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A Study of the Effects of Titanium Dioxide Nanoparticles on the Fluorescent Intensity of Fluorescent Compounds in the Presence of Known Quenchers.

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ABSTRACT

A Study of the Effects of Titanium Dioxide Nanoparticles on the Fluorescent Intensity of Fluorescent Compounds in the Presence of Known Quenchers

by

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Titanium Dioxide is a naturally occurring oxide of titanium. It has a wide range of uses in commercial products for providing whiteness and opacity. It has photocatalytic properties and can also be used to produce electricity in its nanoparticles form. This research is focused on investigating the effect of titanium dioxide nanoparticles in analysis of compounds using luminescence-based techniques. Quenching, which is one of the basic problems of fluorescent measurements, was studied in the presence of molecular oxygen and methyl iodide. The rutile phase of titanium dioxide nanoparticles was synthesized by the acid hydrolysis of titanium isobutoxide at low temperatures with nitric acid. The crystalline powder was dissolved at different concentrations and used to monitor the fluorescence intensities of carbazole, pyrene, and fluoranthene in the presence of methyl iodide and oxygen. Quenching by molecular oxygen was studied by comparing the fluorescence intensities of compounds with and without degassing the solutions. Titanium Dioxide was found to exhibit interesting effects on the fluorescent intensities of these compounds in the presence of quenchers.

DEDICATION

To God Almighty who made this possible, and to my beloved family.

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CHAPTER 1

INTRODUCTION

Luminescence is the name given to three related optical methods: fluorescence, phosphorescence, and chemiluminescence. Luminescence techniques are widely used in the analysis of many compounds in recent years. Their diverse application in other fields has made luminescence methods almost indispensible. Luminometry has several advantages over other analytical techniques. Excellent sensitivity and selectivity makes luminescence very popular. Luminescence is 100,000 more sensitive than absorption spectroscopy. A wide dynamic range and inexpensive instrumentation are also some of features that make this technique highly recognized. The technique is classified according to the means by which energy is supplied to excite the luminescent molecule. This technique is hence an excellent way to study unique properties of compounds.

Fluorescence

Molecules excited by interactions with photons of electromagnetic radiation give rise to fluorescence. This normally occurs when the release of electromagnetic radiation is from the singlet energy state. Every fluorescent molecule has two distinctive spectra; the excitation spectrum and the emission spectrum. The fluorescent spectrum of a compound results from the reemission of radiation absorbed by the molecule. Fluorometric methods can detect concentration of substances as low as one part in 10 billion, a sensitivity 1000 times greater than most spectrophotometric methods. High sensitivity is however a result of direct measurement of emitted radiation. Hence a zero background signal is measured, which is not the cases in spectrophotometric methods, as their measurements are done indirectly as a difference between incident and transmitted beams. Fluorescence measurements are also very specific. This is mainly because very few compounds fluoresce compared to absorbing ones. This is however because not all compounds that absorb radiation essentially emit (1). Fluorescence is a short-lifetime phenomenon with its emission occurring between the same states with similar multiplicities. The primary demerit of fluorescence as an analytical technique is its serious dependence on environmental factors, such as temperature, concentration, and pH. The

fluorescent lifetime of most fluorophores is about 10^{-9}s. Within this range collisional interactions also occur around 10^{-4}-10^{-3} M. Thus collisional deactivation of the excited state is mostly a competitive process at room temperature. Other competing deactivation processes mostly known as radiationless transitions also lead to reduction of fluorescence. Vibrational relaxation, and internal conversion are the results of the collisional interactions that lead to fluorescence quenching.

Phosphorescence

This is the delayed emission of light after the absorption of light. A change in electronic spin accompanies phosphorescence, with longer excited state. One unique feature of phosphorescence is the persistence of the emitted radiation after the excitation beam is removed, which is not the case with fluorescence. Phosphorescence is hence associated with the phenomenon of "after glow". The delayed time scale of re-emission is due to the forbidden energy state transitions in quantum mechanics. Phosphorescence, just as fluorescence, adds to one of the wonders of nature. Humans have taken advantage of this wonder and created items for vital use and entertainment, some of which are glow sticks for parties or matching bands, highway exist signs, pathway markings, and glow sticks used for military purposes amongst others (2-4).

Chemiluminescence and Bioluminescence

Production of visible illumination by a chemical reaction is called Chemiluminescence. Such chemical reactions occurring in living organisms are called bioluminescence. Three conditions are normally required for Chemiluminescence to occur; the chemical reaction must release sufficient energy to populate an excited energy state; the reaction pathway must favor the formation of an excited state complex; and the excited state product must be capable of emitting a photon itself or transferring its energy to another molecule that can emit Chemiluminescence occurs when an energetic (exothermic) reaction produces a molecule in an electronically excited state. That molecule, as it returns to the ground state, releases its energy as a photon of light. The rate of production of light and concentration of chemiluminescent molecule, often coupled to concentration also of a catalytic reagent, imposes limits on the amount of time that this luminescence can be usefully observed from a sample volume. (5) Some samples will generate

a relatively bright signal for a short period of time (until the entire chemiluminescent reagent is used up); others will yield a weaker signal over a longer period. In some occurrences the excited species is the product of a reaction between an analyte and a suitable reagent, mostly strong oxidants. These normally result in an emission spectrum characteristic of the oxidation product of the analyte or the reagent rather than the analyte itself. However, in other instances the analyte is not directly involved in the reaction. Instead, the analyte inhibits or has a catalytic effect on the chemiluminescence reaction. Technically the chemical reactions results in an electronically excited species that emits a photon in order to reach the ground state. Chemiluminescence and bioluminescence methods are highly sensitive, hence offer important advantages for chemical analysis. It is very easy to measure low levels of light emission using these techniques and the calculation of the theoretical detection limits from the efficiency of chemiluminescence is quite simple.

Currently developed methods for atmospheric ozone and nitric oxide provide a good example of the advantages of chemiluminescence generating reactions for chemical analysis. Ozone can be determined either by its reaction with rhodamine-B adsorbed on an activated silica gel surface or by its gas phase-phase reaction with ethylene. The rhodamine-B method however has all the advantages of Chemiluminescence that are linear response up to 400ppb, sensitivity up to less than 1-ppb, simple instrumentation in the form of a gas flow system for pulling sample over the surface and a photomultiplier tube to measure Chemiluminescence intensity. This method however needs frequent re-calibration as the sensitivity of the chemiluminescence surface changes with time as the rhodamine is consumed.

Nitric oxide can be determined determined by involment of ozone in the chemiluminescence reaction;

$$NO+O_3 \rightarrow NO_2^* + O_2 \qquad (1)$$

$$NO_2^* \rightarrow NO_2 + hv \qquad (2)$$

As shown in the reactions 1and 2, the total oxides of nitrogen ($NO+NO_2$) is determined by reducing NO_2 to NO with carbon before reacting with ozone. The NO_2 concentration is equal is equal to the difference between the total oxides of nitrogen and the NO concentration.

Bioluminescence is an extensively dispersed phenomenon in nature, mostly found in marine organisms. The most widely studied bioluminescent orgamisms are the firefly, marine bacteria such as Beneckea harveyi and Photobacterium fischeri, the comb jelly Renilla, and the jelly fish Aequorea. Studies of other organisms are limited by their availability. Some of these contain bioluminescent substances that have considerable analytical potentials, for instance the Pholas dactylus. Their scarcity coupled with their complex nature of extraction and purification of the active bioluminescent substances has not allowed the serious consideration of such substances as reagents for routine analysis. Rapid growth in genetic engineering techniques is leading to the reappraisal of these bioluminescent substances as they could be prepared in large amounts by such techniques in theory. In the firefly, an enzyme called luciferase triggers a reaction that energy emitted as light, normally a flashing beacon from the insects lower abdomen. Fireflies reactions below;

$$\text{Luciferase}+LH_2+\text{MgATP}\rightarrow\text{luciferase: } LH_2\textit{: AMP}+\text{Pyrophosphate} \quad (3)$$

$$\text{Luciferin: } LH_2\text{AMP}+O_2 \rightarrow \text{Oxyluciferin} +CO_2 +\text{AMP}+\text{Light.} \quad (4)$$

In equation 3 and 4, firefly luciferase catalyses the ATP-dependent oxidation of D-luciferin (LH_2). Initially a luciferase:luciferyl adenylate (LH_2:AMP) complex is formed and this reacts with oxygen to produce oxyluciferin(the emitter), carbon dioxide, AMP, and light. Firefly luciferase is prepared by extraction of firefly tails and purified by crystallization. (6)

Luciferin is also contained in the tails of fireflies but is more conveniently obtained by chemical synthesis. Both luciferase and Luciferin are available at chemical suppliers.

These light emitting reactions have been explored by chemists in a large number of laboratories and clinical tests. Some of its applications are in: lights from emergency “light sticks” used by campers and glowing necklaces seen at concerts and sporting events.

Types of Fluorescence Quenching

Fluorescence quenching occurs when the fluorescent intensity of a given substance is decreased. A variety of processes can result in quenching. Normally it is engineered by the presence of a competing de-activating process resulting from specific interaction between a fluorophore and another substance present in the system. The substance that causes this phenomenon is called a quencher. Different types of quenchers and their mechanisms are discussed below.

Collisional or Dynamic Quenching

This type of quenching results from collisional encounters between a fluorophore and a quencher. It requires molecular contact. In Collisional dynamic quenching, the quencher diffuses towards a fluorophore during the lifetime of its excited state. Upon contact the quencher facilitates non-radiative transition to the ground state. Collisional or dynamic quenching reveals a quencher concentration- dependence which is described by the Stern-Volmer equation;

$$F_0/F=1+Kq\tau_0[Q] =1+K_D[Q], \quad (5)$$

In equation 5, F_0/F are the fluorescence intensities in the absence and presence of the quencher. Kq is the bimolecular quenching constant that describes the quenching process. It is proportional to the effectiveness of the quencher and the accessibility of the fluorophore to collisions with the quencher. τ_0 is the lifetime of the fluorescent state in the absence of a quencher; [Q] is the concentration of the quencher. K_D is the Stern-Volmer quenching constant, also called the quenching rate constant. K_D represents quenching by collision. The D is the sum of the diffusion coefficients of fluorophore and (D_f) and the quencher Dq. A large K_D value thus represents effective collision between fluorophore and quencher. Dynamic quenching is hence effective when there is little hindrance to the collision between fluorophore and quencher. Small values of K_D typically reflect steric hindrance of quencher-fluorophore collisions. K_D is replaced by Ksv when quenching is not by dynamic quenching. A plot of F_0/F versus [Q] yields a straight line with a slope K_D and an intercept on the y-axis (7).

