

PHOTOPHYSICAL STUDIES OF DDP DYE WITH glucose IN AQUEOUSE SOLUTION

Submitted in partial fulfilment of the requirements for the award of
Bachelor of Science in Chemistry

By

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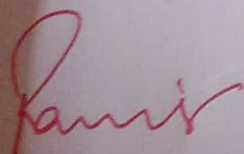
This is to certify that this Project Report is the bonafide work of DHIVYA.S (40030006) who carried out the project entitled "PHOTOPHYSICAL STUDIES OF DDP DYE WITH GLUCOSE IN AQUEOUS SOLUTION" under my Supervision from December 2022 to April 2023.

S. Gayathri
Guide

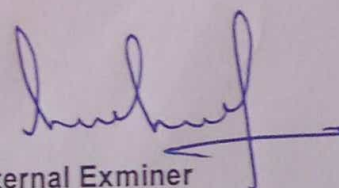
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DECLARATION

I S.DHIVYA (40030006) hereby declare that the project report entitled "PHOTOPHYSICAL STUDIES OF DDP DYE WITH GLUCOSE IN AQUEOUS SOLUTION" done under the guidance of Dr.S.GAYATHRI Ph.D., Associate professor, Department of Chemistry, SATHYABAMA INSTITUTE OF SCIENCE AND TECHNOLOGY is submitted in the partial fulfilment of the requirements for the award of bachelor of science degree in chemistry.

DATE 08.05.2023

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SIGNATURE OF THE CANDIDATE

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ABSTRACT

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

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

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CHAPTER-1

INTRODUCTION

1.1. PHOTOPHYSICS OF PROBES IN SELF ASSEMBLIES AND MOLECULAR AGGREGATES

The concept of photophysics and photochemistry in microheterogeneous systems are of utmost importance in the field of chemistry and biology^[25]. Photo physical studies serve as a vital link in establishing and elucidating the structure and properties of the probe molecules in the microheterogeneous environment in their ground and excited states^[10]. 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^[22,11]. 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^[21]. Micelles form self assemblies in aqueous solution and act as an ideal medium to probe the photo physical properties. Likewise, urea interaction with fluorophores^[11] has been carried out in both aqueous and non-aqueous solvents, and the influence of urea-solvent hydrogen-bonding properties on the photophysical 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 urea and alkyl substituted urea molecular assemblies.

Studies involving the role of hydrogen-bonding interaction and the hydrophobic interaction involving urea on organized micelles^[11-20], reversed micelles^[20-22], vesicles^[11], mono-layers^[23], polymers^[24] and with cyclodextrins^[25,30] have been well established. In spite of reports available on the properties of urea and alkyl urea derivatives, there is no concrete evidence about the mechanism and the

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^[106] 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 biomolecule, or solutes situated in a highly heterogeneous environment are divided into two major classes^[10]; 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 aminoacids that are fluorescent in nature.

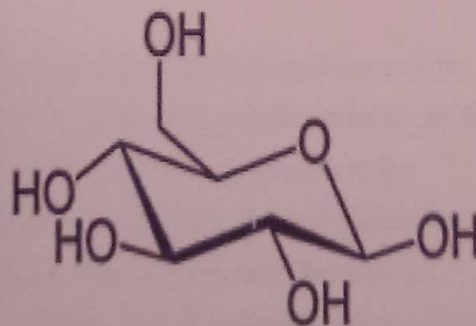
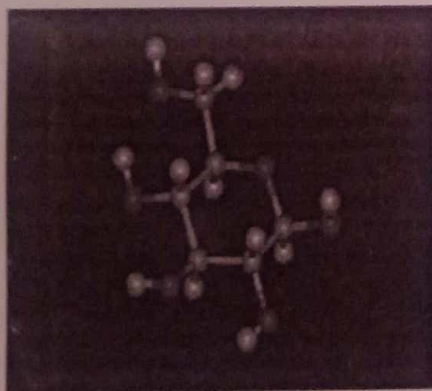
It is advantageous to use the intrinsic fluorophores for these investigations in order to avoid complicated labeling with an extrinsic dye. The fluorescence spectral properties of protein have been largely confined to the tryptophan amino acid. Tryptophan can be preferentially excited above 295 nm in 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 group^[22,29] Tyrosine and phenylalanine

are not sensitive to the microenvironment as compared to that of tryptophan and does not provide valuable information regarding the dynamics of protein folding. For more than four decades fluorescence instrumental methods^[11,25] has been involved in elucidating the structure of protein, which is attributed to the tryptophan fluorescence properties

