Photophysics of Biological Active Chromophores Confined in Graphene-Oxide Mediated Reverse Micelles

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By

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Dedicated to my parents
for their
Persistent love &
unconditional support

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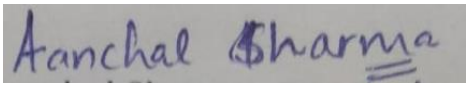

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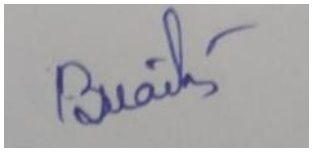

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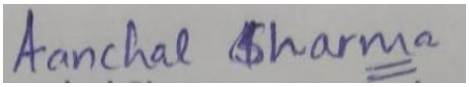

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ABSTRACT

In this thesis, the entrapment of anti-fungal, anti-bacterial, non-fluorescent cationic dye molecules Crystal Violet (CV) and Acridine Orange (AO) in presence of bio-mimetic confined Reverse-Micelle aggregates has been studied. The photophysical characteristic behaviour of CV and AO has been carried out by varying different kinds of surfactants (named as Aerosol-OT, SDS, SOS) forming reverse micelles. The main objective is to improve the solubility and fluorescence properties of the studied fluorophores in presence of bio-mimic confined aqueous and non-aqueous reverse micelles and deciphers different kinds of non-covalent solute-solvent interactions. To interpret the result, steady state absorption and fluorescence emission techniques have been carried out. In methanol, CV and AO molecule exhibits non-fluorescent in nature. The value of fluorescence quantum yield (Φ) is $\sim 10^{-4}$. But after the confinement of the molecules (CV and AO) in different reverse micelle mediums, they become highly fluorescent in nature. It has also been detected that in presence of different reverse micelle aggregates, both CV and AO molecules exhibits remarkable enhancement of absorption and fluorescence emission spectral behaviour. Moreover, the role of the GO in aqueous medium was also explored. Presence of GO in RMs assemblies caused quenching in absorption, fluorescence emission and the fluorescence quantum yield values. This result clearly interpreted that GO acts as quencher and have ability to release the studied molecules from the RMs assemblies. For that reason, the highly fluorescent nature of the molecules again turns into non-fluorescent in presence of GO. The work involved in this thesis might be valuable for potential targeted drug-delivery implications, detection analysis, sensors in physiological systems.

LIST OF CONTENTS

PAGE

- Acknowledgement i
- Certificate ii
- Declaration iii
- Abstract iv
- List of Contents v-vii
- List of Tables viii
- List of Figures ix

Chapter-1

1.INTRODUCTION **Pages 1-6**

1.1 Dyes

1.2 Crystal Violet (CV)

1.2 Biological importance of CV

1.3 Acridine Orange (AO)

1.4 Biological importance of AO

1.5 Confined medium

1.6 Reverse-Micelles (RMs)

1.7 Graphene-Oxide (GO)

Chapter-2

2. LITERATURE REVIEW **Pages 7-10**

2.1 Basic literature review on Surfactant and CV and AO

2.2 Research gap

2.3 Aim of this project

Chapter-3

3. MATERIALS AND METHODS **Pages 11-16**

3.1 Materials

3.1.1 Pharmaceutical compound

3.1.2 Different surfactants and chemicals used

3.2 Experimental methods

3.2.1 Preparation of Graphene Oxide

3.2.2 Preparation of CV and AO solution

3.2.2.1 Preparation of AOT-H_2O/GO-H_2O RMs

3.2.2.2 Preparation of SDS-H_2O/GO-H_2O RMs

3.2.2.3 Preparation of SOS-H_2O/GO-H_2O RMs

3.2.2.4 Preparation of AOT-MeOH/GO-MeOH

3.3 Experimental Techniques

3.3.1 Ultraviolet-Visible absorption measurements

3.3.2 Fluorescence measurements

3.3.3 Determination of fluorescence quantum yield

Chapter-4

4. RESULTS AND DISCUSSIONS **Pages 17-30**

4.1 Steady-state absorption studies

4.1.1 CV molecule in solvents (water and GO-water)

4.1.2 CV molecule in AOT- water RMs

4.1.3 CV molecule in AOT-GO-water RMs

4.1.4 CV molecule in SDS-Water RMs and SDS-GO-water RMs

4.1.5 CV molecule in SOS-Water RMs and SDS-GO-water RMs

4.1.6 AO molecule in solvents (water and GO-water)

4.1.7 AO molecule in AOT-water RMs

4.1.8 AO molecule in AOT-GO-water RMs

4.1.9 AO molecule in AOT-water RMs and GO-methanol RMs

4.2 Steady-state fluorescence studies

4.2.1 CV molecule confined in water RMs and GO-water RMs

4.2.2 AO molecule confined in water RMs and GO-water RMs

4.2.3 AO molecule confined in methanol RMs and GO-methanol RMs

4.3 Fluorescence quantum yields of CV and AO molecules in different systems

Chapter-5

5. CONCLUSION **31**

LIST OF TABLES

Pages

Table 3.1: Preparation of water RMs and GO-water RMs 13

Table 3.2: Preparation of methanol RMs and GO-methanol RMs 14

Table 4.1: Fluorescence quantum yield values (Φ) of CV in different systems 29

Table 4.2: Fluorescence quantum yield values (Φ) of CV in different systems 30

LIST OF FIGURES

Pages

Figure 1.1: Schematic representation of two isomeric form of Crystal Violet. 2
Figure 1.2: Schematic representation of Acridine Orange Dye. 3
Figure1.3: Schematic Representation of micelles and reverse micelles 6
Figure 3.1: Structures of AOT, SDS and SOS 11
Figure 3.2: Perkin Elmer Lambda 35 UV/Vis spectrophotometer 14
Figure 3.3: Schematic block diagram of UV-Vis spectrophotometer 15
Figure 3.4: Perkin Elmer LS 55 spectrofluorimeter 16
Figure 3.5: Schematic Block Diagram of spectrofluorimeter 16
Figure 4.1: UV-Vis absorption of CV in water and GO-water 17
Figure 4.2: UV-Vis absorption of CV in water and AOT-isooctane mixture (w_0) 18
Figure 4.3: UV-Vis absorption of CV in AOT-water RMs 19
Figure 4.4: UV-Vis absorption of CV in AOT-GO-water RMs 20
Figure 4.5: UV-Vis absorption of CV in SDS-water RMs and SDS-GO-water RMs 21
Figure 4.6: UV-Vis absorption of CV in SOS-water RMs and SOS-GO-water RMs 22
Figure 4.7: UV-Vis absorption of AO in water and GO-water 23
Figure 4.8: UV-Vis absorption of AO in AOT-water RMs 23
Figure 4.9: UV-Vis absorption of AO in AOT-GO-water RMs 23
Figure 4.10: UV-Vis absorption of AO in AOT-methanol RMs and AOT-GO-methanol RMs 24
Figure 4.11: Fluorescence emission of CV in AOT-water RMs and AOT-GO-water RMs 25
Figure 4.12: Fluorescence emission of CV in SDS-water RMs and SDS-GO-water RMs 25
Figure 4.13: Fluorescence emission of CV in SOS-water RMs and SOS-GO-water RMs 26
Figure 4.14: Fluorescence emission of AO in water RMs and GO-water RMs 26
Figure 4.15: Fluorescence emission of AO in methanol RMs and GO-methanol RMs 27

CHAPTER-1

INTRODUCTION

1.1 Dyes

Dyes are one of the most important classes of unsaturated organic substances,which absorbs in visible region of electromagnetic spectrum. Generally, they exhibit different colours because of the presence of different chromophores & auxochromes. [1]The dyes are broadly classified into two classes (i) Natural dyes and (ii) Synthetic dyes. They also have wide applications in different fields such as printing ink, textile dyeing, and photography as well as in the area of biochemistry & analytical chemistry [2-4]**.** In this project, two synthetic cationic dyes (i) Crystal Violet (CV) & (ii) Acridine Orange (AO) have been used.

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1.2 Crystal Violet (CV)

Crystal-Violet (CV) is also termed as Gentian-Violet or Methyl-Violet. CV belongs to the family member of triphenylmethane derivatives, which was first synthesized by **Charles Lauth** in 1861, under the name of **'Violet de Paris'.** Crystal-Violet is basically defined as hexamethylrosaniline, which is completely a symmetric molecule in which every amino group have two methyl-group. This is a trimer of dimethyl-aniline containing 6 methyl-groups & is a highly coloured Dye. In Earlier times, this is also known as (Methyl-Violet 10B) or (Methyl-Violet 2B).[5] Crystal Violet is an inexpensive cationic & a basic Synthetic dye having molecular formula ($C_{25}H_{30}N_3Cl$) and molecular weight (407.986). The dye molecule is poorly metabolized by microbes. [6] CV molecule modulates its change of the colour (from violet to yellowish green) depending upon the concentration of the acid. [7] In aqueous medium, the fluorescence property of CV is very-week because of the very fast non-radiative deactivation pathways due to the presence of phenyl-rings. [8] In aqueous solution, the studied molecule exhibited two absorption maxima, which was due to the presence of two equilibrated isomeric forms (planar form and distorted or Pyramidal form). [8] Both the forms of CV molecules are shown in **Figure 1.1:-**

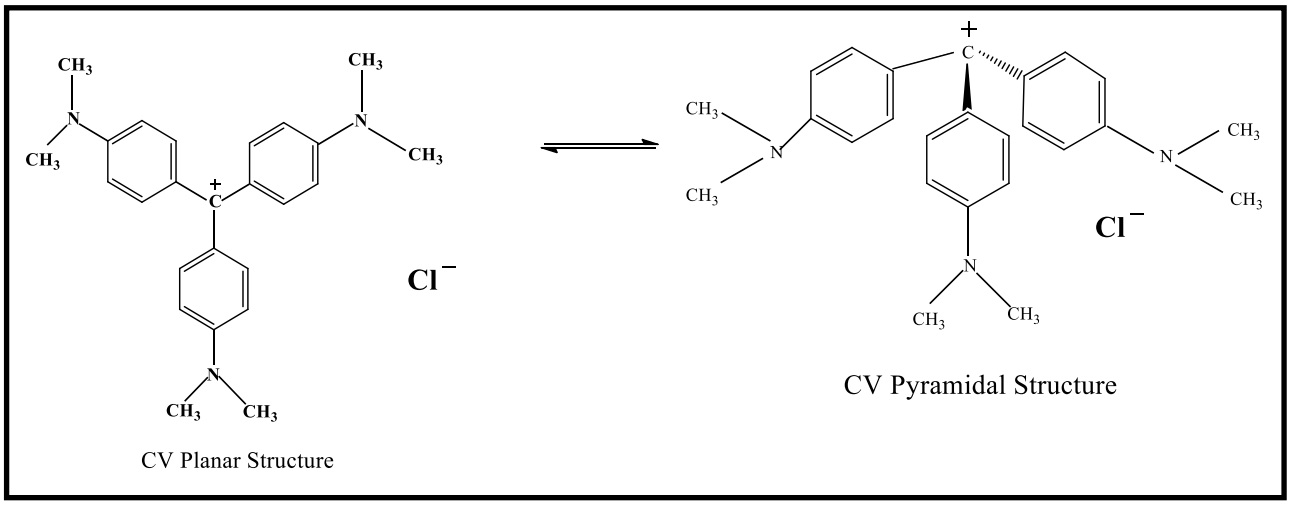

Figure 1.1: Schematic representation of two isomeric form of Crystal Violet.