Static Quenching

This quenching occurs as a result of the formation of a non-fluorescent ground state complex between the fluorophores and the quencher. Upon absorption of radiation this complex returns to the ground state almost immediately without the emission of a photon. The association constant for the quencher-fluorophore complex describes the effectiveness of a static quencher. The association constant has an expression:

$$K_s = [F\text{-}Q]/ [F] [Q], \quad (6)$$

where [F-Q] is the concentration of the non-fluorescent complex and [F] is the concentration of the uncomplexed fluorophore.

Comparism of Dynamic and Static Quenching

Static and Dynamic quenching are basically distinguished by their fluorescent lifetime measurements. Temperature is also used to distinguish between these two quenching mechanisms. Dynamic quenching depends upon diffusion, and because higher temperatures result in larger diffusion coefficients, the bimolecular quenching constant is expected to increase with increasing temperatures. Diffusion rate increases with temperature and this leads to an increase in dynamic quenching at higher temperatures. In contrast however, complex formation tends to be inversely proportional to temperature. Increased temperature mostly results in decreased stability of complexes, thus lower values of quenching constants. As a result, static quenching tends to be higher at lower temperatures (8).

One other unique feature in distinguishing dynamic and static quenching is by careful examination of the absorption spectra of the fluorophores. Collisional quenching only affects the excited states of the fluorophores, and thus no change in the absorption spectra are predicted. In contrast, ground state complex formation frequently results in perturbation of the absorption spectrum of the fluorophores (9).

Concentration Quenching

Absorption is necessary for fluorescence to occur. Hence, fluorescence intensity is proportional to the molar absorptivity. This implies the more highly absorbing a substance the greater the fluorescence. However, when absorption is too much, no light can pass through to cause excitation. Thus, at low concentrations, when the absorbance is less than about 0.05, a linear relationship exists between fluorescence and concentration. At very high concentrations of a sample, front-face absorption occurs. In front-face absorption, the portion of the sample nearest the radiation source absorbs so much radiation due to high number of molecules. The result is that less radiation is available for the rest of the solution. The incident radiation is thus attenuated as it goes through the sample towards the viewing area of the cuvet. This leads to a depression in fluorescence intensity of the sample. This phenomenon is known as Inner filter effect or concentration quenching. Concentration of solutions should therefore be taken into consideration in the preparation samples for fluorescence analysis, in order to avoid this phenomenon.

Heavy Atom Substituent Effects

Research has shown that the halide ions are good chemical quenchers (7, 8). Iodine, chlorine, and bromine, which are also heavy atom elements, reduce the fluorescent intensity of fluorophores. This is as a result of intersystem crossing to an excited triplet state, promoted by spin-orbit coupling of the excited singlet fluorophores and the halogen. Intersystem crossing is very competitive with the presence of this substituent. The pattern of quenching ability of these ions was found to be iodine>bromine>chlorine, an order which is the same as their ionization energies (10). This could be attributed to the increase in size of the ions as they go down the group 7, with the radius of sphere of action getting bigger too, with iodine having the biggest size, thus more prone to collision, leading to quenching. Quenching by these halide ions are mostly by dynamic or collisional quenching or energy transfer. Methyl Iodide was used as the quencher for this research.

Dark Chemical Quenchers

Dark chemical quenchers are substances that absorb excitation energy from fluorophores and dissipate the energy as heat, as opposed to a typical fluorescent quencher that re-emits much of this energy in the form of light. Dark quenchers are largely used in molecular biology in conjunction with fluorophores. When a fluorophore and a dark quencher are close together, such as in a molecule or protein, the fluorophores emission is suppressed. This effect is used to study molecular geometry and motion (11).

Dark quenchers also normally known as dyes with no native fluorescence, (non-fluorescent dyes) offer a solution to background noise, caused by quencher fluorescence, due to overlap between the quencher and the fluorescence spectra (12). They exhibit lower background fluorescence, causing larger signal-to-noise ratio with great dynamic range. In the absence of secondary fluorescence arising from a dark quencher, multiple fluorophores can be simultaneously spectrally resolved, making dark quencher probes amendable to multiplex assays. In addition, dark quenchers enable multiplexing when two or more reporter-quencher probes are used together(13).

Types and Applications of Dark Quenchers

Fluorescence resonance energy transfer (FRET) is a highly distance-dependent interaction between a reporter dye in an excited state and a quencher in its ground state. Energy is transferred from one molecule (the fluorophore) to the other (the quencher) without the emission of a photon. In order for efficient FRET quenching to take place: a) the fluorophore and quencher molecules must be close to each other (approx. 10 - 100 Å) and, b) the absorption spectrum of the quencher must overlap with the emission spectrum of the fluorophore.

Dark quenchers are mostly used in fluorescent dye-quencher probes requiring suppression of the dye's fluorescence under one set of circumstances. Common examples of dark quenchers are Dabcyl and the three Black Hole Quenchers (BHQ). Taken together, the absorption spectra of these four dark quenchers span the entire visible range, which provides a researcher with broad flexibility in choice of fluorescent dye, along with the ability to search a sample for multiple targets in a multiplex quantitative, polymerase chain reaction (PCR) format.

Fluorescent quenchers, such as tetramethyl-6-carboxyrhodamine (TAMRA) dye are typically used in fluorescence resonance energy transfer (FRET)-based applications. FRET probes contain a donor (fluorescent dye)-acceptor (fluorescent quencher) pair in close proximity. After absorbance of light by the donor moiety the donor's fluorescence emission energy is absorbed (quenched) by the acceptor moiety and subsequently emitted at the acceptor's emission wavelength. The result is a final fluorescence emission at a substantially longer wavelength than would be expected if only the donor moiety were present. FRET probes thus are useful in cases where a substantial shift in final emission wavelength is desirable. This type of FRET-based system is typically used to determine intra- and inter-molecular distances at very high resolution (1-10 nm). For instance, FRET oligo probes have been used to measure the dynamic changes in intermolecular distances between tRNAs bound at the A and P sites of ribosomes during mRNA translation (14). FRET oligo primers have also been used to obtain direct evidence of strand slippage during in vitro synthesis of poly (dG)-poly (dC) duplexes by the Kleno exo- fragment of DNA polymerase I (15).

Mechanism of Energy Transfer Quenching

In this, mechanism sometimes known as resonance energy transfer quenching applies. Energy from an excited state is transferred to an acceptor molecule. The transfer normally occurs without the emission of a photon, but the process is somehow related to absorbance. The rate transfer depends on the following processes:

1. Spectral overlap between the emission spectrum of the donor and the absorption spectrum of the acceptor molecule.
2. The quantum yield of the donor.
3. The relative orientation of the transition dipoles of the donor and acceptor.
4. Distance between donor and acceptor.

Resonance energy transfer is essential during photosynthesis, as light molecules use this mechanism to transfer collected energy to the photosynthetic reaction centre. It is also applied in the study of proteins resonance energy quenching is used the measure the distance within or between molecules. These studies are either carried out with single donor or acceptor functions or by the use of instruments capable of time resolved measurements.

Oxygen Quenching

Oxygen is one of the most notorious of all quenchers. It is a ubiquitous quencher of both fluorescence and phosphorescence. Dissolved oxygen has a very large diffusion coefficient especially in polar solvents. The mechanism by which oxygen quenches fluorescence has been the subject of considerable research (17) and it is clear that contact between the oxygen molecule and the fluorophores is a requirement for quenching. The studies so far published show that quenching by oxygen is by diffusion –controlled process in which virtually every collision with the excited fluorophores is effective in quenching (18). Unsubstituted aromatics are severely affected by oxygen. The enhancement of intersystem crossing by oxygen is the basic route of oxygen quenching. Oxygen is also an effective triplet state quencher because oxygen gas is triplet in its ground state. Fluorescence intensity is enhanced with the removal of oxygen. This is achieved by bubbling an inert gas through the solution for about 5-10 minutes or by a freeze-thaw cycle. In this study helium gas was bubbled through the sample solution in order to study the extent of oxygen quenching of the fluorophores (19).

Other Quenchers

Aromatic and aliphatic amines are efficient quenchers of most unsubstituted aromatic hydrocarbons (20). Anthracene fluorescence is for example effectively quenched by diethylaniline. The mechanism of quenching is based on the formation of a charge transfer complex. The excited state fluorophores accepts an electron from the amine. In nonpolar solvents fluorescence emission from the charge transfer complex (exciplex) is frequently observed, and this process is often regarded as an excited state reaction rather than quenching. In polar solvents however the exciplex emission is often quenched so that the fluorophores-amine interaction appears to be that of simple quenching (21). Other collisional quenchers include hydrogen peroxide, acrylamide, BrO^-_4, I^-, nitrous oxide, nitromethane, and some olefins. Many halogen-containing substances act as collisional quenchers. Examples of such are chloroform, trichloretanol, bromobenzene, methylmercuric chloride, and a variety of substances containing more than a chorine atom. Indole, carbazole, and their derivatives(22) are uniquely sensitive to quenching by chlorinated hydrocarbons and by electron scavengers such as protons, histidine, cysteine, NO^-_3, fumarate, Cu^{2+}, Pb^{3+}, Cd^{3+}, and Mn^{2+}. Indole, tryptophan, and its derivatives are

quenched by succinimide, dichloroacetamide, pyridinium hydrochloride, imidazolium hydrochloride, methionine, Eu^{3+}, Ag^{+}, and Cs^{+}. A variety of quenchers are hence essential in the study of protein fluorescence, especially regarding the surface accessibility of tryptophan residues and the permeation of proteins by the quenchers. The last but not the least group of quenchers includes purines, pyrimidines, N-methyl-nicotinamide, N-alkyl pyridinium, and picolinium salts. An example is the fluorescence of both flavin adenine dinucleotide(FAD) and reduced nicotinamide adenine dinucloetide (NADH) are quenched by the adenine moiety. Flavin fluorescence is quenched by both static and dynamic processes, whereas the quenching of dihydronicotinamide appears to be primarily dynamic. These aromatic substances appear to quench by the formation of charge-transfer complexes. Depending however upon the structure involved, the ground state complex can be reasonably stable. Hence both dynamic and static quenching are frequently observed. Because most substances act as quenchers, fluorophore-quencher combinations are identified for a desired purpose. Thus the occurrence of quenching depends on the mechanism, which in turn depends on the structure of the individual molecules (23).