STRUCTURE OF GLUCOSE

Glucose (Glc), a monosaccharide (or simple sugar), is the most important carbohydrate in biology. The cell uses it as a source of energy and metabolic intermediate. Glucose is one of the main products of photosynthesis and starts cellular respiration in both prokaryotes and eukaryotes^[15]

Two isomers of the aldohexose sugars are known as glucose, only one of which (D-glucose) is biologically active^[19]. This form (D-glucose) is often referred to as **dextrose** (dextrose monohydrate), especially in the food industry.^[18] This article deals with the D-form of glucose. The mirror-image of the molecule, L-glucose, cannot be used by cells.^[11]



STRUCTURE

Glucose ($C_6H_{12}O_6$) contains six carbon atoms and an aldehyde group and is therefore referred to as an aldohexose^[19]. The glucose molecule can exist in an open-chain (acyclic) and ring (cyclic) form (in equilibrium), the latter being the result of an intramolecular reaction between the aldehyde C atom and the C-5 hydroxyl group to form an intramolecular hemiacetal. In water solution both forms are in equilibrium, and at pH 7 the cyclic one is the predominant.^[13] As the ring contains five carbon atoms and one oxygen atom, which resembles the structure of pyran, the cyclic form of glucose is also referred to as glucopyranose. In this ring, each carbon is linked to an hydroxyl side group with the exception of the fifth atom, which links to a sixth carbon atom outside the ring, forming a CH_2OH group^[15]

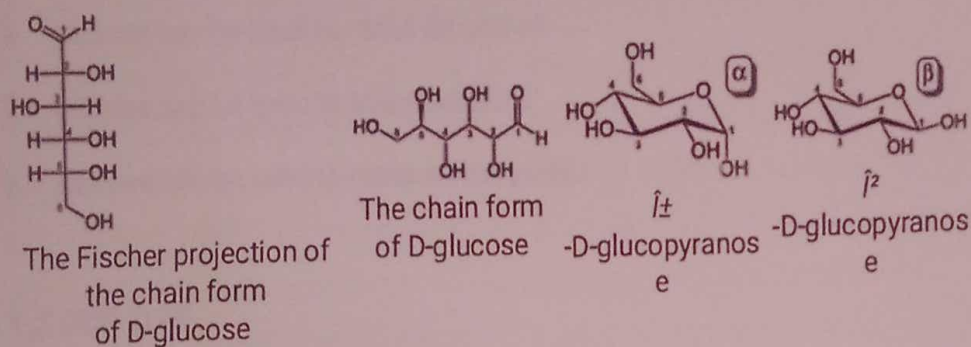
ISOMERS

Aldohexose sugars have 4 chiral centers giving $2^4 = 16$ optical stereoisomers. These are split into two groups, L and D, with 8 sugars in each.^[11,14] Glucose is one of these sugars, and L and D-glucose are two of the stereoisomers^[3,8]. Only 7 of these are found in living organisms, of which D-glucose (Glu), D-galactose (Gal) and D-mannose (Man) are the most important. These eight isomers (including glucose itself) are all diastereoisomers in relation to each other and all belong to the D-series.^[14,15]

An additional asymmetric center at C-1 (called the *anomeric carbon atom*) is created when glucose cyclizes and two ring structures, called anomers, can be formed: α -D-glucose and β -D-glucose.^[22, 25] They differ structurally in the orientation of the hydroxyl group linked to C-1 in the ring. When D-glucose is drawn as a Haworth projection, the designation α means that the hydroxyl group attached to C-1 is below the plane of the ring, β means it is above. The α

and β forms interconvert over a timescale of hours in aqueous solution, to a final stable ratio of $\alpha:\beta$ 36:64, in a process called *mutarotation*^[25].

GLUCOSE SHIFTING FROM FISCHER PROJECTION TO HAWORTH PROJECTION.



FUNCTION

We can speculate on the reasons why glucose, and not another monosaccharide such as fructose (Fru), is so widely used in evolution/the ecosystem/metabolism^[17]. Glucose can form from formaldehyde under abiotic conditions, so it may well have been available to primitive biochemical systems. Probably more important to advanced life is the low tendency of glucose, by comparison to other hexose sugars, to non-specifically react with the amino groups of proteins. This reaction (glycation) reduces or destroys the function of many enzymes^[12,13]. The low rate of glycation is due to glucose's preference for the less reactive cyclic isomer. Nevertheless, many of the long-term complications of diabetes (e.g., blindness, kidney failure, and peripheral neuropathy) are probably due to the glycation of proteins or lipids. In contrast, enzyme-regulated addition of glucose to proteins by glycosylation is often essential to their function^[11].