1.2 Biological importance of CV

Crystal violet is majorly used as biological stain in animal and veterinary medicine.[6] Apart from that, the dye molecule also plays an important role as antiviral, antifungal, antibacterial, anti-microbial and anti-tumour activities. CV is also used for curing several diseases named as thrush, Impetigo, burns, pinworm, trench-mouth and systematic fungal infections. [5] CV is basically dissociates as (CV^+) and (Cl^-), which penetrate both Gram positive & Gram negative bacterial cells.

1.3 Acridine-Orange (AO)

The IUPAC nomenclature of acridine orange (AO) is (N,N,N′,N′-tetramethylacridine-3,6-diammine hydrochloride). It is one of the synthetic cationic dyes, which is readily soluble in water and alcohol (polar solvents). AO was firstly synthesized in 1889 and its first biological application was reported in 1940. AO is also known as acridine orange base, basic orange 14. AO has numerous applications in the field of biology. Since, the dye molecule contains lone pair of electrons on the heteroatom (-N atom), which has tendency to accept proton from. More briefly, the dye molecule acts as proton acceptor. Therefore, for biological systems such as cell-organs, AO molecule penetrates into the membrane of the cell-organs. Moreover, the photophysical property of AO is very much sensitive to the

local environments of the surrounding media. The photophysical properties of the dye molecule significantly modulate depending upon the pH values of the medium. AO shows two absorption maxima in aqueous medium. The first absorption maximum appears at 470 nm (due to the presence of dimeric absorption of AO) along with the second absorption maxima at 490 nm (due to monomeric absorption of AO) respectively. [9] The emission spectra of AO is observed at ~527 nm. [10] The pK_a value of AO is reported as 10.4. [9]. **Figure 1.2** represents the schematic representation of Acridine Orange.

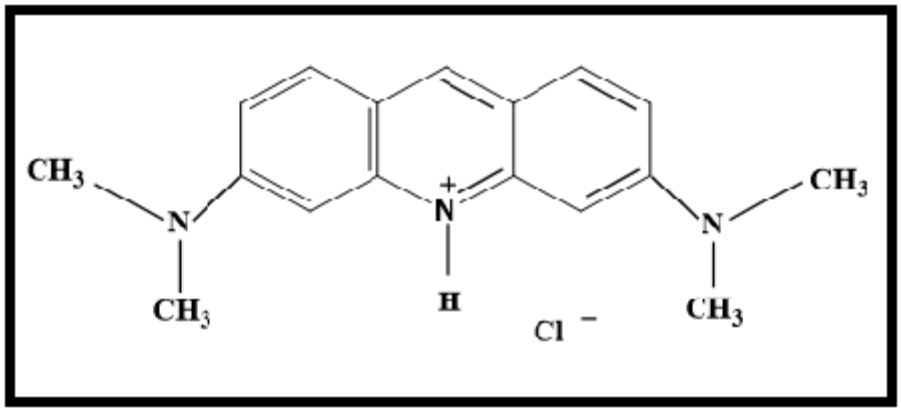

Figure 1.2: Schematic Representation of Acridine Orange Dye.

1.4 Biological Importance of AO

Since the pK_a value of AO is 10.4, therefore the molecule is basic in nature. As a result, the dye molecule is frequently used as a probe to study various biological systems such as micelles, micro emulsions, nucleotides etc.[9] AO also shows biological activities such as anti-tumour activity, pH detection activity, photo-sensitizing etc. [11] The low molecular-weight (M.W. 265 g/L) and simple structural form of AO has capability to rapid flow into cytoplasm through the plasma membrane. Due to the ionic form, AO molecule has also tendency to interact with biomolecules (such as RNA and DNA).[11] One of the most important biological application of AO is to destroy the cancer cell by using photon-energy. The photon energy will excite AO molecule to generate the active oxygen species, which will aids to oxidize the fatty acid of lysosomal membrane.[11] So in this way, AO molecule exhibiting excellent selective property towards the anti-cancer cell activity. For this reason, AO molecule is recognized by the name of "Magic-Bullet" for the treatment of cancer.

[11]Moreover, AO molecule is also extensively used in photodynamic therapy due to its photo-sensitizing ability. [12]

1.5 Confined Medium

Confined medium has specific, selective and well-defined boundaries, in which several chemical and biological processes takes place. Basically, in confined medium, the motion of the molecules and their surrounding media becomes restricted in a small region or volume. In other words, free motion of the solute and solvent gets restricted due to confinement. The confined environment is substantially different from that of bulk environment. In bulk medium, the motion of the molecules becomes irregular and random in nature. Confined medium has tendency to impose some active chemical species inside its aggregated domain, in which different organized assemblies like micelles, reverse micelles, lipids, bile-salts etc. gets agglomerated. These are the suitable model system for bio mimic confined medium. This kind of confined medium is termed as "heterogeneous" from microscopic view or frequently termed as "micro-heterogeneous". Hence this is a very interesting task to investigate the various photo-physical and dynamics in bio-mimic heterogeneous systems. Moreover, most of the physicochemical & biological phenomena occur in confined medium rather than in homogeneous environment.

1.6 Reverse-Micelles (RMs)

In polar environments, beyond a particular concentration, known as critical micelle concentration (*CMC*), monomer of surfactant molecules assembled together to form micelles **(Figure 1.3)** in polar solvents. The hydrophilic head groups of the surfactants oriented towards the exterior of the polar solvents while the hydrophobic tail groups are oriented towards the interior of the non-polar environments **(Figure 1.4)**. Reverse-micelles are nano-meter sized polar droplets, which possess hydrophobic exterior towards the non-polar solvents and hydrophilic interior to the polar solvents pools. Reverse micelles are formed in non-polar environment. As they are electrostatically neutral, so they have tendency to collide smoothly with hydrocarbon-tail group with minimum efficiency. That's why reverse-micelles are considered as dynamics in nature. [14] Also the polar solvent molecules behave differently in reverse-micelle than in bulk solvents. The internal nano-shaped diameter of reverse-micelle is well-suited with the size of confined biological-

assemblies. [13, 15, 16, 17] RMs are generally used as template for bio-mimic confined models. They also have multiple applications such as cell-membrane, chemical-catalysis, pharmaceuticals and medicine and also have numerous applications in both science and technology. [18-23]One of the most excellent characteristic property of revese-micelle is its capability to encapsulate impartially large amount of polar solvent molecule in their hydrophilic core which will be further able to solubilizes organic fluorophores, proteins and DNA, RNA without any change in their isotropic nature. [15,24] In general, while performing studies on fluorophorescofined within RMs, it is necessary to use a non-polar solvents that will not be able to solubilize the fluorophores. This will clearly indicates that fluorophore is confined within the central solvent pool of reverse micelle and not in exterior part of the solvent. In the present work, we prepare reverse micelle, by using an anionic surfactant named as Aerosol-OT (AOT) (Sodium-bis-2-ethyl-hexylsulphosuccinate), dissolved in non-polar solvent (iso-octane) beyond its *CMC* value. From few decades, AOT is used as a most commonly used surfactant because of the following reasons:-

(a) This is non-toxic in nature and is preferably used in pharmaceuticals and medical-applications.

(b) AOT contains both polar and non-polar phases. That's why the molecules are solubilized by this system.

(c) Three different phases gets formed inside the central core of reverse-micelle (i) Non-polar phase (ii) Polar phase (iii) Interface between polar and non-polar phase.

(d) By controlling the concentration of polar solvents or surfactants within the system, the size of the reverse-micelle can be controlled.[25] In actual practice, the size of reverse-micelle central core is distinguished by the polar to surfactant concentration ratio i.e. w=[polar solvent]/[surfactant].[16]

(e) We choose Aerosol-OT because AOT molecules have ability to form cluster in the presence of non-polar solvents such as cyclohexane, n-heptane, iso-octane, in which hydrophilic polar head group (SO_3^{2-}) will interact with central core of reverse-micelle. As a result they get protected by the interaction with the bulk non-polar solvent.[26]

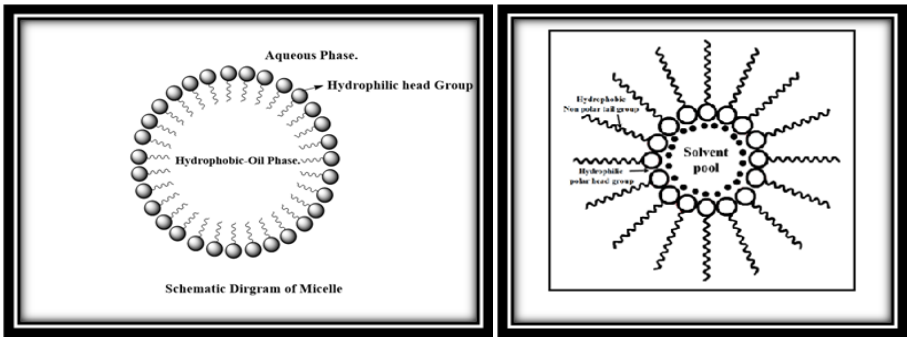

Figure 1.3: Schematic Representation of micelles and reverse micelles.

1.7 Graphene-Oxide (GO)

Graphene-Oxide, a 2D allotrope of carbon, is rapidly growing importance across various scientific fields, because of its different and unique physical and chemical properties. [27] This is perfectly a single layered sheet of graphene, in which every carbon atom is sp^2 hybridized. Graphene oxide (GO) is a functionalized graphene which is bonded with oxygen atom in the form of carboxyl, carbonyl, hydroxyl or epoxy, that imparts hydrophilic in nature. [27,28] GO is excellent solubilized in aqueous medium because of the high specific area, high chemical stability and high optical transmittance properties. This kinds of features makes GO tremendous attention from fundamental as well as from applied research point of view.[29] Furthermore, it is necessary to point out that GO and graphite oxide can be produced by same chemical process, but both are different in its structure.[30] Graphite oxide can be defined as group of layers of graphene-oxide. [31] Also we can say that graphite exfoliation will give single layered graphene oxide. Moreover in UV-Visible, GO molecule exhibited two absorption maxima. The first absorption maxima is obtained at 230 nm which is due to the π-π* transition and the second maxima is obtained around 300 nm which is due to the n-π* transition of carbonyl (C=O) group. [32] Moreover due to the large surface-area and hydrophilic character, graphene oxide has a variety of applications. It has been used as a drug delivery, exploited in cellular imaging, Catalysis, photovoltaics, antibacterial and in electrical bio sensing. [27] Inspired from various applications of GO in inter-disciplinary fields, we are interested to execute photophysical investigation of some selective flouophores confined in graphene oxide medium.