Applications of Fluorescence Quenching

Quenching of fluorescence has been explored in biochemical and bioanalytical research (24). In the study of proteins and other macro-bimolecules, some of the quenchers that have been tried and found useful for bioanalytical studies are iodide, oxygen, and acryl amide. A great number of quenchers are still being studied for their usefulness.

Iodide

Iodide and its applications in quenching tryptophan fluorescence using model compounds and the protein lysozyme have been studied by Lehrer, a biochemist (25). He found iodide to be an efficient dynamic quencher due to its size and charge. In the protein studied, iodide was found to quench mainly the surface tryptophanyl fluorescence. Iodide is very sensitive to local charge densities. Hence the nature of the microenvironment can be estimated because the presence or absence of charged groups in the vicinity of the fluorophore can affect the local concentration of iodide and hence alter the quenching efficiency. The viscosity of the microenvironment can be estimated for dynamic quenching because the viscosity of the solvent

influences the quenching rate by affecting the diffusion rate of quenchers towards the fluorophore. Diffusion within the protein shows less dependence on the viscosity of the environment. Denatured protein is mostly quenched by iodide with 100% efficiency. Hence the quenching of protein fluorescence by iodide can indicate the degree of exposure and accessibility of the fluorescent residue. Structural information can be inferred from these data.

Oxygen

Oxygen has been proven to be an efficient quencher of proteins by most researchers (26). Oxygen, a small, neutral, and polar molecule, has been found to quench dynamically and effectively with a large quenching constant. Oxygen devoid of charge effects eliminates uncertainties in its usage where charge effects may play a role in observing fluorescence quenching. Oxygen in this regard is contrasted with iodide quenching in the interpretation of quenching data by oxygen of the ethidium bromide DNA complex. This complex contains positive charged ethidium bromide and negatively charged phosphate groups that can affect the local concentration of charge-sensitive quenchers due to attraction of opposite charges. Some researchers have unambiguously concluded that the fluorophore is intercalated and protected by the helix and that of oxygen must collide with the fluorophore for quenching to occur (27). Molecular oxygen is an important quencher for many fluorophores because of its high solubility in aqueous solutions and organic solvents. Fluorescence quenching by molecular oxygen is usually a diffusion-controlled mechanism (28). Lakowicz and Weber also proposed non-rigid conformations for proteins. However the rather undergo very rapid fluctuations on a nanosecond time scale. They hence concluded that oxygen effectively quenches tryptophanyl residues that were known to be buried deep into the known protein structures. Hence some of these conformations allow oxygen to diffuse into the proteins.

Acrylamide

The use of acrylamide as a quencher of indole fluorescence was investigated by Eftink and Ghiron (29). They found that acrylamide is an efficient quencher and quenches by both dynamic and static quenching. It's a neutral quencher like oxygen. However it is larger, more polar, and very soluble aqueous solution. In their evaluation of acrylamide as a quencher, they

used an indole-micelle complex to stimulate a simple a simple protein structure. They were able to obtain information about the microenvironment of the fluorescence probe and the general structure of the complex. An additional advantage of acrylamide over other neutral and non-polar quenchers like oxygen and trichloroethanol is that it does not interact with proteins to any significant extent. In another study of trichloroethanol as a quencher, Eftink et al. (30) found that tricloroethanol can localize in the polar region of the macromolecules resulting in observable changes in quenching efficiencies.

Some Recent Applications of Fluorescence Quenching

The myriad applications of fluorescence quenching is still being explored and used in all areas of analytical chemistry, biomedical, and biochemistry in recent years. Fluorescence quenching methods have been seen to be useful in obtaining information about conformation and dynamic changes of proteins in complex macromolecular systems (31).

A study by Limei et al. employed the mechanism of fluorescence quenching for the detection of antigen. In the study, a highly specific fluoroimmunoassay system for antigen detection was developed using gold and magnetic nanoparticles (32). The assay used was based on the fluorescence quenching of fluorescein isothiocyanate resulting from gold nanoparticles coated with monoclonal antibody. The analytical properties of the gold nanoparticles were explored by coating the magnetic nanoparticles with anti-α-fetoprotein polyclonal antibodies. A sandwich-type-immunocomplex was formed, with the α-fetoprotein captured by the magnetic nanoparticles probes. The supernatant liquid form the immunocomplex containing unbound gold nanoparticles probes was used to quench the fluorescence. The fluorescence intensity of fluorescein isothiocyanate observed was at 516 nm, which was proportional to the α-fetoprotein concentration. The results gave the limit of detection 0.17 nm for the α-fetoprotein. From their finding Limei et al. concluded on the possibilities of extension of the fluoroimmunoassay system to detect target molecules with matched antibodies with potential applications in immunoassay and disease diagnosis.

Fluorescence quenching mechanisms has also been explored by Zhuang et al. (33) to study protein folding at a single molecule level. In the study titin molecules were used as a

model system. Fluorescence resonance energy transfer (FRET) was used to fold titin molecule with multiple dye molecules attached to a native folded state. Fluorescence from the dye molecules was quenched in the native state due to the closeness of the dye molecules. The titin molecule was unfolded after this state which led to a dramatic in the fluorescence intensity. The folded and unfolded states of the titin molecules were clearly differentiated hence permitted the measuring of the folding dynamics of the individual titin molecules in real time. Their finding hence demonstrated the use of fluorescence quenching for signal folding and unfolding of a small protein with only one immunoglobulin domain.

Another useful application of fluorescence quenching is its use in optical halide sensing (38). A review article by Chris D. Geddes outlines some of the unique applications of fluorescence quenching in optical halide sensing. He discusses the versatility of the analytical technique for the determination of halide ions or organic halides. Some of the advantages of this technique over other methods such as ion-selective electrode were mentioned in that halide concentrations can be determined in very small sample volumes with no pre-treatment of samples required, and measurements can be non-evasive and very quick and sometimes continuous. The possibility of the determining the halide concentration using either intrinsic or extrinsic fluorescence probes is another advantage of halide sensing by fluorescence quenching. The addition of extrinsic probe molecules to livingsystems and some industrial processes is however not desirable and therefore, for physiological samples, intrinsic fluorescent probes, such as protein tryptophans or even yellow or green fluorescent proteins, are sometimes preferred. An alternative to the introduction of extrinsic probes has been to immobilize halide sensitive fluorophores onto or within a support that, when immersed into the desired sample, readily allows the diffusion and therefore the sensing of aqueous halide ions, or, in the case of halothane, gaseous alkyl halide (38).

CHAPTER 2

EFFECTS OF SELECTED SUBSTANCES ON LUMINESCENCE

Surfactants and Micellar Mediums

Surfactants have different levels of impact on the fluorescence of compounds, mainly due to their ionic properties. Surfactants in the form of micelles serve as organized media that have the tendency of protecting the molecules of a compound from impurities and quenching characteristics. Micelles have the tendency to arrange solutes into their interior or on their colloidal surface. When solutes move from aqueous medium to the micellar medium, changes in several properties such as solubility, reactivity, and spectroscopic characteristics result. These change in properties lead to increase in fluorescence intensity due to increase in sensitivity. The relative viscosity of micellar microenvironments also inhibit quenching by molecular oxygen (40).

Research by Charles Odame Ankra of the chemistry department, under Dr Ho (June,2009), came out with the finding that surfactants such as CTAB, SDS, and Triton X-100, singly and in combination with titanium dioxide enhanced the fluorescence of fluorophores, such as Anthracene and fluoranthene, but they quenched that of carbazole and phenanthrene. Surfactants are generally known to affect the fluorescence intensity through micelle formation. Micelles enhance fluorescence by increasing sensitivity, reduction in number of potential interferences and result in greater experimental convenience (41).

Research by Kim et al. (42), showed that surface modification of CdS and CdMnS quantum dots by micelles using the reverse-micelle method enhanced the luminescence properties of the quantum dots. In their findings, before the surface modification a broad luminescence band from defects was observed for CdS quantum dots. After the modification however intensity of the band-edge was remarkably enhanced. They also reported an increase in the intensity of Mn^{2+} luminescence after its surface was modified by CdMnS quantum dots. A study of the quantum yield of CdS quantum dots with broadband luminescence also recorded an increase in quantum yield after treatment with surfactant. The quantum dots were synthesized by

classic inverse micelle method using dioctyl sulfosuccinate sodium salt in heptanes. The quantum yield of CdS quantum dots was doubled to a 20% instead of the 10-13% quantum yield without the modification (43). Other research by Robert Leif and others concluded that luminescence of lanthanide ion complexes increased by using the micellar solution medium to enhance luminescence. The Resonance Energy Transfer Enhanced Luminescence (RETEL) effect was used as a mechanism of luminescence enhancement (44). Two methods were used to achieve the enhancement; firstly a complex of a second lanthanide ion was added in a micellar solution. In the other method a dry preparation by evaporation of a homogeneous solution containing an added complex of a second lanthanide ion in excess of an unbound antenna ligand was obtained. Both methods lead to an increase in luminescence by the RETEL method.