USES OF GLUCOSE

glucose is required for respiration. As we will discuss later, glucose is a reactant in the reaction for respiration, which produces energy. ...

- ☐ glucose can be stored as starch. ...
- ☐ glucose can be used to make fat and oil. ...
- ☐ glucose can be used to make cellulose. ...
- ☐ glucose can be used to make amino acids.

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 intermolecular 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^[3,4]. DDP dye has a strong acceptor group dicyanomethylene- $C(CN)_2$ in 4th position and donor moieties in 2nd and 6th positions (Scheme 1). 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^[4]. Most of the photophysical studies were carried out with DCM dye only due to the presence of Twisted Intramolecular Charge Transfer (TICT) behavior^[5]. The striking

difference is that DDP dye does not exhibit TICT behaviour. Further the quantum yield and the photophysical properties of these dyes are governed by the number of donor and acceptor moieties^[9,7]. 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 glucose and alkyl glucose derivatives.

Interestingly, DDP dye is soluble in water compared to that of DCM dye (Scheme 1) and possesses significant advantage over DCM dye in the field of photophysical, 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^[8,2] 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^[5,4]. The variation in the ground and excited state properties of DDP dye in the presence of glucose and alkyl glucose derivatives is probed by fluorescence spectral technique. The hydrogen-bonding interaction of glucose-solvent and its influence on the fluorescence emission and lifetime of DDP dye is compared with other alkyl glucose derivatives.

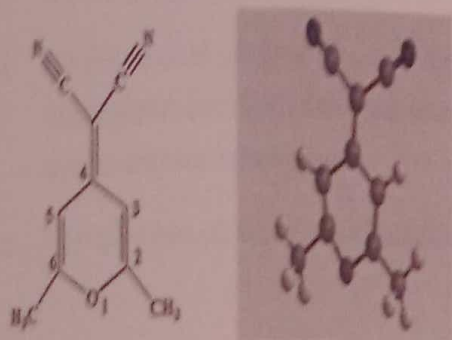
In our present study, we focus on the variation in the photophysical properties of an ICT based dye, 4-Dicyanomethylene-2,6-dimethyl-4H-pyran (DDP) in the presence of glucose. Further, a complete mechanism of the origin of fluorescence emission and lifetime of DDP dye in the presence of water soluble non fluorescent solutes are very limited in the literature to the best of our knowledge,^[7,5]

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- 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 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.



Scheme 1

- Further the electroluminescent and photophysical 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 microenvironment formed by the

adlayer around the close vicinity, and the electronic interface provides valuable information on the electrochemical properties of these probes. Considering the high sensitivity of fluorescence and the salient structural information that it can provide, photophysical 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.^[10]

- ☐ On the other hand, although redox reaction can be carried out in the bulk solution, this technique provides very little control and the idea about the processes taking place to a larger area.
- ☐ Spectroscopic outcomes-provides better knowledge for understanding the nature of the interaction.
- ☐ Photophysical studies of a well-known water-soluble fluorescent solute like sugars (glucose) with DDP dye are largely governed by the solute and the solute-solvent interactions.
- ☐ Like glucose, glucose 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

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:

Sayed 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 malonitrile, 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 upconverting 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.

CHAPTER-3

AIM AND SCOPE

3.1 AIM

The Aim of the project is to study the photophysical studies of DDP dye with glucose with aqueous solution.

3.2 SCOPE

- ▣ Sugar solution(glucose) in different concentration.
- ▣ Hydrogen-bonding interaction of Guest-Host molecule.
- ▣ organized self-assemblies in water
- ▣ Photophysical properties of DDP – glucose 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

- ▣ DDP dye and add water to the remaining part of solution.

This process is classified under the physisorption because of weak forces, reversible even after reacting with atmosphere by filtration.

CHAPTER-3

OBJECTIVE OF 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 photophysical 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.