CHAPTER-2

LITERATURE REVIEW

2.1 Basic literature review on surfactant and CV and AO

D.Liu*et.al.* (1998), investigated the basis hydrolysis of crystal violet (CV) in mixed reverse micelles formed with anionic surfactant Aerosol-OT and non-ionic surfactant (Brij 30, lgepal). They reported that hydrolysis of CV caused significant modulation in the reverse-micelles (RMs) compared to that in aqueous medium. As a result, the equilibrium constant (*K*) dramatically changed in RMs. **(33)**

Ghosh *et. al.* (2012), investigated the interaction of crystal violet with different types of surfactants (like cationic, anionic and non-ionic) using steady state absorption and fluorescence emission techniques. They reported that the studied dye molecule (CV) existed as dimer form in pre-micellar concentration whereas in micellar concentration monomer form was present. According to their work, it was comprehended that the studied dye molecule (CV) interacts more strongly in anionic surfactants (due to electrostatic interaction) compared to cationic and non-ionic surfactants. It has also been found from the report that increasing the number of hydrophobic alkyl tail groups in cationic surfactants, the efficiency of interaction gradually decreases. For that reason, the binding interactions of CV gradually decreases from DTAB (n=12)→TTAB (n=14)→CTAB (n=16). In fact, CV molecule was not solubilised in CTAB micelles as well as the molecule did not exhibit interaction due to its repulsive interaction. The interaction of the studied molecule was also investigated in presence of non-ionic micelles. It was observed that in non-ionic micelles, the absorbance values of CV molecule gradually increases due to the interaction between tri-phenyl moiety of CV molecule and polyoxy ethylene sorbitan moiety of non-ionic micelles, which causes non-polar hydrophobic with solute-solvent interaction. Moreover, due to the presence of cis-double bond character of Tween 80, it becomes structurally bend and more restrict environment. As a result, CV molecule can't interact reasonably with Tween 80 surfactant. **(8)**

Feng *et. al.*(1998), investigated the binding constant and effect of energy transfer between acridine orange (AO) dye molecule with Bovine serum albumin (BSA). They executed the study by using steady state absorption and fluorescence techniques. Moreover, they studied the effect of micelle on the acridine orange association state, which confirmed that at lower concentration range of sodium dodecyl sulphate (SDS) micelles, the studied dye molecule present as a dimer state while at higher concentration range of SDS, AO molecule present as monomeric form. **(34)**

Ganguly *et. al*. (2010) demonstrated the interaction of cationic dye acridine orange (AO) in aqueous micellar dispersion of surfactants and in different polar and non-polar solvents. They studied the absorption and fluorescence spectra of AO with cationic (CTAB), anionic (SDS) and non-ionic (Triton X-100) surfactants. It was observed that interaction between AO with respective micelles caused hydrophobic interaction as well as electrostatic interaction. In binding with neutral surfactant (Triton X-100) and cationic surfactant (CTAB), AO molecule exhibited hydrophobic interactions while the studied molecule (AO) showed electrostatic interactions in presence of anionic surfactant (SDS). It was also found from the report that absorption and fluorescence characteristics properties of AO molecule are very sensitive towards the polarity of different solvents. With the increase of polarity of the solvent, stokes shift will also increases. This is due to the reason that in the more polar solvent, AO will have more solvation due to which it will be more ionized. **(35)**

The structure of AO is resemblance with the structure of lumichrome (LCM). Maity *et. al*. (2013) executed the photophysics of LCM in both aqueous (water RMs) and non-aqueous RMs (methanol, DMF, EG and glycerol RMs). They also studied the confinement of LCM in presence of respective neat solvents and demonstrated a comparative study of LCM in solvent media as well as reverse micellar media. It was found that the photophysics of neat solvents significantly dierent than that of RMs environments. On gradual increasing the solvent pool of the RMs (*w* values), LCM exhibited quenching of the fluorescence as well as bathochromic shift in their emission maxima. The fluorescence quantum yield value gradually increases with their respective '*w*' values.

Maity *et. al*. (2015) studied the photophysics of the dual behaviour of crystal violet lactone (CVL) entrapped in reverse micelles. They also concluded that with increasing 'w' values of respective RMs, the photophysical properties of the molecule significantly changed. Moreover, they also compared their results with respective neat solvents and concluded that CVL molecule confined RMs assemblies, turned to more fluorescence in nature while in neat solvents CVL molecule exhibited less fluorescence property. The structure of the studied crystal violet (CV) molecule is somewhat analogous with that of crystal violet lactone (CVL). **(36)**

2.2 Research gap

Based on the literature review the following research gaps have been identified:

(1) .CV and AO are used as biological stain in animal and veterinary medicine and can be used as antiseptic, anti-fungal, anti-bacterial. The studied molecule exhibited comparatively less solubility in aqueous medium as well as in other solvents. Therefore, the main target is to enhance its solubility property in the confined environments.

(2). Since, CV and AO molecules are non-fluorescent/less fluorescent in nature in aqueous solution and other solvents. Therefore, the objective is to improve their fluorescence properties in presence of bio-mimic confined environments such as in the presence of aqueous and non-aqueous reverse micelles. As a result, these molecules can be used for detection analysis and sensing agent in physiological system.

(3). Very limited work is reported on the photophysics of CV molecule. Only interaction between CV with different types of surfactants (cationic, anionic and non-ionic) and bile-salts were reported till date.

(4). Since, reverse micelle has excellent impact on cell membrane and also have ability to encapsulate large amount of polar solvent molecule in their hydrophilic core. But there is no literature report on the interaction of CV and AO in reverse micelles.

(5). There is no literature report that how the photophysics of CV and AO will change on interaction of different kinds of surfactant (AOT, SDS, SOS) mediated reverse micelles. (water, GO-water, methanol, GO-methanol).

To the best of our knowledge, there is no report on the photophysics of fluorophore in GO-mediated RMs.

2.2 Aim of this project

Very limited studies on interaction of CV and AO in confined environments have reported in literature.[37,38] The project work is explored mainly on spectroscopic interactions of two bio-molecules namely acridine orange (AO) and Crystal violet (CV) in research, in order to enhance the solubility as well as to modulate the fluorescence property (because CV and AO is non-fluorescent in aqueous medium). So by making CV and AO fluorescence active might be useful constructive tool for sensors (biological sensing), detection analysis in biological implications. Our aim of the project is to develop various bio-mimic confined media with graphene oxide (GO) for drug delivery application because in literature, there is no report on the photophysical exploration of chromophores confined in GO-mediated RMs. For that reason, we were interested to explore the photophysical work in this field.

CHAPTER-3

MATERIALS AND METHODS

3.1 Materials

3.1.1 Pharmaceutical compound

Crystal violet (CV) and Acridine orange (AO) was purchased from Loba Chemie and used as such as received. The purity of the compounds was more than 97%. Freshly prepared solution of CV and AO (in methanol) was used every time, in order to avoid any degradation and aggregation. The concentration of the studied solution of CV and AO was maintained at 10^{-5}(M) and10^{-6}(M) respectively throughout the experiment.

3.1.2 Different Surfactants and Chemicals used

Different anionic surfactant Aerosol-OT (Dioctyl Sodium Sulfosuccinate), SDS (sodium dodecyl sulphate), SOS (sodium octyl sulphate), Graphite powder were purchased from LobaChemie and was used as such they was received. All the surfactants used are of high purity except SDS which was purchased from Sigma-Aldrich (≥97%). Iso-octane was procured from LobaChemie. Methanol (HPLC grade) was obtained from SDFCL. **Figure 3.1**represents the structures of AOT, SOS and SDS respectively.

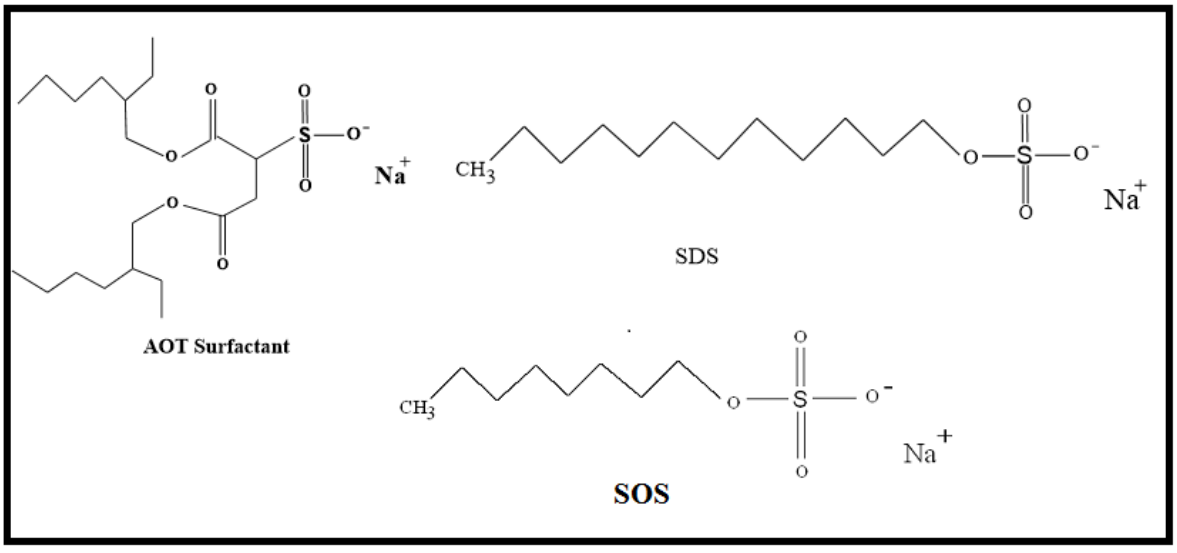

Figure 3.1: Structures of AOT, SDS and SOS.