Beta- Cyclodextrins Effects on Luminescence

Cyclodextrins are cyclic oligosaccharides obtained from degradation of starch by Bacillus macerans. They were first isolated in the late nineteenth century. Applications for cyclodextrins and their derivatives are sought in various areas of chemistry, including the sensing of organic molecules (45). Their ability to form complexes has lead to its wide array of applications. Beta-cyclodextrin is added to compounds as complexes to enhance their luminescence. In a study by Turnball and Walker, beta-cyclodextrin was added to para amino benzoic acid (PABA). The fluorescence intensity was increased upon complexation of the para amino benzoic acid with beta-cycodextrin at 298K. This was attributed to the prevention of Collisional deactivation of the para amino benzoic acid. At a lower temperature of 77K however, the fluorescence intensity was reduced due to the vibrational deactivation modes available to the complex relative to the free PABA. The luminescence effects were interpreted as inclusion complexation of the PABA anion (46).

Another study by Vazquez et al, recorded a substancial enhancement of the fluorescence emission of aflatoxins by complexation with cyclodextrin. In their study fluorescence properties of four main aflatoxins; B_1, B_2, G_1, and G_2 in solution was investigated alone and in the presence of various cyclodextrin derivatives. An enhanced fluorescence emission of the aflatoxins with an unsaturated furan ring (B_1 and G_1) in the presence of acqueous solutions of α,-β-heptakis-di-O-methyl-β-cyclodextrin and hydroxypropyl-β-cyclodextrin was observed. The selectivity of the

interaction was attributed to the partial involvement of a non-inclusion process because of the high molar ratio of cyclodextrin to aflatoxin needed for the fluorescence enhancement (47).

Titanium Dioxide Nanoparticles

Titania is an oxide of titanium, TiO_2, that occurs naturally. This naturally occurring oxide can be mined and used as a source of commercial titanium. Titania exists in three basics: tetragonal rutile (stable at room temperature), tetragonal anatase (metastable for kinetic reasons), and orthorhombic brookite (not commercially viable). Each of the above forms exhibits different physical properties such as refraction or chemical or photochemical reactivity that allows its use in particular applications normally requiring a specific particle size. (48)

Applications and Uses of Titania

Titanium dioxide is widely used white pigment in the world. It's not toxic and chemically stable, used mainly to achieve opacity and whiteness of commercial products. Titania has a myriad of applications.

Titanium dioxide is the most widely used white pigment as a result of its brightness and high refractive index of about 2.7. Titania is an efficient opacifier in its powder form, mostly used as pigment to provide whiteness and opacity to common products such as paints, papers, plastics, inks, food , drugs (in pills and tablets), and also in most toothpastes. In food it is mostly used to whiten skimmed milk, which increases its palatability (49).

Titania is used in sunscreen due to its high refractive index, its strong ultraviolet light absorbing capability, and its resistance to discoloration under ultraviolet light. This property enhances its stability and ability to protect the skin from ultraviolet radiation. Sunscreens designed for infants and adults with sensitive skin contain titanium dioxide or zinc oxide as they cause less skin irritation (50).

Titanium dioxide has a wide range of uses as a photocatalyst and catalytic support. It is also used as a heterogeneous catalyst in solar cells and gas sensors. The rutile and anatase phase exhibit photocatalytic properties. However the anatase form shows higher photocatalytic efficiency compared with the rutile phase. (51) Titanium dioxide, when spiked with nitrogen

ions or doped with metal oxides such as tungsten trioxide, also exhibit photocatalytic properties under either visible or ultraviolet radiation (52). The strong oxidative potential of the positive holes oxidizes water to create hydroxyl radicals. It also oxidizes oxygen or organic materials directly. Titanium dioxide is hence added to cements, paints, windows, tiles, and other products for its deodorizing, sterilizing, and anti-fouling properties. It is also used as a hydrolysis catalyst. (53).

Luminescence Characteristics of Titanium Dioxide

Titanium dioxide in its different phases exhibit luminescence characteristics under various conditions. Research by Zhang and others reported anatase titanium dioxide nanocrystals possessed interesting luminescence characteristics and have potential applications in photocatalysis and photoelectric chemical conversion using visible light (54). Another study by E. Zaleta-Alejandre et al. (2009) recorded strong luminescence properties of a mixture of tetragonal anatase and rutile crystals. The unique properties were determined by x-ray diffraction. The crystals exhibited strong luminescence under ultraviolet and electron beam excitation. These characteristics were attributed to f-f transitions (55).

New Developments and Future Trends

Quantum Dots Nanoparticles

Quantum dots are semiconductors whose electronic characteristics are closely related to the size and shape of the individual crystal. Generally, the smaller the size of the crystal, the larger the band gap, the greater the difference in energy between the highest valence band and the lowest conduction band. Therefore, more energy is needed to excite the dot, and concurrently, more energy is released when the crystal returns to its resting state (56).

Researchers have studied quantum dots in transistors, solar cells, LEDs, and diode lasers. They have also investigated quantum dots as agents for medical imaging and hope to use them as qubits (57). For instance, in fluorescent dye applications, this equates to higher frequencies of light emitted after excitation of the dot as the crystal size grows smaller, resulting in a color shift from red to blue in the light emitted. In addition to such tuning, a main advantage with quantum dots is that, because of the high level of control possible over the size of the crystals produced, it

is possible to have very precise control over the conductive properties of the material. Quantum dots of different sizes can be assembled into a gradient multi-layer nanofilm (58).

Fullerenes as a Cushion for Nanoparticles

Nanoparticles are known as promising building blocks for future applications. Depositing them however on surfaces or in a matrix is a major task (59). Stefanie et al. (60) have suggested a double layer of spherical C_{60}, carbon molecules called fullerenes as an ideal substrate for these microscopic particles. They discussed that by tuning the size and composition of the nanoparticles, one can 'tailor' the chemical, optical, or magnetic properties, and obtain features different from any bulk material. But for an application of this potential in the fields of catalysis, magnetic storage technology, or optoelectronics, one has to deposit the nanoparticles on surfaces or in matrixes. However during this process, the interaction with the surface or matrix can destroy the unique properties of the nanoparticles. Therefore it is important to develop techniques for a 'gentle' yet secure fixation of nanoparticles. The nanoparticles were deposited on a layer of spherical C_{60}carbon-molecules and their properties investigated. It was shown that a double layer of fullerenes on a metal surface is an ideal substrate for the fixation of nanoparticles. The size and shape of the particles stayed unchanged for days even at room temperature, which is a high demand for nanoscale processes. On a single layer of fullerenes, however, the nanoparticles shrank fast and disappeared within a few hours. Using atomic simulations this was traced back to temporary contacts bridging the fullerene layer and transporting atoms from the nanoparticles to the supporting metal surface. On the basis of these results it might be possible to control the contact between nanoparticles by thin films that can either be penetrated or stay isolating. The report demonstrated how to deposit nanoparticles on surfaces without destruction of their geometric structure and also characterized a decay process for nanoparticles by the penetration of nanoscopic barriers in detail. These findings improve significantly the understanding of nanoparticle stability, which is an important step towards the application of tailor-made nanosystems (61).

Research Proposal

The unique characteristics of luminescence as an analytical tool, due to its selectivity and sensitivity, drive the desire to explore its applications and properties in the presence of different

substances. This research was developed to explore the luminescence properties of chosen compounds in the presence of substances that are known to exhibit luminescent characteristics. The myriad characteristics and applications of titanium dioxide leads to curiosity about what more it can do. Previous research showed that titanium dioxide to an extent enhanced the fluorescent intensity of some chosen fluorescent aromatic compounds on its own and also in the presence of surfactants. This discovery lead to the quest to investigate the possible effects of titanium dioxide in the presence of a known quencher on selected organic compounds. Beta-Cyclodextrin, also known to possess luminescence characteristics, was explored to investigate its properties in the presence of chosen fluorophores.

The following scope of analysis and findings were hence developed to discover the effects of titanium dioxide nanoparticles on the fluorescent intensities of the selected organic compounds in the absence and presence of a known quencher.

Scope of Research

1. To explore the quenching of the above aromatic compounds in the presence of methyl iodide and also titanium dioxide nanoparticles.
2. To study the effects of the presence of oxygen and degassed solutions of the solutions of these fluorophores in varying concentrations of methyl iodide and varying concentrations of titanium dioxide nanoparticles.
3. To study the effects of the fixed concentrations of methyl iodide (higher and low) on the quenching of the solutions of the fluorophores with varying concentrations of titanium dioxide nanoparticles.
4. To study the effects of the fixed concentrations of titanium dioxide nanoparticles (higher and low) on the quenching of the solutions of the fluorophore with varying concentrations of methyl iodide.

CHAPTER 3

EXPERIMENTAL PROCEDURE

In this chapter the materials and methods used to synthesize titanium dioxide nanoparticles, prepare various working solutions, and determine fluorescent intensities of various compounds are described.

Reagents Used

All reagents used were of highest purity grade obtained from commercial sources hence no further purifications were needed.

1. Titanium isobutoxide Ti $(OC_4H_9)_4$, 97% Aldrich (Milwaukee,WI).
2. Methyl Iodide (99% Alfa Aesar) A Johnson Matthey Company(Ward Hill, MA)
3. Carbazole, pyrene and fluoranthene from Aldrich Chemical Company (Milwaukee, WI).
4. 95% Ethanol, Laboratory grade from Fischer Scientific (Pittsburgh, PA)
5. Deionized Water, from US Filter Company Pittsburg, PA).

Synthesis of Rutile TiO_2

TiO_2 nanocrystals were prepared by following the procedures described by Tang et al. (62). This procedure involves the combination of titanium isobutoxide and nitric acid in water. Titanium isobutoxide $Ti(OC_4H_9)_4$ was added drop- wise to 2 M nitric acid (HNO_3) with continuous stirring of the solution until a transparent solution was obtained. The molar ratios of Ti $(OC_4H_9)_4$: (HNO_3): H_2O were 1: x: 50 with $2 \leq x \leq 5$ ratio held constant. This ratio ensured that pure rutile phase TiO_2 was obtained, as the rutile phase can easily be converted to the anatase phase when the solution is too acidic. The transparent solution was hydrolyzed by heating to a temperature of about 50°C in a water bath. A two-layered solution (an upper organic and a lower sol) was obtained after hydrolysis. The sol was separated from the organic layer by decantation and heated to about 50°C without stirring for about 6-7 hours. A transparent gel was obtained and dried in the oven overnight at temperatures of about 50°C. White crystalline TiO_2 was obtained and used for analysis (64).