- ☒ Photophysical 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 photophysical 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

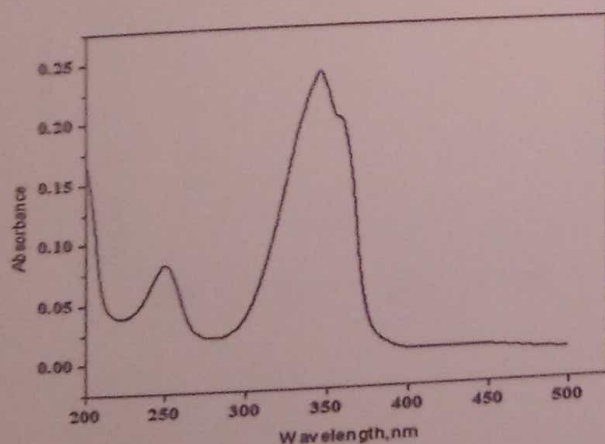
UV ABSORPTION STUDIES DYE ALONE

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.

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.

5.1 ABSORPTION GRAPH FOR DDP .



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.

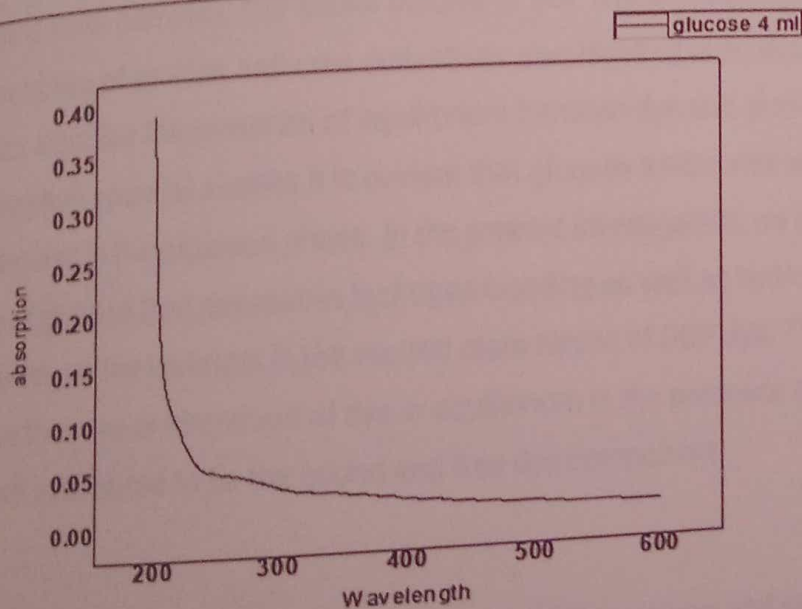
In this graph we will see that absorption peak is at 430nm.

5.2 ABSORPTION GRAPH ONLY FOR SUGAR SOLUTION

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. This is in



- ◆ 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(glucose) it does not show any peaks or high point in

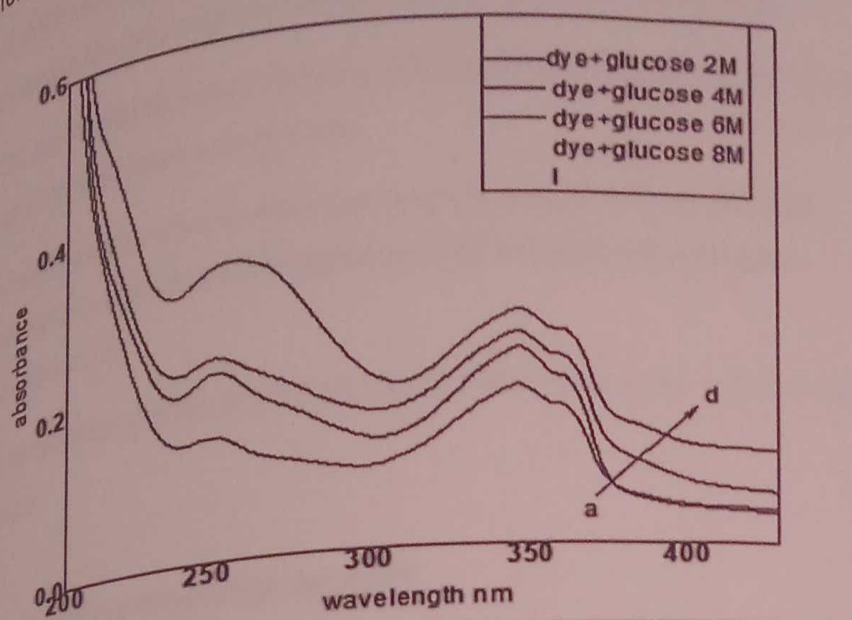
ABSORPTION GRAPH FOR GLUCOSE SOLUTION WITH DDP DYE