3.2 Experimental methods

3.2.1 Preparation of Graphene Oxide (GO)

Graphene oxide (GO) was prepared by using Hummer's method. Finely powered graphite (0.5 g), $NaNO_3$ (0.5g), concentrated H_2SO_4 (23mL) were added to an Erlenmeyer flask (50 mL) and then allowed to cool this mixture in an ice bath such that the temperature of the system was below 20^0C.After cooling, the mixture was stirred continuously for 4 hours with the help of magnetic stirrer. Then, in an ice bath, 3g $KMnO_4$was added to the reaction mixture in a prolonged time. The mixture was again stirred for an hour. At this stage, distilled water (100 mL) was added in 4 slots of 25 ml volume and the entire mixture was transferred to another Erlenmeyer flask (100 mL). The mixture was then allowed to cool at room temperature followed by addition of distilled water (10 mL) with stirring. Finally, 30%of H_2O_2 (5 mL) was added drop-wise using fume hood. After this, the mixture was washed with 5% HCl and distilled water to obtain Graphene oxide (GO). The graphene oxide obtained was dried in oven overnight to get dry graphene oxide. This was stored in micro centrifuge tubes at room temperature for later use. Aqueous Graphene oxide solution was prepared by addition GO (1mg) in water (10 mL) and centrifuged for 10 minutes at 5000 rpm kept temperature 25^0C.

3.2.2Preparation of CV and AO solution

The stock solution of CV and AO was prepared in methanol solution. From the stock solution, required amount of aliquots (20 µl) was pipette out by using micro-litre pipette in a cuvette and completely dried. After that, 0.1(M) different surfactants (AOT, SOS and SDS) were added followed by the addition of 2 ml isooctane to prepare respective 'w_0'values, where, 'w_0' = [polar solvent]/[surfactant].

3.2.2.1 Preparation of AOT-H_2O/GO-H_2O RMs

In order to prepare this solution, firstly 20 µl of the dye solution was pipette out and dried for a long time, so that methanol will get evaporate. After that, the calculated amount of 0.1(M) AOT surfactant was added in isooctane (2 ml). Then calculated amount of water/ GO-water solution was added step-wise from the lower concentration of 'w_0' to higher concentration range.

3.2.2.2 Preparation of SDS-H_2O/GO-H_2O RMs

To prepare this solution, firstly 20 µl of the dye solution was pipette out in a cuvette and the solution was completely dried. After that, calculated amount of 0.1(M) SDS surfactant was added in isooctane (2 ml). Then, calculated amount of water/GO-water solution was added step-wise from the lower concentration of 'w_0' to higher concentration range.

3.2.2.3 Preparation of SOS-H_2O/GO-H_2O RMs

In a similar method, 20 µl of the studied stock solution was pipette out and dried by using drier. After that, calculated amount of 0.1(M) SOS surfactant was added in 2ml isooctane. Then, calculated amount of water/GO-water solution was added step-wise from the lower concentration of 'w_0' to higher concentration range.

The following volume of the solution (water, GO-water) was added to prepare various solvent pools (w) inside different RMs.

Table 3.1: Preparation of water RMs and GO-water RMs

System Name	*'w'=[polar solvent]/[surfactant]*	Volume of solvent added(µl)
AOT/SDS/SOS-water RMs and GO-water RMs	$w0$	0
	$w1$	3.5
	w_3	10.5
	w_5	17.5
	w_{10}	35
	w_{20}	70

3.2.2.4 Preparation of AOT-MeOH/GO-MeOH

In order to prepare this solution, firstly 20 µl of stock solution of the studied dye was pipette out and completely dried. After that, calculated amount of 0.1 (M) AOT surfactant was added in 2 ml of isooctane to prepare 'w_0'. Then, calculated amount of MeOH/GO-MeOH was addedto prepare methanol RMs such that the solvent pool of the RMs can gradually increases from lower size to higher size. Similarly, the following

volume of the solution (methanol, GO-methanol) was added to prepare various solvent pools (*w*) inside AOT-RMs.

Table 3.2: Preparation of methanol-RMs and GO-methanol RMs

System Name	*'w'=[polar solvent]/[surfactant]*	Volume of solvent added(µl)
AOT-MeOH RMs and GO-MeOH RMs	w_0	0
	w_1	8.1
	w_2	16.2
	w_3	24.3
	w_5	40.4
	w_8	64.8

3.3 Experimental Techniques

3.3.1 Ultraviolet–Visible absorption measurements

Absorbance measurements were performed on Perkin Elmer Lambda 35 UV/Vis spectrometer**(Figure 3.2)** using 1cm path length of quartz cuvette.

Figure 3.2 Perkin Elmer Lambda 35 UV/Vis spectrophotometer.

Adequate energy of light (~200 nm to ~800 nm) covering the range of UV and visible region was required as an excitation source. Henceforth, deuterium and halogen lampsare used in UV region and in visible region and are used as a light source in this absorption spectrophotometer. Also the spectra were recorded at 400-800 nm

wavelength range. **Figure 3.3** represents the simple block diagram of the UV-Vis spectrophotometer instrument. After taking the absorption, the plot of intensity of absorbance with the variation of wavelengths (in nm) is termed as absorption spectra. Then the Beer-Lambert law will represents the linear correlation between absorbance (A) and concentration of an absorbing species (c) as follow:

$$A = \log \frac{I_0}{I} = \varepsilon_\lambda .c.l$$

The parameters *'I_0'* and *'I'* represents the intensities of the incident and transmitted light respectively. The optical path length is designated by *'l'* which is kept fixed at 1cm. *'ε_λ'* is the molar extinction coefficient at a particular wavelength.

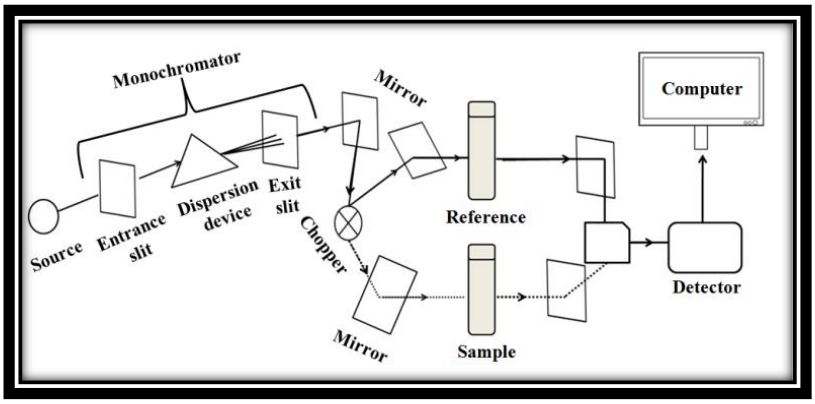

Figure 3.3: Schematic block diagram of UV-Vis spectrophotometer.

3.3.2Fluorescence measurements

Fluorescence spectra of the sample solution were recorded using Perkin Elmer LS 55 **(Figure 3.4)**fluorescence spectrometer and 1 cm path length of quartz cuvette.

The emission spectra of CV solution were measured at two different excitation wavelengths (λ_{exi}= 550 nm and 590 nm) while the emission spectra of AO solution was measured at 465 nm and 490 nm respectively. Two different excitation wavelengths were chosen because all the studied fluorophore (both CV and AO) displayed shoulder band (465 nm for AO and 550 nm for CV) followed by absorption maxima (490 nm for AO and 590 nm for CV). The emission slit widths were fixed at 5 nm and 2.5 nm respectively. The scan time was fixed at 600 nm per minute. **Figure 3.5** represents schematic diagram of spectro-fluorimeter.

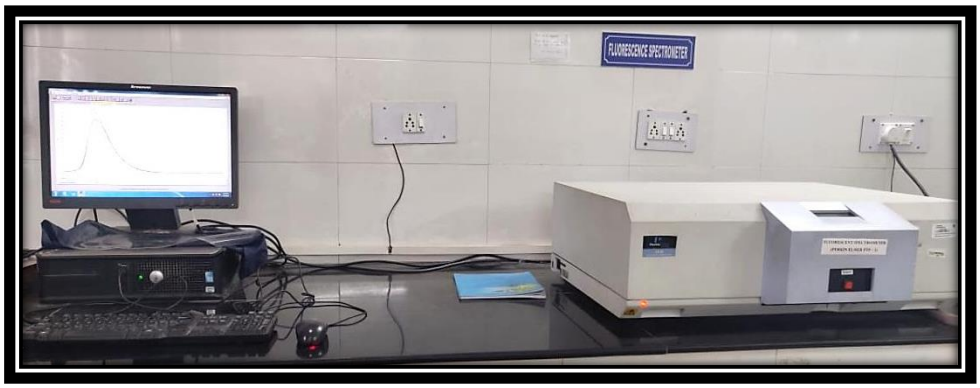

Figure 3.4: Perkin Elmer LS 55 spectrofluorimeter.

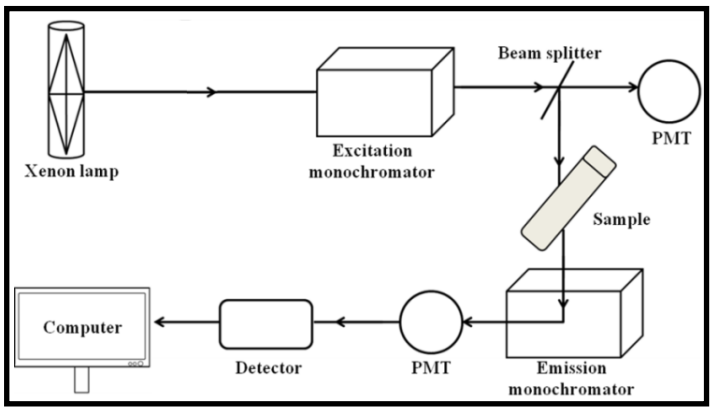

Figure 3.5: Schematic Block Diagram of spectrofluorimeter.

3.3.3 Determination of fluorescence quantum yield (Φ)

The term fluorescence quantum yield (Φ) is used to measure the ability of photon emission through fluorescence.In general, fluorescence quantum yield is often defined as the ratio of the number of photons emitted to the number of photons absorbed, which gives the idea about the nature of the fluorescence property of the fluorophore. The fluorescence quantum yield values are calculated from the fluorescence emission intensity (integrated area) and the absorbance value at the particular wavelength of excitation. The fluorescence quantum yield can be mathematically expressed as: [39]

$$\Phi_S = \Phi_R \times \left(\frac{A_S}{A_R} \times \frac{(Abs)_R}{(Abs)_S} \times \frac{n_S{}^2}{n_R{}^2}\right)$$

Where, 'Φ_S' represents the fluorescence quantum yield of studied dye molecules (CV) and AO) and 'Φ_R' represents the fluorescence quantum yield of reference (quinine sulphate), '*Abs*' denotes absorbance, '*A*' denotes the area under the fluorescence emission, '*n*' is the refractive index of the solvent used. Quinine sulphate solution in 0.1 (N) was used as the reference solution (Φ_R = 0.546).[40]

CHAPTER-4

RESULTS AND DISCUSSIONS

4.1 Steady-state absorption studies

4.1.1 CV molecule in solvents (water and GO-water)

In polar solvents (water and methanol), CV molecule showed absorption maxima at 590 nm followed by a shoulder band at 550 nm. Lucke *et al.*[8] suggested that the emergence of the shoulder band was due to the formation of dimeric structure. Later Garcia Rio et al. [41] observed that two shoulder bands are due to the presence of two ground state isomers formed in aqueous medium. One is pyramidal or distorted form (C_3- symmetry) and second one is propeller form. As a result it is confirmed that in polar solvent CV molecule exist in two isomeric forms. Moreover, the absorption as well as the wavelength of CV molecule gets dramatic change in reverse micelles with the variation of their *'w'* values. Therefore, it can be stated that the photophysical properties of CV molecule significantly changes and senses the environment of the medium.