Preparation of Working Solutions

The working solutions were prepared using 95% ethanol as the solvent. All the compounds used in the preparations were organic in nature with the exception of TiO_2. TiO_2 has good dispersibility in water but is insoluble in ethanol. The 5% water in the ethanolic solution ensured even distribution of the nanoparticles in the solution. Stock concentrations of all fluorophores were prepared and further diluted for desired concentrations for each analysis.

1. Carbazole: 0.083g of carbazole ($C_{12}H_9$, 167.21 g/mol) was dissolved in an amount of 95% ethanol solution and made up to 50 ml to obtain a 1.0×10^{-2} M stock solution and was kept in the refrigerator until analysis. Further dilutions of the stock solution were made for various studies.
2. Pyrene: 0.010 g of pyrene ($C_{16}H_{10}$, 202.25 g/mol) was dissolved in an amount of 95% ethanol and made up to 50 ml to obtain a 1.0×10^{-2} M stock solution and was kept in the refrigerator until analysis. Further dilutions of the solution were made for various studies.
3. Fluoranthene: 0.010 g of fluoranthene ($C_{16}H_{10}$, 202.26 g/mol) was dissolved in an amount of 95% ethanol and made up to 50 ml to obtain a 1.0×10^{-2} M stock solution and was kept in the refrigerator until analysis. Further dilutions of the solution were made for various studies.
4. Iodomethane: 1.0×10^{-2} M working solution was prepared by pipetting 30 µL of the 16.05 M stock solution and diluted to 50 ml with 95% ethanol in a volumetric flask. This was kept in the refrigerator until measurements were done.
5. TiO_2 Nanoparticles: 0.040 g of TiO_2 (80 g/mol) was dissolved in 95% ethanol and made up to 50 ml to afford a 10^{-2} M solution. This was kept for analysis, with further dilutions made as desired.

Fluorescence Instrumentation

A Perkin Elmer Model 650 Spectrometer (Perkin Elmer Corporation, Waltham, Massachusetts) was used. It has a xenon arc lamp as its radiation source with a photomultiplier tube as a detector. Signals were recorded on a built-in readout attached.

It has an excitation and emission wavelength selectors that were varied to obtain the appropriate signal level for each sample analyzed. The excitation and emission wavelengths used for all aromatic compounds studied were: for carbazole, excitation wavelength of 290 nm and emission wavelength of 350 nm; for pyrene, excitation wavelength of 300 nm and emission wavelength of 371 nm; and for fluoranthene, excitation wavelength of 322 nm and emission wavelength of 395 nm. A fused silica fluorescence cuvette was used for all measurements. The excitation and emission slits were both 2 nm wide all measurements. Background corrections were done using a 95% ethanol blank solution and all final readings were corrected with this.

Data Analysis

All measurements were conducted in triplicates and the data reported with average values. Data were processed using Microsoft Office Excel 2007 software for Windows Vista (Microsoft Corp., Redmond, WA). The results obtained from the experimental measurements, were tabulated, graphed, and analyzed using Excel.

CHAPTER 4

RESULTS AND DISCUSSION

The results of fluorescence measurements on the selected compounds alone and in the presence of added methyl iodide and titanium dioxide of various concentrations are presented and discussed in this chapter. Various concentrations of the selected fluorophores were prepared as well. To these fluorophores were added the various concentrations of methyl iodide and titanium dioxide, either alone or together, to establish how these compounds and their concentrations affect the fluorescent intensities of the select fluorophores. The solutions of these fluorophores were also degassed to establish whether oxygen quenching had occurred and to what extent.

Dynamic Quenching of Fluorophores

The first study we would like to do is to confirm that the quenching of the fluorophores that occurred involving methyl iodide and titanium dioxide nanoparticles is that of dynamic quenching mostly. Table 1 is the ratios of the results obtained for carbazole solutions with various concentrations of methyl iodide to them, and Figure 1 is the corresponding plot of the ratios calculated.

Table 1: Ratios of fluorescent intensities of solutions of carbazole with varying concentrations of methyl iodide added compared to one without any methyl iodide present

Log Molar Concentration Of Methyl Iodide	I_{f0}/I_f
-5.0	1.09
-4.0	1.08
-3.0	1.28
-2.0	2.22
-1.0	4.31

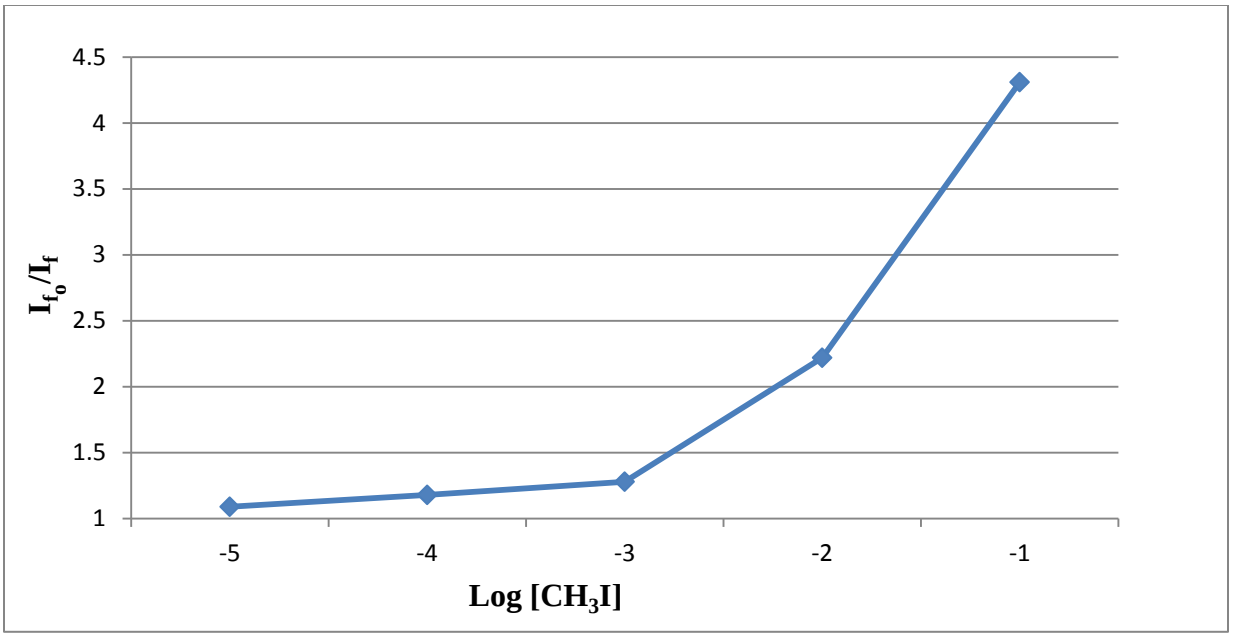


Figure 1: Stern-Volmer plot for carbazole showing the ratios of fluorescence intensity in the absence (I_{f0}) and presence (I_f) of methyl iodide

The Stern-Volmer plot for carbazole in Figure 1 indicates that quenching by methyl iodide did not occur significantly at very low concentrations. The fluorescent intensities did not change much in the presence of quencher as the ratios were pretty much constant until a reasonably high concentration of methyl iodide was reached. Then when the concentration was above 1.0×10^{-3} M dynamic quenching did occur. The plot is not as linear as it should be. This is most likely due to experimental error, such as volume measurements and other quenching process such as oxygen quenching that might also occurred to some extent.

The same study was conducted for pyrene solutions. Table 2 tabulated the data obtained for pyrene in the presence of varied concentrations of methyl iodide. The corresponding Stern-Volmer plot is shown in Figure 2. Again, at the lowest concentration of methyl iodide quenching was not linear. Because the quenching even at this low concentration was quite noticeable, it most likely was due to oxygen quenching as pyrene was well known to be susceptible to oxygen quenching (76). However, the quenching was linear after methyl iodide was higher than 1.0×10^{-4} M. The linear plot after that concentration clearly indicates that pyrene was dynamically quenched by methyl iodide.

Table 2: Ratios of fluorescent intensities of solutions of pyrene with varying concentrations of methyl iodide added compared to one without any methyl iodide present

Log Molar Concentration Of Methyl Iodide	I_{f0}/I_f
-5.0	1.21
-4.0	1.28
-3.0	1.46
-2.0	1.76
-1.0	2.02

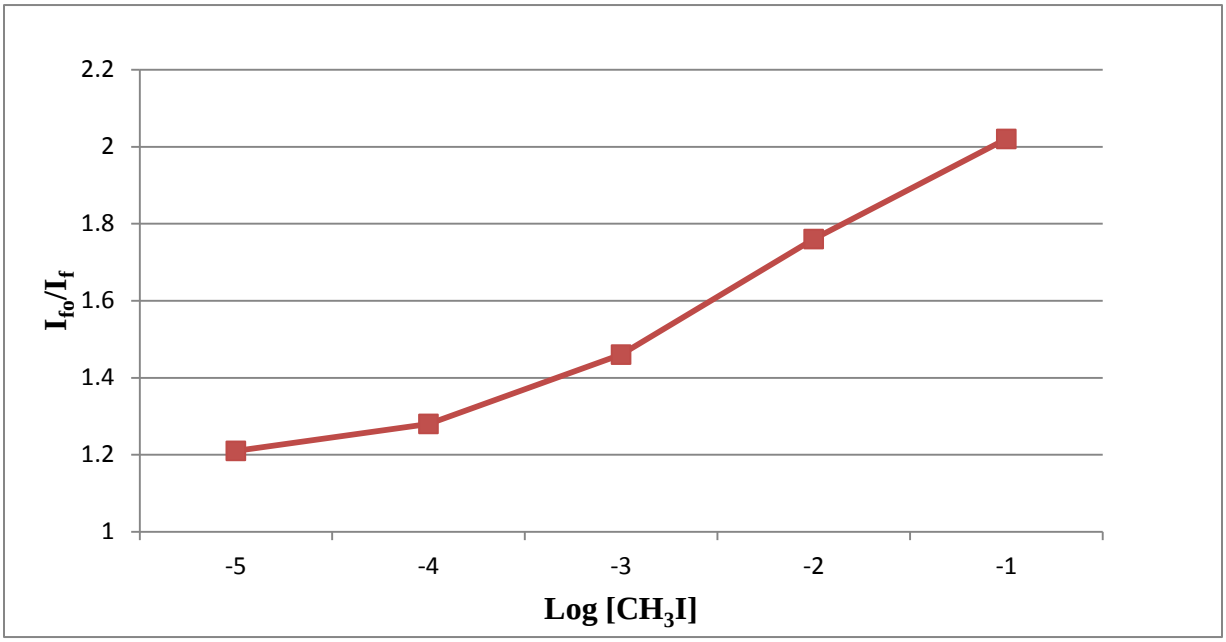


Figure 2: Stern-Volmer plot for pyrene showing the ratios of fluorescence intensity in the absence (I_{f0}) and presence (I_f) of varying concentrations of methyl iodide

The same study was finally carried out for fluoranthene solutions. Table 3 shows the ratio data obtained for fluoranthene in the absence and presence of varied concentrations of methyl iodide. The corresponding Stern-Volmer plot is shown in Figure 3.

