules in the aqueous phase. In the present investigation, we account for the role of maltose 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 maltose which associated to be the bound and free dye component

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

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

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

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

Brown mark line shows the absorbance peak of 8ml of maltose 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-5

EMISSION STUDIES

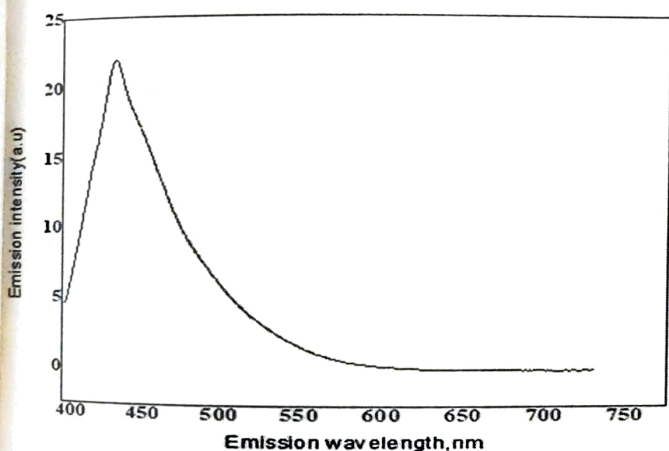
The emission spectrum of the chemical element or compound is the spectrum of the frequencies of electromagnetic radiation emitted due to an atom or molecule transitioning from a high energy state to lower energy state.

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.

5.1 EMISSION GRAPH FOR ONLY DYE

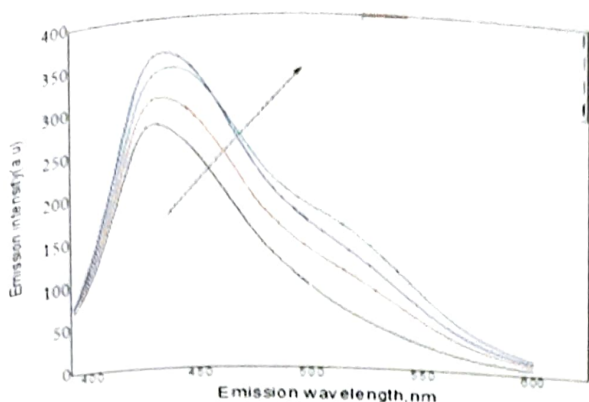


In the graph, the Emission of a characteristic energy is shown as a peak on a graph. In this case, the height and width of the peak represent the line's intensity.

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

5.2 EMISSION GRAPH FOR SUGAR SOLUTION MALTOSE ALONE

5.3 EMISSION GRAPH FOR DDP DYE WITH MALTOSE 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 maltose.

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

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

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

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

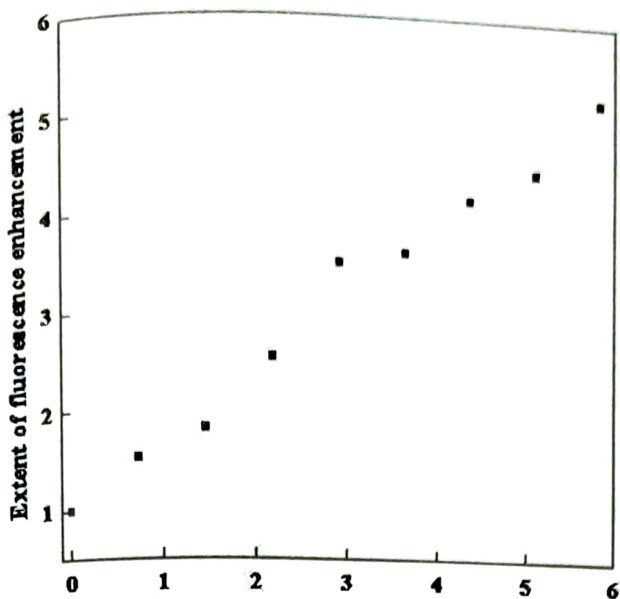
The graph above explains us that the DDP dye enhances the solution, the same way higher the volume of sugar solution with DDP dye shows the large hydrogen bonding interaction.

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 maltose to DDP dye results in a fluorescence enhancement. A broad emission maximum around 430 to 440 nm resulted on the addition of maltose as observed in the case of maltose, 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 maltose resulted in broad emission maxima at $435 \pm \text{nm}$ with a larger fold of enhancement at the longest wavelength emission. The emission spectral studies of maltose 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 maltose. 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 maltose. Interaction of Photoinduced Electron Transfer (PET) based acridinedione dyes with maltose 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 maltose. Maltose 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 maltose. 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, which is discussed in detail in section 3.3.



It is well known that maltose 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 maltose 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 maltose elucidate the importance of binding sites in maltose ^[13,14]. The most preferred site for the binding of fluorescent probes in maltose is the hydrophobic exterior rather than the hydrophilic domain. The most probable location of the dye in maltose 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 maltose. Interaction of maltose 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 maltose as applied to other probe-protein systems. The binding constant of DDP dye with maltose is obtained from the plot of $(F_N - F_0)/(F_X - F_0)$ versus $1/[\text{MALTOSE}]$. The binding constant (K) calculated from the slope and the intercept is found to be $5.070 \times 10^{-3} \text{ M}^{-1}$ for DDP dye which is found to be similar to that of the ICT based probes interaction with maltose as proposed earlier. The binding constant value of

DDP dye with maltose 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 maltose influence the emission spectra of DDP dye wherein the presence of microheterogeneous population of the amino acids definitely governs the emissive nature of the dye. The fluorescence enhancement of DDP dye in the presence of maltose is larger in terms of fluorescence enhancement. The influence of maltose-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 MALTOSE

In general, the mechanism of fluorescence enhancement (FE) of fluorophore in the presence of maltose 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 maltose 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 maltose 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 maltose 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 maltose. 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 maltose. This was established based on the fluorescence lifetime amplitude distribution of the various lifetime components of DDP dye. Interestingly, the addition of maltose (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 maltose 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 fluorophore. The presence of maltose 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 maltose. The nature of the solute influences the excited state properties of DDP dye is illustrated from this present study.