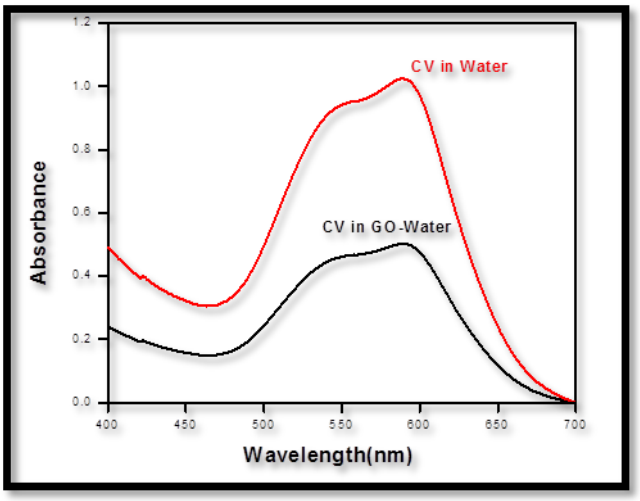

Figure 4.1: UV-Vis absorption of CV in water and GO-water.

In GO water solvent, CV molecule exhibited two bands which appeared at 550 nm and 588 nm respectively. The absorption spectra of CV molecule behave differently in aqueous suspension of GO medium compared to that of neat water medium. The absorbance of CV

molecule significantly quenched (50%) in GO-water medium compared to that of neat water **(Figure 4.1)**. The decrease of the absorbance in ground state can be rationalized on the basis that CV molecule possessed cationic dye whereas GO also behave as electron-deficient in character. As a result, due to repulsive interaction, the absorbance value markedly quenches.

4.1.2 CV molecule in AOT- water RMs

The absorption spectra of the CV molecule get dramatic change in AOT-isooctane mixture (w_0). CV molecule exhibited significant bathochromic shift as well as enhancement of the absorbance values. In AOT-isooctane mixture, the absorbance maxima of CV molecule exhibited two prominent peaks at 516 nm and 602 nm respectively. In neat water, CV molecule showed absorption maxima at 590 nm and the shoulder band at 550 nm respectively. This kind of the significant shift in absorption spectra indicated that the studied molecule (CV) is quite hydrophobic as well as cationic in nature. AOT surfactant is also anionic in nature and isooctane is non-polar medium. Therefore, it can be assumed that CV molecule confined in non-polar microenvironment, causes dye-surfactant electrostatic interaction as well as hydrophobic interaction. As a result, the solubility of the dye molecule significantly enhances which results modification in the absorption spectra.

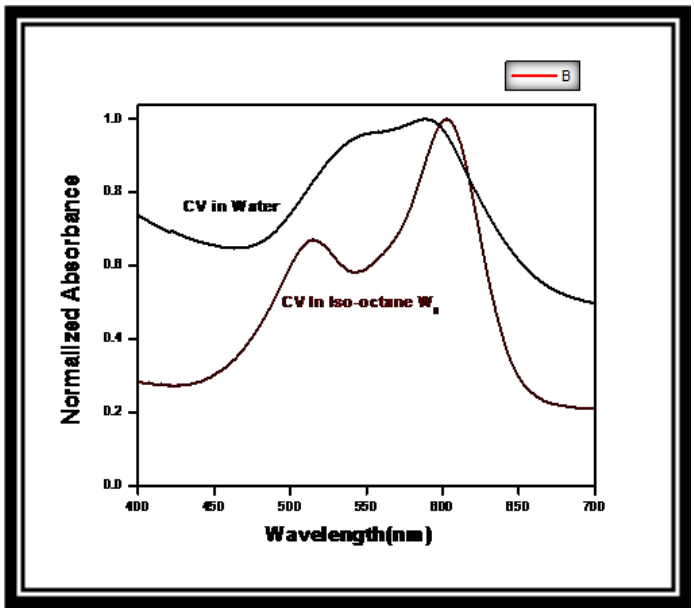

Figure 4.2: UV-Vis absorption of CV in water and AOT-isooctane mixture (w_0).

In AOT and iso-octane medium at *'w_0'* the studied dye molecule gives two prominent band at 516 and 603 nm with the significant increase in absorbance values. The absorbance value gets significantly increased because of the hydrophobic interaction between CV and Non polar isooctane medium. The following **Figure 4.2** shows the absorption spectra of CV in water only and CV in water RM medium. Moreover with gradual addition of *'w'* values from lower *'w_0'* to higher *'w_{30}'*, the absorption value of CV molecule increased along with the hypsochromic shift in the absorption maxima **Figure 4.3**.

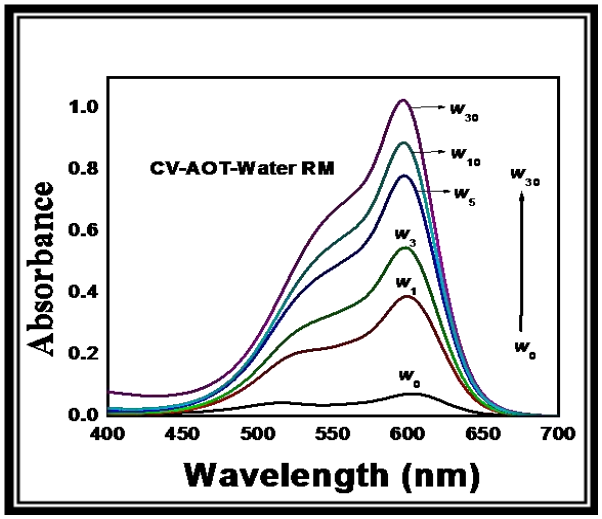

Figure 4.3: UV-Vis absorption of CV in AOT-water RMs.

4.1.3 CV molecule in AOT-GO-water RMs

It was previously described that CV molecule showed two absorption bands at 550 nm and 588 nm respectively. But, the same dye molecule (CV) present in GO-water RMs, results significant modulation in the absorption spectra. CV molecule confined in GO-water RMs showed two prominent absorption bands at 515 and 604 nm respectively. Moreover, the absorbance value significantly enhances **(Figure 4.4)**. With increase in '*w*' values of the GO-water RMs, hypsochromic shift was observed along with enhancement of the absorbance. It was observed that the absorbance value ~4 times increases in GO-water RMs, which indicated that in RMs media, the dye molecule gets more solubilized compared to neat GO-water solvent. On a closer look, compared the results of water RMs, it was observed that the absorbance value of CV molecule ~14 times enhances in water RMs with increasing the

solvent pools of the RMs. The hypsochromic shift in the absorption maxima of CV in both the AOT based RMs (water RMs and GO-water RMs) was observed. This can be rationalized on the basis that since the studied dye molecule (CV) possessed hydrophobic in nature and the RMs medium also have hydrophobic micro-environment due to presence of isooctane solvent. Therefore, in the ground state, hydrophobic interaction plays a major role, which causes hypsochromic shift and increases the solubility of CV molecule. As a result, the absorbance value enhanced. **Figure 4.4** represents the absorption spectra of CV molecule in GO-water RMs.

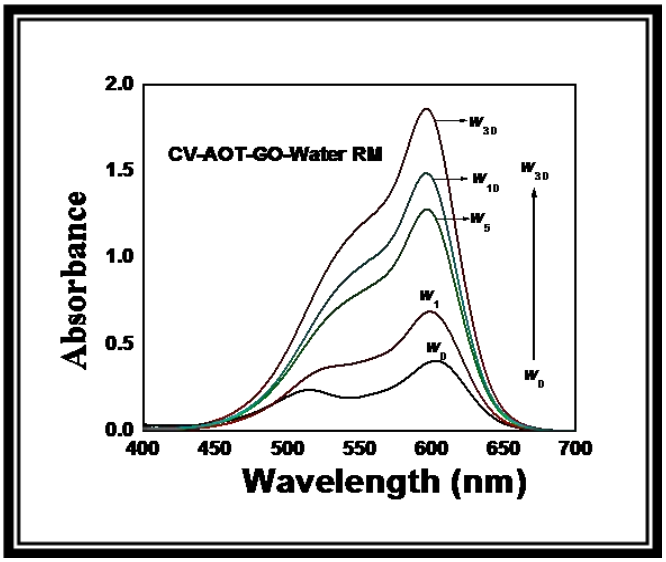

Figure 4.4: UV-Vis absorption of CV in AOT-GO-water RMs.

4.1.4 CV molecule in SDS-Water RMs and SDS-GO-water RMs

In order to know the effect of anionic surfactant, the absorbance of CV molecule was recorded in presence of SDS-isooctane mixture. With increasing the 'w_0' value in both the water and GO-water RMs, the absorbance value subsequently enhances along with the hypsochromic shift in the absorption maxima. Similar kind of observations (like AOT RMs) was also found. In water RMs and GO-water RMs at higher 'w_0' values ('w_0' = 30), the absorbance value increases ~4.3 folds and ~1.9 folds respectively compared to SDS-isooctane mixture. **Figure 4.5** represents the absorption spectra of CV molecule in SDS based water RMs and GO-water RMs.

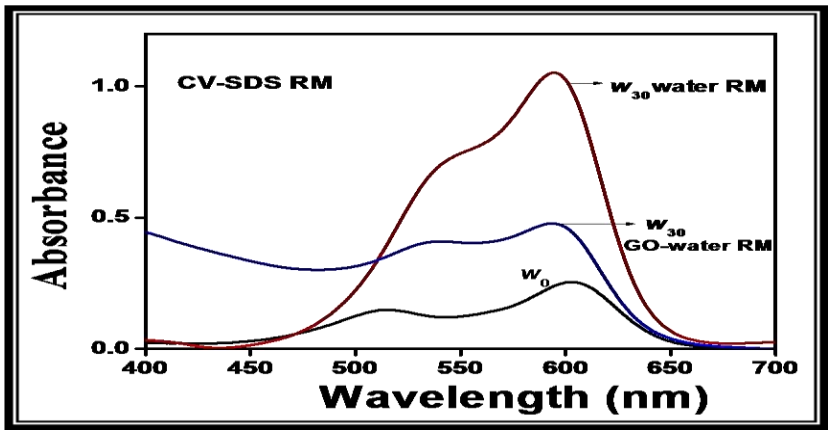

Figure 4.5: UV-Vis absorption of CV in SDS-water RMs and SDS-GO-water RMs.