Table 3: Ratios of fluorescent intensities of solutions of fluoranthene with varying concentrations of methyl iodide added compared to one without any methyl iodide present

Log Molar Concentration Of Methyl Iodide	I_{f0}/I_f
-5.0	1.16
-4.0	1.21
-3.0	1.26
-2.0	1.31
-1.0	1.46

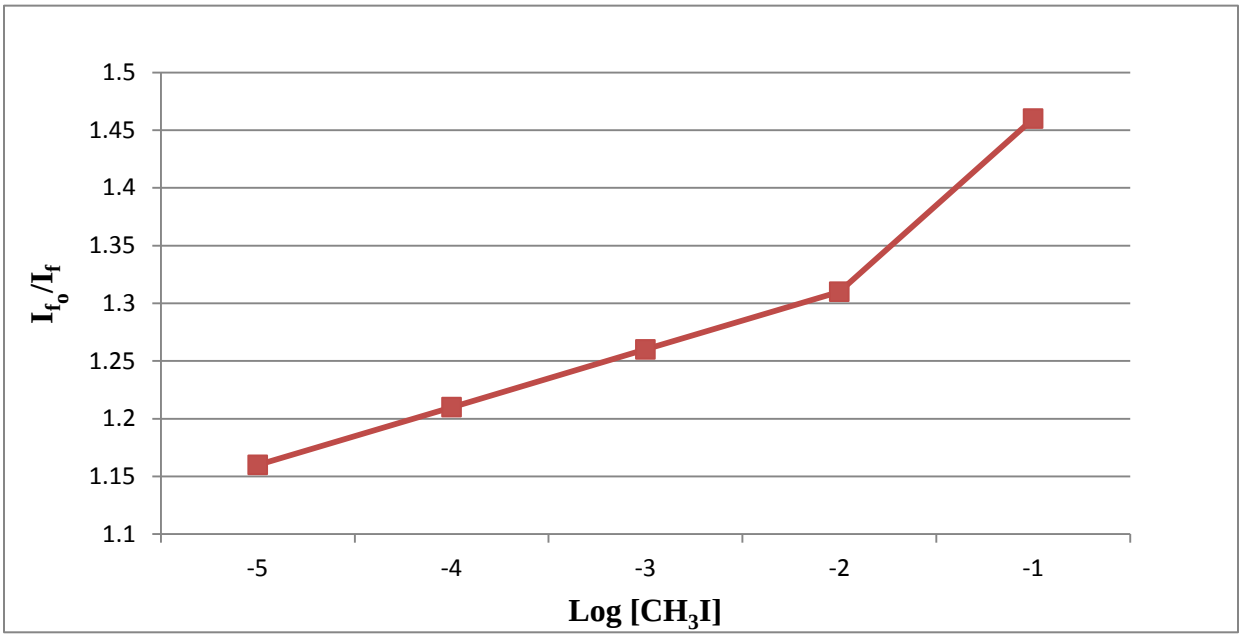


Figure 3: Stern-Volmer plot of the ratios of the fluorescence intensities for fluoranthene in the absence (I_{f0}) and presence (I_f) of methyl iodide

The Stern-Volmer plot can be used to figure out the kind of fluorescence quenching that is taking place in the solutions by methyl iodide. Also the possibility that at high quencher concentrations a fraction of the fluorophores were adjacent to the quencher at the moment of excitation and thus the chance that they were immediately deactivated was high. Research findings by Lakowicz, (7) concluded that positive deviations were observed when the extent of quenching was large. He discussed further that oxygen molecules adjacent to the fluorophore at the moment of excitation also contributed to such positive deviations. The large deviation could

also be an indication of inner-filter effect at higher concentrations of the fluorophore-quencher mixture if the excitation wavelength used could also be absorbed by the quencher.

The plot for fluoranthene in Figure 3 was interesting in that it was linear at the lower concentration range and that the slope abruptly changes when the concentration of methyl iodide was 1.0×10^{-2} M. The initial linearity in the curve at lower concentrations of methyl iodide confirms the occurrence of dynamic quenching. The positive deviation from the normal linear plot of Stern-Volmer observed at 1.0×10^{-2} M methyl iodide seems to point to the presence of some level of quenching by molecular oxygen, and there may be some degree of inner-filter effect

Studies of Oxygen Quenching

Oxygen is a well-known ubiquitous dynamic quencher, as it is small, has a very high diffusion coefficient, and is present everywhere. This set of studies would like to find out the extent to which it quenches the fluorophores on top of the added methyl iodide and titanium dioxide. Table 4 shows the results obtained for carbazole with various concentration of methyl iodide added in the presence and absence of oxygen (by degassing) and Figure 4 is the corresponding plot of the results.

Table 4: Fluorescence intensities found in normal and degassed solutions of carbazole with varying concentrations of methyl Iodide

Log Molar Concentration of Methyl Iodide	Fluorescent Intensity of Normal Carbazole Solution	Fluorescent Intensity of Degassed Carbazole Solution
-5.0	76.4	84.8
-4.0	70.6	81.0
-3.0	65.8	77.4
-2.0	37.8	44.5
-1.0	19.8	23.3

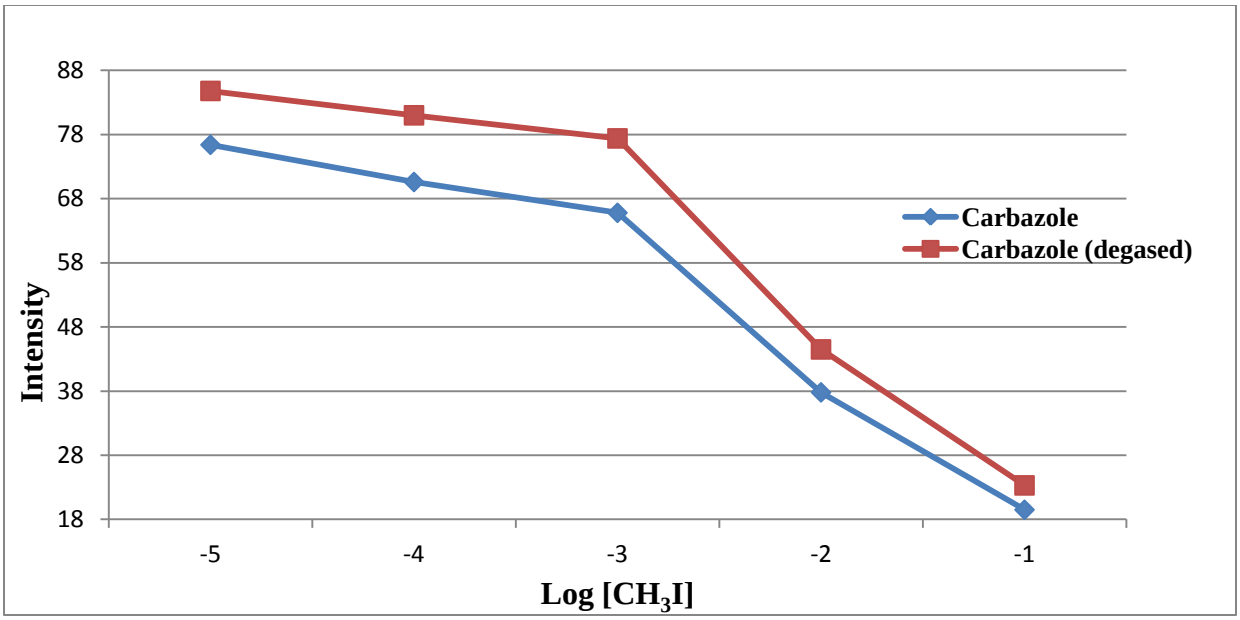


Figure 4: Plots showing the effect of methyl iodide of varying concentrations on the fluorescence intensity of normal (blue) and degassed (red) solutions of carbazole

The results from Table 4 and Figure 4 showed an initial gradual decrease in the fluorescent intensity of carbazole in the presence of methyl iodide. However when the concentration of the quencher was increased to 1.0×10^{-3} M, a sharp decrease in intensity was observed. This severe quenching of carbazole can be attributed to the charge-transfer interactions between the electronically excited carbazole and the ground state quencher acceptor. These charge-transfer interactions lead to the delocalization of electrons, leading to the formation of a hydrogen bonded complex, causing intense quenching by methyl iodide (76). Carbazole is also quenched by molecular oxygen because an increase in the signal intensities was observed after the solution was degassed. Also the two lines are somewhat parallel, and the amount of oxygen quenching was from the range of about 8% at 1.0×10^{-5} M to about 13% at 1.0×10^{-1} M. Oxygen quenching of carbazole could be linked to the complex formation between oxygen molecules and the molecules of carbazole at the ground state (63).