DDP dye exhibits two characteristic absorption peaks at 248 and 348 nm accompanied with a shoulder around 360 nm as reported [42]. Addition of glucose 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 the strong concentration of glucose. The increase in the absorbance around 280 nm is attributed to absorbance of glucose. Further, an isosbestic point in the absorption spectrum of DDP dye with glucose (figure 3) is correlated to the formation of a ground state complex. Our earlier studies of DDP dye with hydrogen-bonding assemblies of amides and urea derivatives also resulted in an isosbestic point which signifies the presence of equilibrium between dye and glucose. From absorption spectral studies it is evident that glucose associates with dye molecules in the aqueous phase. In the present investigation, we account for the role of glucose 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 glucose which associated to be the bound and free dye component

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

the dye shows the peak in 430nm and the sugar solution alone doesn't show any peak.

The dye is characterized under the microenvironment and Inter Charge Transfer.
 Now absorption for our solution prepared with glucose 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 glucose.

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.

EMISSION STUDIES

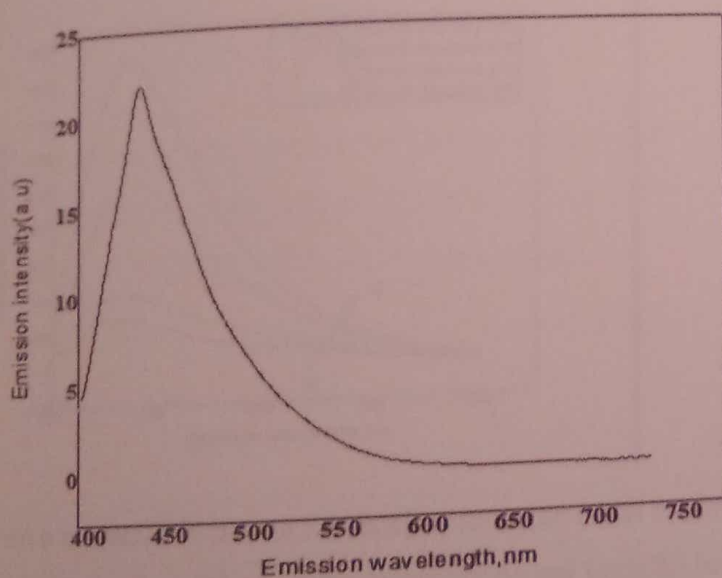
The emission spectrum of the chemical element or compound is the spectrum of the frequencies of electromagnetic radiation emitted due to an atom or molecule transitioning from a high energy state to lower energy state.

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.

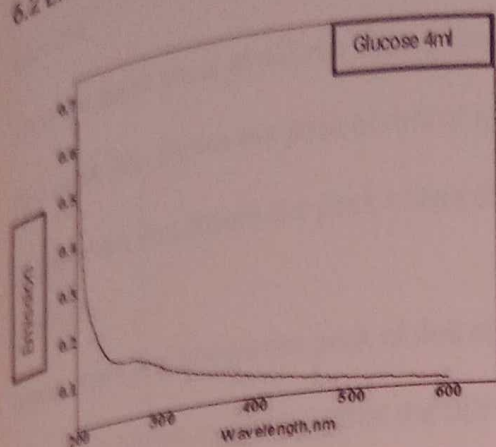
6.1 EMISSION GRAPH FOR ONLY DYE



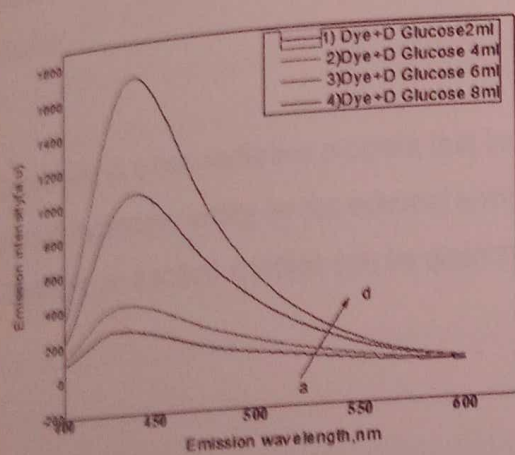
In the graph, the Emission of a characteristic energy is shown as a peak on a graph. In this case, the height and width of the peak represent the line's intensity.