4.1.5 CV molecule in SOS-Water RMs and SDS-GO-water RMs

Similarly, the absorbance of CV molecule was also recorded in presence of SOS-isooctane mixture. With increasing the 'w_0' value in both the water and GO-water RMs, the absorbance value subsequently enhances along with the hypsochromic shift in the absorption maxima (similar phenomenon like AOT and SDS). In water RMs and GO-water RMs at higher 'w_0' values ('w_0' = 30), the absorbance value increases ~1.9 folds and ~1.3 folds respectively compared to SDS-isooctane mixture. **Figure 4.6** represents the absorption spectra of CV molecule in SOS based water RMs and GO-water RMs.

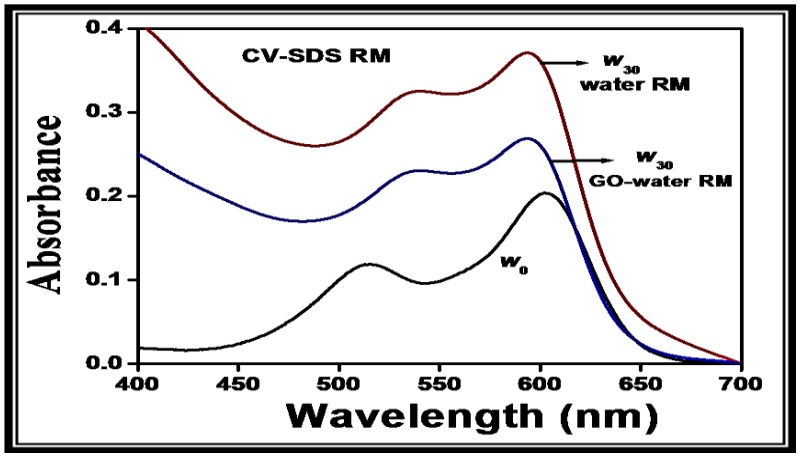

Figure 4.6: UV-Vis absorption of CV in SOS-water RMs and SOS-GO-water RMs.

4.1.6 AO molecule in solvents (water and GO-water)

In aqueous solution, the absorption maxima of AO molecule appeared at 465 and 490 nm respectively, which was due to the formation of dimer and monomer form of AO respectively. But in case of GO-water medium, only one absorption band was observed at 488 nm. (Figure 4.7). Moreover, the absorbance of AO molecule in GO-water medium significantly quenched. this reduced wavelength will indicates that the repulsive interactions were take place between cationic dye molecule(AO) and partial positively charged GO-water medium.

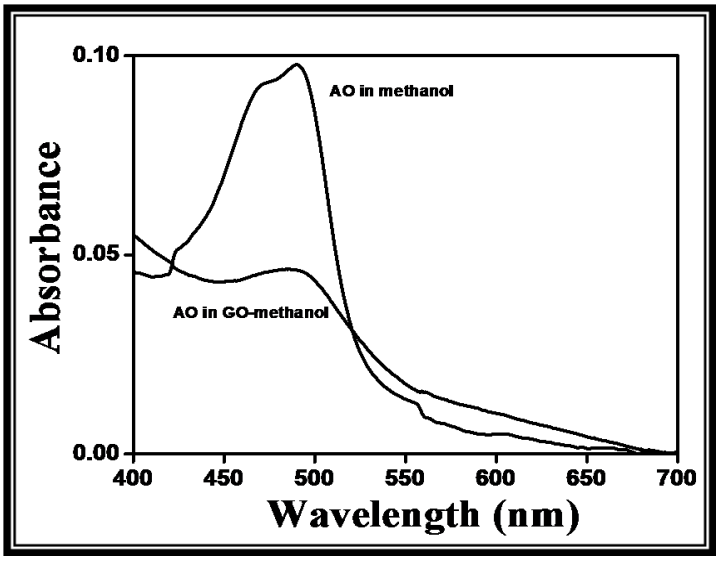

Figure 4.7: UV-Vis absorption of AO in water and GO-water.

4.1.7 AO molecule in AOT-water RMs

In AOT-isooctane mixture (w_0), AO molecule exhibited absorption maxima at 488 nm only, which is due to the monomer form. With gradual increase of the *'w_0'* values (from w_1 to w_{30}), the absorbance values gradually increases along with the bathochromic shift in the absorption maxima (Figure 4.8). The absorbance value of AO molecule ~13 times increases at higher water pool inside the water RMs. This result clearly indicated that increasing the micro polarity of the medium, the dye molecule senses more polar environment.

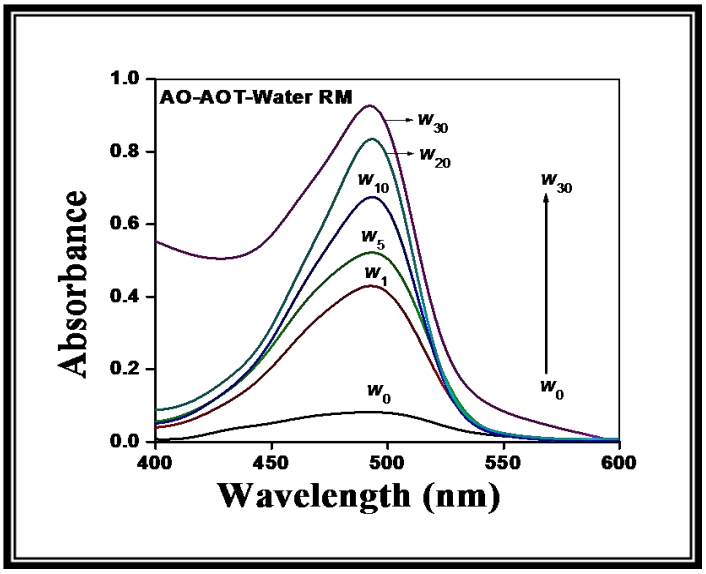

Figure 4.8: UV-Vis absorption of AO in AOT-water RMs.

4.1.8 AO molecule in AOT-GO-water RMs

With gradual increase of the *'w_0'* values (from w_1 to w_{30}), the absorbance values gradually increases along with the hypsochromic shift in the absorption maxima (Figure 4.9). The absorbance value of AO molecule ~3 times increases at higher water pool inside the GO-water RMs. This result clearly indicated that increasing the micro polarity of the medium, due to repulsive/hydrophobic interaction, hypsochromic shift was observed.

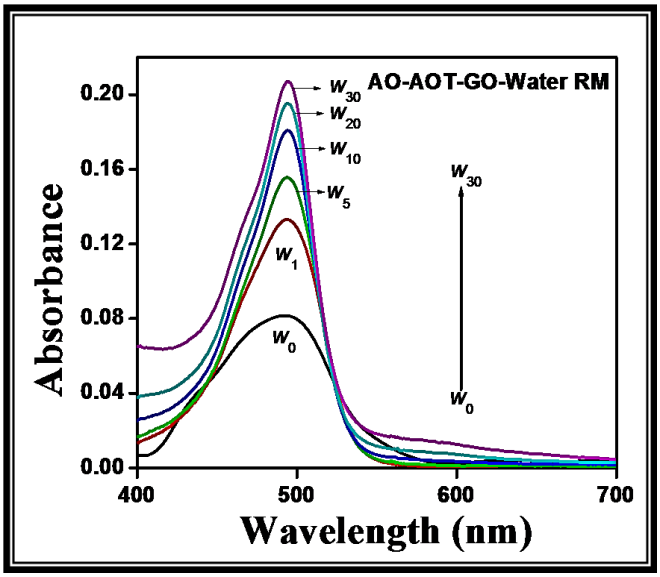

Figure 4.9: UV-Vis absorption of AO in AOT-GO-water RMs.

4.1.9 AO molecule in AOT methanol RMs and GO-methanol RMs

In case of non–aqueous RMs, similar kind of observation was also found. AO molecule confined in AOT-methanol RMs and AOT-GO-methanol RMs, exhibited enhancement of the absorbance values. But, the enhancement of the absorbance value in case of methanol RMs is more compared to GO-methanol RMs at higher *w* values. In case of methanol RMs and GO-methanol RMs, the increment of absorbance values of AO molecule was found to be 1.5 times and 1.1 times respectively (Figure 4.10). Moreover, with increase of the solvent pool of the RMs (*w*), AO molecule confined in methanol RMs exhibited bathochromic shift while in GO-methanol, hypsochromic shift was observed. Figure 4.9 shows the absorbance plot of AO in methanol and GO-methanol RMs respectively.

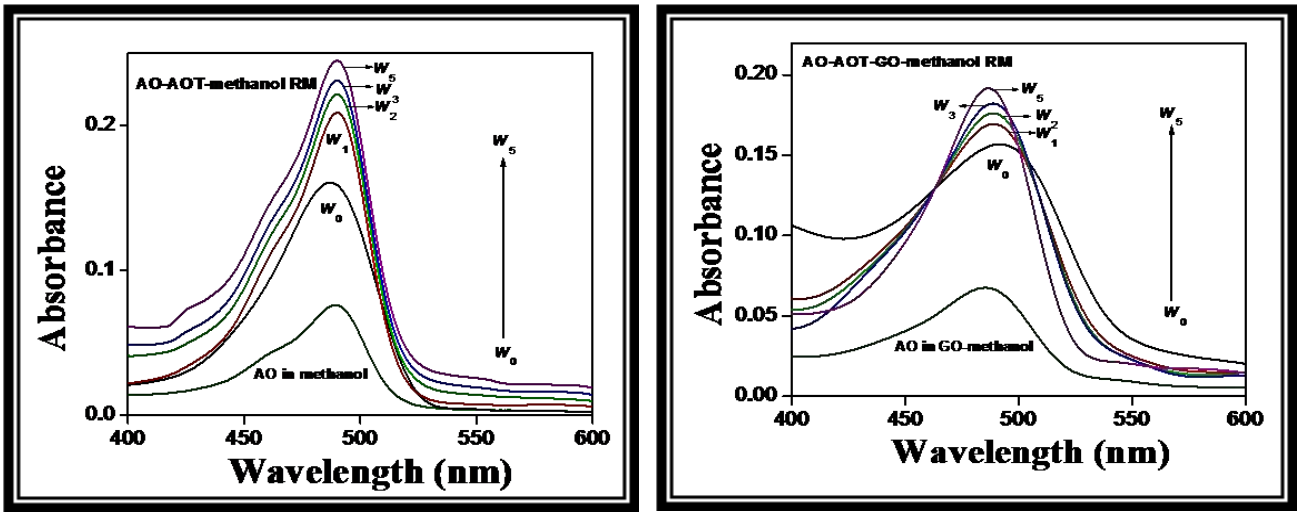

Figure 4.10: UV-Vis absorption of AO in AOT-methanol RMs and AOT-GO-methanol RMs.