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 maltose. The fluorescence enhancement reveals that the excited state properties are largely governed by the concentration of the protein.

REFERENCE

- [1] Woods, L. L. Some Further Reactions of 2,6-Dimethyl-4-pyrone. *J Am Chem Soc.* 1958, 80, 1440-1442. DOI: 10.1021/ja01539a041.
- [2] Hammond, P. R. Laser dye DCM, its spectral properties, synthesis and comparison with other dyes in the red, *Optics Comm.* 1979, 29, 331-333. [https://doi.org/10.1016/0030-4018\(79\)901111](https://doi.org/10.1016/0030-4018(79)901111).
- [3] Marason, E. G. Laser dyes DCM: CW, Synchronously Pumped, Cavity Pumped and Single Frequency Performance, *Optics Comm.* 1981, 37, 56-58. DOI: 10.1016/0030-4018(81)90176-0.
- [4] Zarins, E.; Jubels, J.; Kokars, V. Synthesis of red luminescent non symmetric styryl-4Hpyran-4-ylidene fragment containing derivatives for organic light-emitting diodes, *Adv Mater Res.* 2011, 222 271-274. doi:10.4028/www.scientific.net/AMR.222.271
- [5] Bourson, J.; Doiz, D.; Lambert, D.; Sacaze, T.; Valeur, B. A. A derivative of laser dye DCM highly soluble in alcohols. *OpticsOptics Comm.* 1989, 72 367-370. [https://doi.org/10.1016/0030-4018\(89\)90441-0](https://doi.org/10.1016/0030-4018(89)90441-0).
- [6] Meyer, M.; Mialocq, J. C. Photophysical Properties of the DCM and DFSBO Styryl Dyes Consequences for their Laser Properties, *Laser Chem.* 1990, 10, 277-296. DOI: 10.1155/1990/67392
- [7] Gustavsson, T.; Baldacchino, G.; Mialocq, J. C.; Pommeret, S. Femtosecond Fluorescence Up- Conversion Study of the Dynamic Stokes Shift of the DCM Dye Molecules in Polar and Non-Polar Solvents *Chem Phys Lett A.* 1995, 236, 587-594. [https://doi.org/10.1016/S0009-2614\(99\)00933-1](https://doi.org/10.1016/S0009-2614(99)00933-1)
- [8] Meyer, M.; Mialocq, J. C.; Rougee, M. Fluorescence Lifetime Measurements of the Two Isomers of the Laser Dye DCM *Chem Phys Lett.* 1988, 150, 484- 490.
- [9] Pal, S. K.; Mandal, D.; Sukul, D.; Bhattacharyya, K. Solvation dynamics of 4-(dicyanomethylene)-2-methyl-6-(p-dimethylaminostyryl)-4H-pyran (DCM) in a Microemulsion, *Chem Phys Lett.* 1999, 312, 178-184. [https://doi.org/10.1016/S0009-2614\(99\)00933-1](https://doi.org/10.1016/S0009-2614(99)00933-1)

- [10] Leffler, H.; Carlsson, S.; Hedlund, M.; Qian, Y.; Pofirier, F. Introduction to galectins. *Glycoconjugate J.* 2002, 19, 433–440.
- [11] Rabinovich, G. A.; Liu, F. T.; Hirashima, M.; Anderson, A. An emerging role for galectins in tuning the immune response: lessons from experimental models of inflammatory disease, autoimmunity and cancer. *Scand. J. Immunol.* 2007, 66, 143–158.
- [12] Almkvist, J.; Karlsson, A. Galectins as inflammatory mediators. *Glycoconjugate J.* 2002, 19, 575–581.
- [13] Dunic, J.; Dabelic, S.; Fogel, M. Galectin-3: an open-ended story. *Biochim. Biophys. Acta, Gen. Subj.* 2006, 1760, 616–635.
- [14] Liu, F. T. Regulatory roles of galectins in the immune response. *Int. Arch. Allergy Immunol.* 2005, 136, 385–400.
- [15] Liu, F. T.; Rabinovich, G. A. Galectins as modulators of tumour progression. *Nat. Rev. Cancer* 2005, 5, 29–41.
- [16] Saraboji, K.; Hakansson, M.; Genheden, S.; Diehl, C.; Qvist, J.; Weininger, U.; Nilsson, U. J.; Leffler, H.; Ryde, U.; Akke, M.; Logan, D. T. The carbohydrate-binding site in galectin-3 is preorganized to recognize a sugarlike framework of oxygens: ultra-high-resolution structures and water dynamics. *Biochemistry* 2012, 51, 296–306.
- [17] Kalyanasundaram K (1987) *Photochemistry in microheterogeneous system*. Academic, New York.
- [18] Lakowicz JR (1999) *Principles of fluorescence spectroscopy*, 3rd edn. Kluwer Academic/Plenum, New York.
- [19] Demchenko AP (1991) in *Topics in Fluorescence Spectroscopy: Biochemical Applications* ed. Lakowicz JR, Plenum, New York, pp 65–112.
- [20] Royer CA (2006) Probing protein folding and conformational transitions with fluorescence. *Chem Rev* 106:1769–1784.