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

6.2 EMISSION GRAPH FOR SUGAR SOLUTION GLUCOSE ALONE



6.3 EMISSION GRAPH FOR DDP DYE WITH glucose 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 glucose.

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

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

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

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

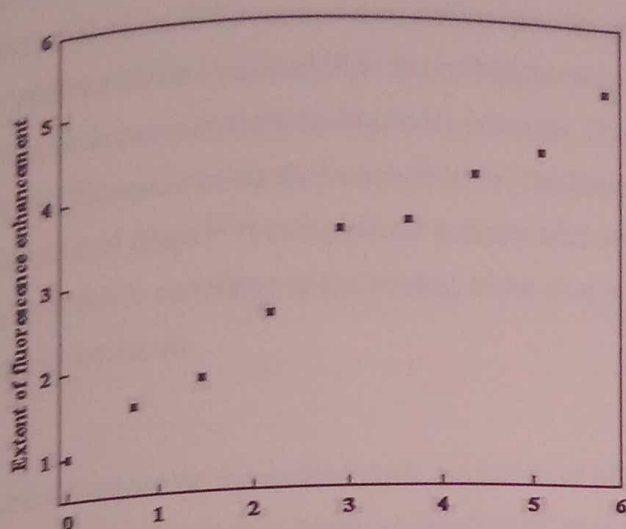
The graph above explains us that the DDP dye enhances the solution, the same way higher the volume of sugar solution with DDP dye shows the large hydrogen bonding interaction.

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 glucose to DDP dye results in a fluorescence enhancement . A broad emission maximum around 430 to 440 nm resulted on the addition of glucose as observed in the case of urea, and a close observation on the emission spectra reveals the presence of two emission peaks of almost similar Tintensity separated by a few nm only. Interestingly, with a further increase in the concentration of glucose 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 glucose 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 glucose. The extent of fluorescence enhancement is accompanied with no considerable shift in the emission towards the red or blue region



It is well known that glucose 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 glucose with fluorescent probes are determined using the modified form of the Benesi-Hildebrand equation [16] which is so far the ideal tool in determining the nature of the interaction involving proteins [12,13]. Studies based on the interaction of probe/drug with glucose elucidate the importance of binding sites in glucose [4,7]. The most preferred site for the binding of fluorescent probes in glucose is the hydrophobic exterior rather than the hydrophilic domain. The most probable location of the dye in glucose 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 glucose. Interaction of glucose with norharmane [22], AODIQ [29, 30], p-(dimethylaminobenzamido-thiosemicarbazone) [12], methylene blue [22,24] 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 glucose as applied to other probe-protein systems [10]. The binding constant of DDP dye with glucose is obtained from the plot of $(F_0 - F)/(F - F_0)$ versus $1/[\text{glucose}]$. The binding constant (K) calculated from the slope and the intercept is found to be 5.070×10^3 M⁻¹ for DDP dye which is found to be similar to that of the ICT based probes interaction with glucose as proposed earlier. The binding constant value of DDP dye with glucose 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 [5,14] 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 glucose 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 glucose is larger in terms of fluorescence enhancement. The influence of urea-solvent hydrogen-bonding properties on the photophysical behavior of fluorescent probe results either in an increase or decrease in the fluorescence emission and lifetime.

MECHANISM OF FLUORESCENCE ENHANCEMENT OF DDP DYE WITH GLUCOSE

In general, the mechanism of fluorescence enhancement (FE) of fluorophore in the presence of glucose in aqueous or buffered solutions is attributed to two different mechanisms [26,11]. These mechanisms are postulated in regard to fluorescence enhancement of extrinsic probes involving globular proteins such

that addition of glucose 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 glucose 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 [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 glucose with fluorescent probes involving a change in the polarity around the fluorophore has been well documented in the literature involving probe-protein interaction [32, 33]. 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 LE state [15, 28]. This mechanism was ruled out since there were no new emissive peaks arising from DDP dye on the addition of glucose. The emission intensity in the spectral range of 435 ± 10 was almost similar which authenticates that the local excited (LE) state emission is stabilized to a larger extent. This was confirmed from 3D emission contour spectral studies.

Apart from these mechanisms, LE state 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 [18] 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 glucose. This was established based on the fluorescence lifetime amplitude distribution of the various lifetime components of DDP dye.

Interestingly, the addition of urea (hydrophilic and hydrophobic moieties) results in a complete change in the microenvironment. The increase in the

fluorescence intensity of DDP dye on the addition of urea 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 LE state emission of the fluorophore. The presence of urea around the dye molecules compete with water molecules in forming hydrogen-bonding interaction such that the variation in the microenvironment of dye is influenced by the concentration of urea. 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 glucose. The fluorescence enhancement reveals that the excited state properties are largely governed by the concentration of the protein.

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