4.2 Steady-state fluorescence studies

4.2.1 CV molecule confined in water RMs and GO-water RMs

In aqueous medium, CV molecule showed unstructured emission band and the remained as non-fluorescent in nature at both the excitation wavelengths. But in AOT-isooctane medium (w_0), the emission intensity significantly enhanced. In the case of both water RMs and GO-RMs gradual increasing the 'w_0' values, the fluorescence intensity significantly

enhanced along with the hypsochromic shift of the emission band (Figure 4.11) was observed. On a closer analysis, it was also found that the extent of the enhancement of fluorescence intensity of CV confined in water RMs is comparatively more than that in GO-water RMs. Similar kinds of observations were also found in SDS-isooctane RMs (Figure 4.12) and SOS-isooctane RMs (Figure 4.13).

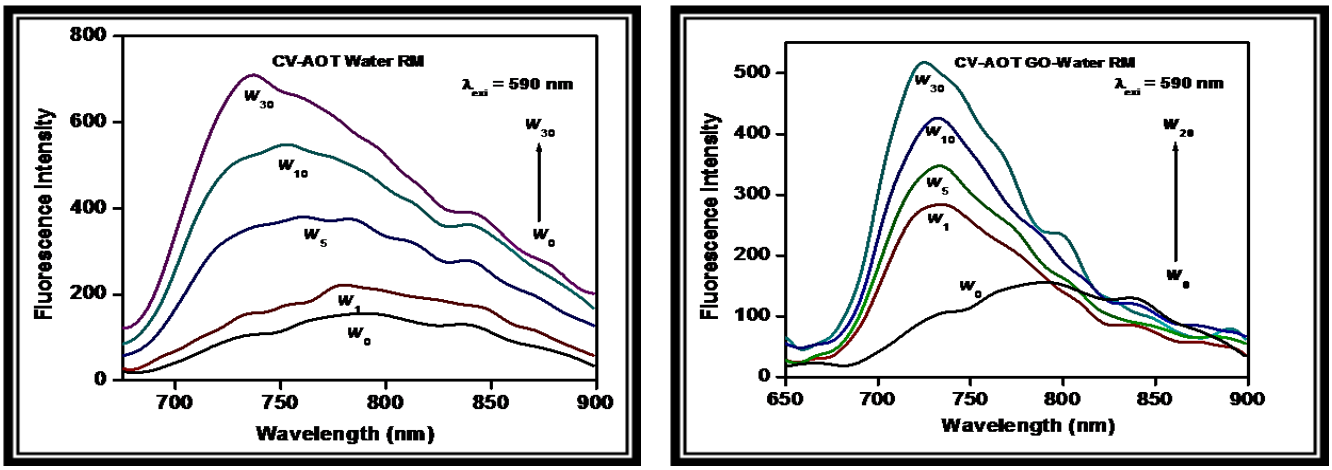

Figure 4.11: Fluorescence emission of CV in AOT-water RMs and AOT-GO-water RMs.

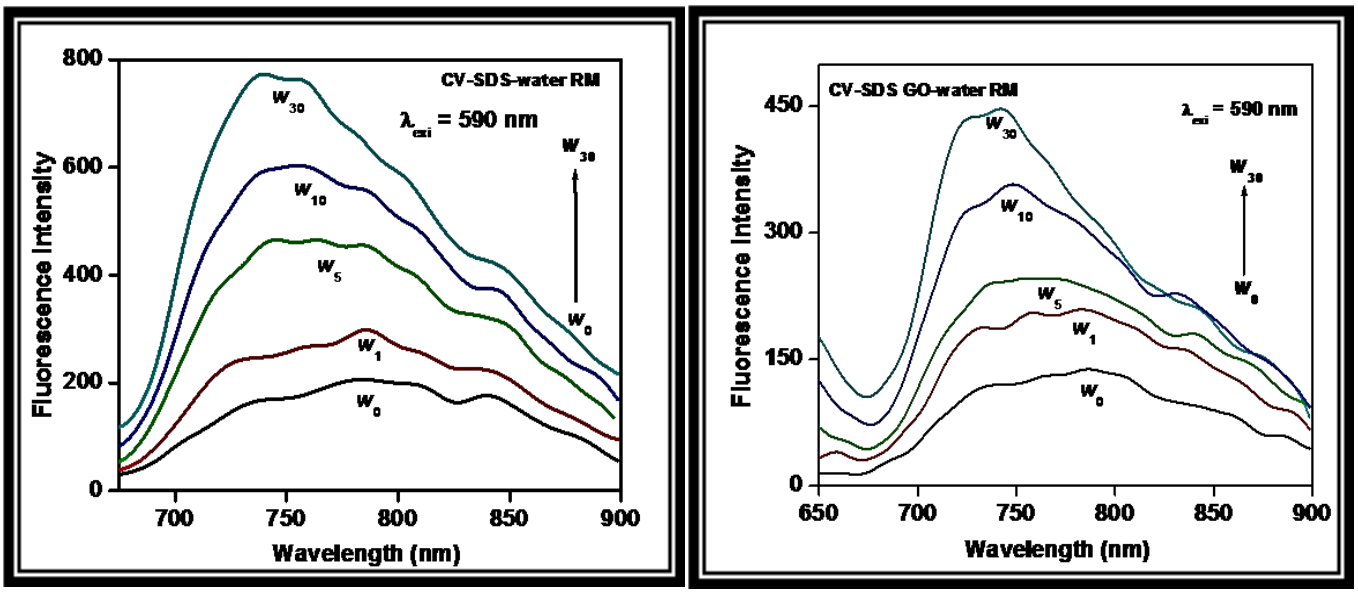

Figure 4.12: Fluorescence emission of CV in SDS-water RMs and SDS-GO-water RMs.

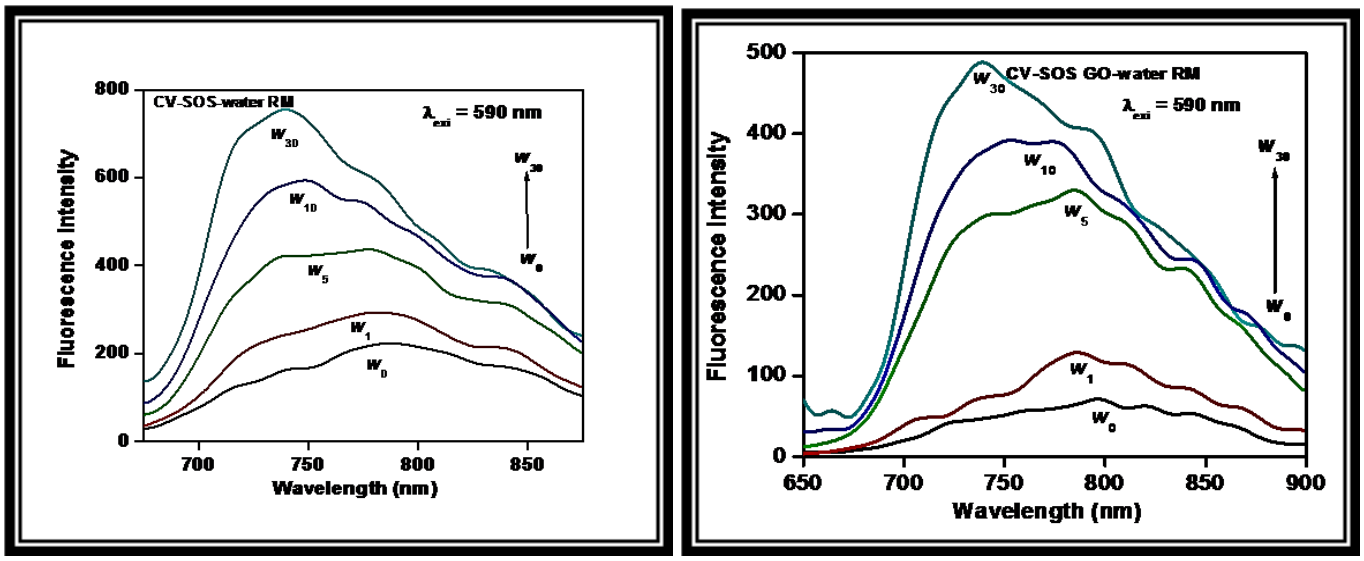

Figure 4.13: Fluorescence emission of CV in SOS-water RMs and SOS-GO-water RMs.

4.2.2 AO molecule confined in water RMs and GO-water RMs

In aqueous solution, the studied molecule (AO) showed the emission maxima at 530 nm. The nature of the emission spectra of AO molecule significantly changed in AOT-isooctane medium. The emission intensity significantly enhanced along with the bathochromic shift in the emission maxima (Figure 4.14).

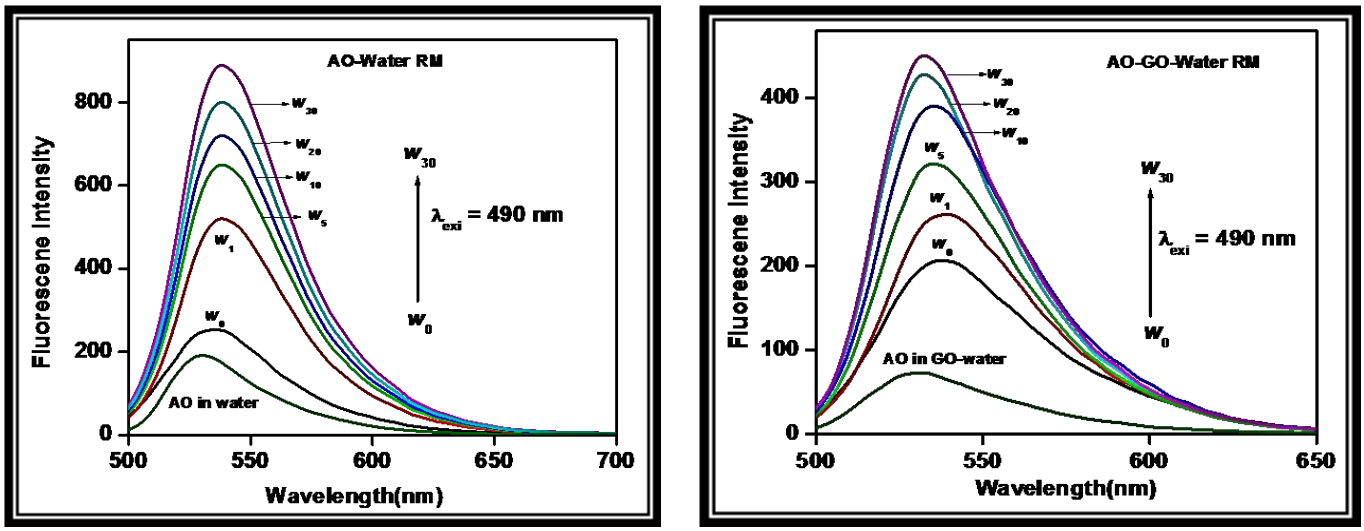

Figure 4.14: Fluorescence emission of AO in water RMs and GO-water RMs.