Table 5 shows the measured fluorescent intensities in normal and degassed solutions of carbazole containing varying concentrations of titanium dioxide. Figure 5 is the plot of the corresponding results obtained

Table 5: Fluorescence intensities found in normal and degassed solutions of carbazole with varying concentrations of titanium dioxide added

Log Molar Concentration of TiO_2 Nanoparticles	Fluorescent Intensity of Normal Carbazole Solution	Fluorescent Intensity of Degassed Carbazole Solution
-5.0	80.4	87.9
-4.0	78.7	87.0
-3.0	75.2	86.0
-2.0	71.0	79.8
-1.0	54.7	70.7

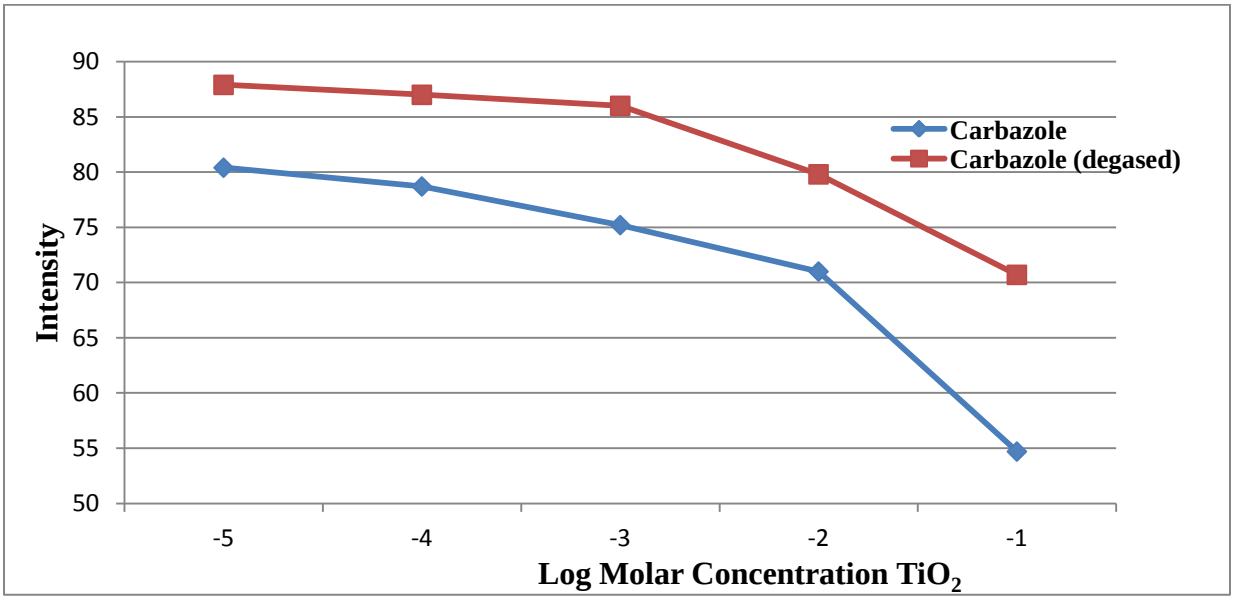


Figure 5: Plots of the intensities of normal (blue) and degassed (red) solutions of carbazole with varying concentrations of titanium dioxide nanoparticles added

A similar trend is observed for the varying concentration of titanium dioxide nanoparticles added under the same conditions as in the case where varying concentrations of methyl iodide were added in the presence and absence of oxygen. The gradual quenching of carbazole by titanium dioxide nanoparticles at two different rates occurring at 1.0 x 10^{-3}M was also seen. This trend of quenching by titanium dioxide is not as abrupt and severe as in the case by methyl iodide. Quenching of carbazole by titanium dioxide nanoparticles could be linked to

the possible charge-transfer complexation between carbazole and titanium dioxide. (72). However quenching observed with titanium dioxide was not as severe as compared to quenching by methyl iodide. Oxygen quenching was again of about 9% to 22% on top of quenching due to TiO_2.

Table 6 shows the results of the same study on normal and degassed solutions of pyrene with varying concentrations of methyl iodide added. Figure 6 is the plot of the corresponding results that show the effects of oxygen quenching on top of quenching by varying concentrations of methyl iodide on the fluorescence of pyrene.

Table 6: Fluorescent intensities of normal and degassed solutions of pyrene with varying concentrations of added methyl iodide

Log Molar Concentration of Methyl Iodide	Fluorescence Intensity of Normal Pyrene Solution	Fluorescence Intensity of Degassed Pyrene Solution
-5.0	58.0	67.5
-4.0	55.0	64.8
-3.0	48.0	55.0
-2.0	40.0	49.8
-1.0	34.8	45.6

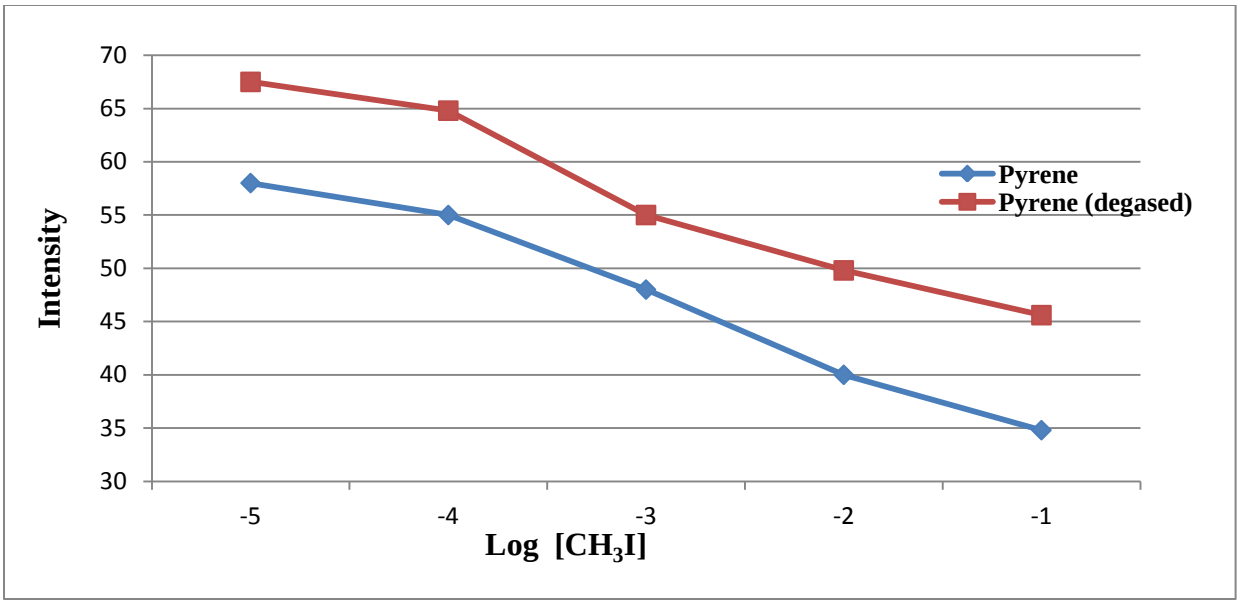


Figure 6: Plot of fluorescent intensities of normal (blue) and degassed (red) solutions of pyrene with varying concentrations of added methyl iodide

The gradual decrease of the fluorescence intensity of pyrene solutions with varying concentrations of methyl iodide added was almost linear with increasing concentration of methyl iodide. The presence of the heavy atom in methyl iodide can lead to quenching of pyrene due to heavy atom effect. The closeness of heavy atom to the fluorophore influences spin-orbit coupling that increases the rate of intersystem crossing (S_1-T_0). Ethanol is a polar solvent and could also influence the quenching efficiency. Dynamic quenching is hence very likely to have occurred. Dynamic quenching by diffusion is about 75% of total quenching of the fluorescence of pyrene (75). Quenching by molecular oxygen was also evident by the increase in fluorescence intensity of pyrene after degassing the solutions even though it was quenched by methyl iodide. In fact, from the difference in the fluorescence intensity of pyrene in normal and degassed solutions, one can estimate that oxygen quenching can account for about 14% of the loss in intensity at the lowest methyl iodide concentration to as high as about 24% at the highest methyl iodide concentration. Oxygen quenching can be attributed to the longer lifetime of pyrene fluorescence in the absence of oxygen, which renders it particularly susceptible to oxygen quenching (64). The higher apparent percentage of quenching at very high methyl iodide concentration may be due to other added factors such as charge interaction or fitter effect.

Table 7 and Figure 7 show the fluorescent intensities obtained on normal and degassed solution of pyrene with varying concentrations of titanium dioxide nanoparticles added now instead of methyl iodide.

Table 7: Fluorescent intensities obtained on normal and degassed solutions of pyrene with varying concentrations of titanium dioxide nanoparticles added

Log Molar Concentration of TiO_2 Nanoparticles	Fluorescence Intensity of Normal Pyrene	Fluorescence Intensity of Degassed Pyrene
-5.0	61.7	68.9
-4.0	59.1	65.6
-3.0	50.0	60.5
-2.0	45.0	52.6
-1.0	41.6	48.0