Moreover, it was also found that with increasing the solvent pool of the RMs, the emission intensity of AO gradually enhanced in both the systems (water RMs and GO-water RMs). But the extent of the fluorescence enhancement in water RMs (~4 times) is comparatively more than GO-water RMs (~2.1 times). In case of water RMs, bathochromic shift in the emission maxima observed compared to neat water medium, whereas hypsochromic shift was observed in case of GO-water RMs. This result clearly depicted that specific solute-solvent interactions as well as hydrogen bonding interactions have predominant role for exhibiting strong bathchromic shift with increase of the solvent pool inside water RMs, while the presence of GO in aqueous RMs repels the fluorophore as well as hydrophobic interactions also took place, which caused hypsochromic shift in the emission spectra.

4.2.3 AO molecule confined in methanol RMs and GO-methanol RMs

Similar kind of emission behaviour was also observed in case of methanol RMs and GO-methanol RMs. The fluorescence emission of AO present in GO-methanol medium significantly quenched compared to neat methanol medium. But, the studied dye molecule present in their respective RMs exhibited strong fluorescence property along with the shift of their emission maxima (Figure 4.15).

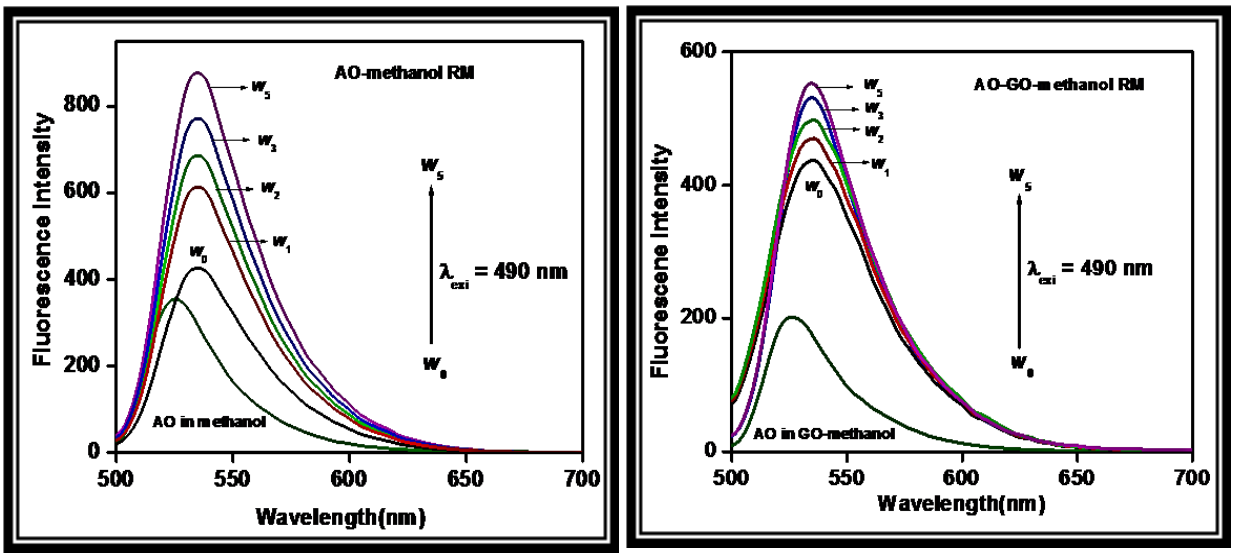

Figure 4.15: Fluorescence emission of AO in methanol RMs and GO-methanol RMs.

In both the systems, it was found that increasing the size of the solvent pools of the respective RMs, the fluorescence intensity of the studied fluorophore (AO) gradually enhanced. It was also found from the figure that the extent of enhancement of the fluorescence intensity of AO confined in methanol RMs is more compared to GO-methanol RMs. Moreover, the bathochromic emission shift of AO molecule confined in methanol-RMs indicated that in specific solute-solvent and hydrogen bonding interactions occurred inside the microenvironment, whereas hypsochromic emission shift of AO confined in GO-methanol RMs indicated repulsive and hydrophobic interactions were present in the microenvironment.

4.3 Fluorescence quantum yields of CV and AO molecules in different systems

The fluorescence quantum yield values of CV molecule in different systems were calculated and tabulated in Table 4.1. In neat water and aqueous GO medium, CV molecule existed as non-fluorescent in nature. Moreover, on a very closer analysis, it was observed that the fluorescent quantum yield of CV in GO-water quenched ~3 times compared to neat water. Astonishingly, it was observed that in different-surfactant based RMs, the fluorescence quantum yield of CV molecule ~100 times increased compared to that of solvent systems. This result clearly indicated that depending on the nature of the microenvironment, the fluorescence property of CV molecule significantly changed. In other words, it can be stated that the non-fluorescent property of the CV molecule turns into highly fluorescent in nature inside RMs. Moreover, it was also found that the fluorescence quantum yield of CV molecule entrapped in water-RMs is significantly high compared to GO-water RMs (varying the nature of the surfactants like AOT, SDS and SOS). In presence of water RMs, with increasing 'w_0' values the bathochromic shift in the emission spectra clearly depicted that CV molecule migrates towards the water pool inside the water-RMs, whereas the hypsochromic emission shift of the emission maxima of CV molecule confined in GO-water RMs indicated that the dye molecule migrates towards the interfacial region of the RMs at higher 'w_0' values. Since, GO have electron-deficient and hydrophobic nature on its surface. Therefore,

due to repulsive interaction and hydrophobic interaction, the quantum yield of CV molecule quenched in GO-water RMs compared to water RMs.

Table 4.1: Fluorescence quantum yield values (Φ) of CV in different systems

System	Φ_{550nm}	Φ_{590nm}
CV molecule in solvents (water and GO-water)		
CV (10^{-5} M) in water	6×10^{-4}	6×10^{-4}
CV (10^{-5} M) in GO-water	2×10^{-4}	2×10^{-4}
CV molecule in AOT-RMs (water RMs and GO-water RMs)		
CV (10^{-6} M)+AOT+isooctane (w_0)	1.8×10^{-2}	1.6×10^{-2}
CV (10^{-6} M)+AOT+H_2O RMs (w_{30})	5.6×10^{-2}	5.3×10^{-2}
CV (10^{-6} M)+AOT+GO-H_2O RM (w_{30})	3.1×10^{-2}	3.0×10^{-2}
CV molecule in SDS-RMs (water RMs and GO-water RMs)		
CV (10^{-6} M)+SDS+isooctane (w_0)	1.2×10^{-2}	1.1×10^{-2}
CV (10^{-6} M)+SDS+H_2O RMs (w_{30})	3.4×10^{-2}	3.3×10^{-2}
CV (10^{-6} M)+SDS+GO-H_2O RM (w_{30})	2.0×10^{-2}	1.8×10^{-2}
CV molecule in SOS-RMs (water RMs and GO-water RMs)		
CV (10^{-6} M)+SOS+isooctane (w_0)	7×10^{-3}	7×10^{-3}
CV (10^{-6} M)+SOS+H_2O RMs (w_{30})	2.3×10^{-2}	2.1×10^{-2}
CV (10^{-6} M)+SOS+GO-H_2O RM (w_{30})	1.2×10^{-2}	1.1×10^{-2}

Similarly, the fluorescence quantum yield values of AO molecule in different solvents and RMs were also evaluated and tabulated in Table 4.2. From the Table, it was observed that the fluorescence quantum yield values of AO molecule noticeably quenched in presence of GO-water and GO-methanol compared to that in neat solvents medium. This result clearly depicted that aqueous suspension of GO acts as a quenching agent. The fluorescence quantum yields of AO molecule significantly enhanced confined in RMs. It was also noticed

that increasing the size of the solvent pools of both the aqueous and non-aqueous RMs, the fluorescence quantum yield values of AO molecule significantly enhanced. Moreover, it was also found that the fluorescence quantum yield values of AO are significantly less in GO-based aqueous and non-aqueous RMs.

Table 4.2: Fluorescence quantum yield values (Φ) of AO in different systems

System	Φ_{465nm}	Φ_{490nm}
AO molecule in solvents (water, GO-water, methanol and GO-methanol)		
AO (10^{-6} M) in water	0.15	0.17
AO (10^{-6} M) in GO-water	0.08	0.06
AO (10^{-6} M) in methanol	0.25	0.28
AO (10^{-6} M) in GO-methanol	0.12	0.11
AO molecule in AOT-RMs (water RMs and GO-water RMs)		
AO (10^{-6} M)+AOT+isooctane (w_0)	0. 38	0.39
AO (10^{-6} M)+AOT+H_2O RMs (w_{30})	0.74	0.76
AO (10^{-6} M)+AOT+GO-H_2O RM (w_{30})	0.45	0.48
AO molecule in AOT-RMs (methanol RMs and GO-methanol RMs)		
AO (10^{-6} M)+AOT+methanol RMs (w_{30})	0.65	0.66
AO (10^{-6} M)+AOT+GO-methanol RM (w_{30})	0.43	0.45

CHAPTER-5

CONCLUSION

In conclusion, the thesis describes the photophysical interactions of two different cationic dyes (CV and AO) confined in different surfactant based RMs (AOT, SDS and SOS) and their comparative study in presence of GO. From steady state absorption spectroscopy and fluorescence emission studies, the solubility and fluorescence property of the studied molecules confined in RMs was significantly improved. As a result, the studied molecule exhibited strong non-covalent interactions inside the RMs assemblies. As a result, the studied fluorophores showed remarkable enhancement in absorption, fluorescence emission as well as fluorescence quantum yield values. This result indicated that the non-fluorescent molecules turns into highly fluorescent in nature inside bio-mimicking RMs assemblies. Moreover, the role of the GO in aqueous medium was also explored. Presence of GO in RMs assemblies caused quenching in absorption, fluorescence emission and the fluorescence quantum yield values. This result clearly interpreted that GO acts as quencher and have ability to release the studied molecules from the RMs assemblies. For that reason, the highly fluorescent nature of the molecules again turns into non-fluorescent in presence of GO. Therefore, the present work may be applicable for drug-delivery vehicles, fluorescent sensing agents in physiological systems.

CHAPTER- 6

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