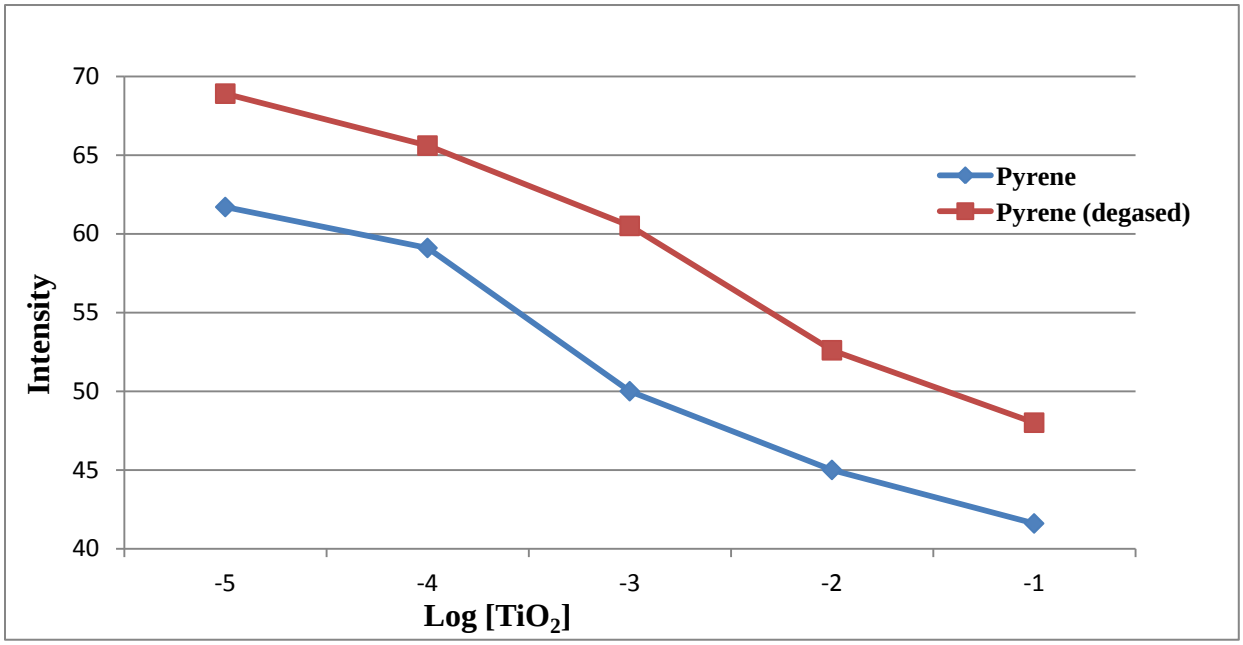


Figure 7: Plots of fluorescent intensities obtained on normal (blue) and degassed (red) solutions of pyrene with varying concentrations of titanium dioxide nanoparticles added

The results shown in Table 7 and plots in Figure 7 indicate a continuous decrease in the fluorescence intensity of pyrene with increasing concentration of titanium dioxide in normal and

degassed solutions. The plots were not entirely linear particularly when oxygen was present because quenching by factors other than titanium dioxide nanoparticles were also taking place. The cause of quenching by titanium dioxide may be the result of complex formation by titanium dioxide and aromatic hydrocarbons upon contact by physical adsorption (65). UV/VIS spectra indicate that the complex formation is by charge-transfer interactions. Comparing the fluorescence intensity seen in solutions with added titanium dioxide nanoparticles with that of pyrene solutions with added methyl iodide, the fluorescence intensity observed with titanium dioxide nanoparticles was slightly higher. This indicates that quenching of pyrene by methyl iodide was stronger. The results also showed that quenching by molecular oxygen were about 10%-13% on top of that due to titanium dioxide compared to that by methyl iodide of 14%-24%. As mentioned before the high concentration of methyl iodide might introduce other factors that quench more.

The results for a similar study of oxygen quenching on top of other quenchers for fluoranthene are shown in Table 8 and plotted in Figure 8.

Table 8: Fluorescence intensity of normal and degassed solutions of fluoranthene with varying concentrations of methyl iodide added

Log Molar Concentration of Methyl Iodide	Fluorescent Intensity of Normal Fluoranthene	Fluorescent Intensity of Degassed Fluoranthene
-5.0	71.1	82.2
-4.0	68.3	79.8
-3.0	65.2	76.7
-2.0	62.8	74.6
-1.0	56.7	72.0

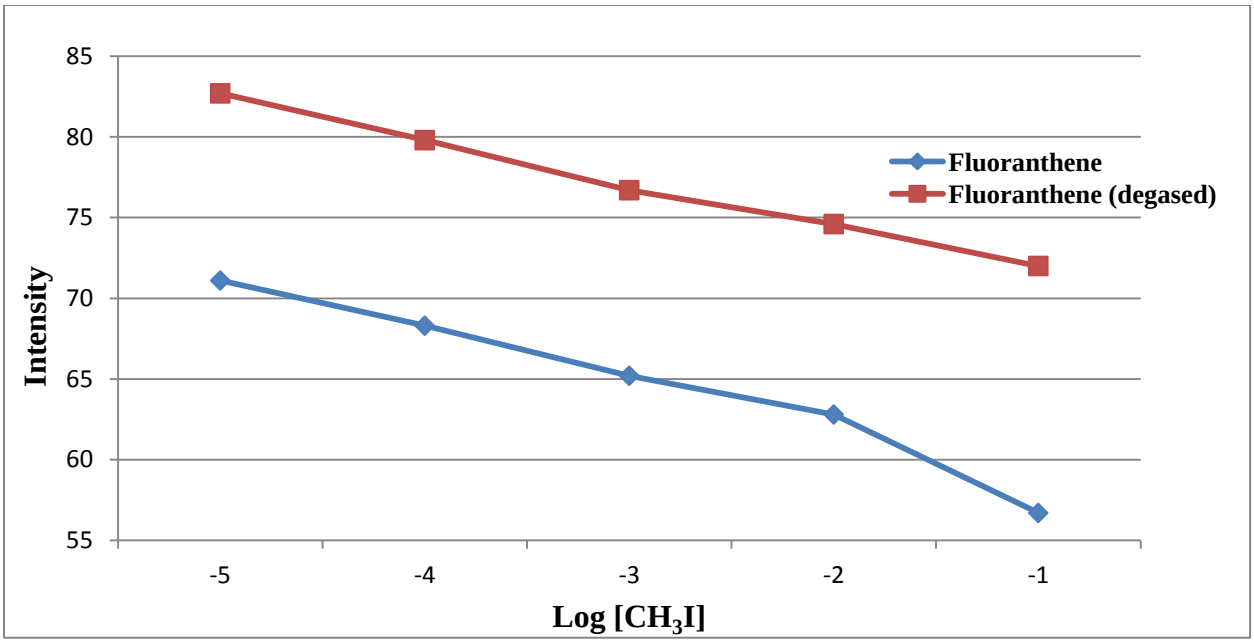


Figure 8: Plot showing the fluorescence intensity of solutions of normal (blue) and degassed (red) solutions of fluoranthene with varying concentrations of methyl iodide added

The intensities of fluoranthene showed a gradual decrease with increasing concentration of methyl iodide. It is interesting to note that fluoranthene was only slightly quenched by methyl iodide. Research done by Berlman et al. (74) reported the anomalous quenching characteristics of fluoranthene in cyclohexane solution. They reported that fluoranthene had a very large Stokes shift and appeared to be less prone to quenching and excimer formation. From the data in Table 8, it appeared that fluoranthene was relatively resistant to quenching by methyl iodide even at high concentrations. Quenching by oxygen was also slightly less in fluoranthene, 13.5%-21% as compared to pyrene. In degassed solution, the plot is quite linear indicating that quenching is mostly due to added methyl iodide alone.

Table 9 is the fluorescent intensities obtained from normal and degassed solutions of fluoranthene with varying concentrations of titanium dioxide added. Figure 9 is the plot of the corresponding results

Table 9: Fluorescent intensities obtained from normal and degassed solutions of fluoranthene with varying concentrations of titanium dioxide nanoparticles added

Log Molar Concentration of TiO_2 Nanoparticles	Fluorescent Intensity of Normal Fluoranthene	Fluorescent Intensity of Degassed Fluoranthene
-5.0	81.5	115
-4.0	76.0	99.5
-3.0	72.1	87.8
-2.0	57.2	72.2
-1.0	46.1	53.5

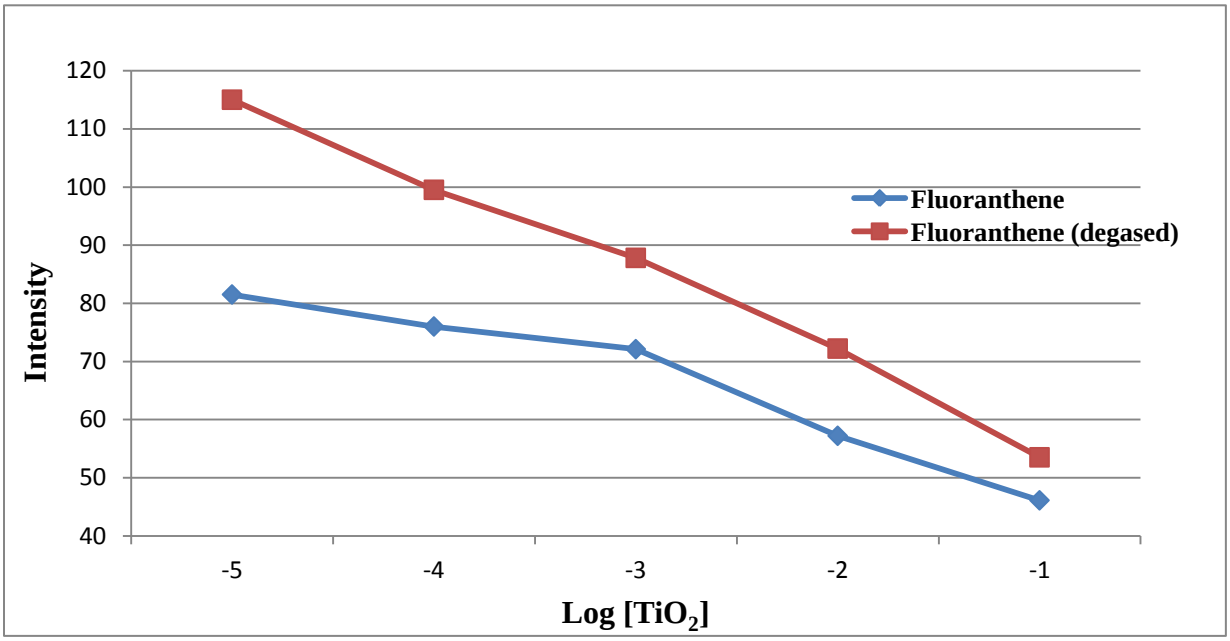


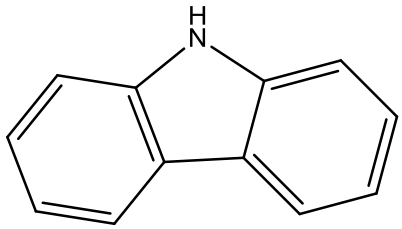
Figure 9: Plots of Fluorescent intensities obtained from normal (blue) and degassed (red) solutions of fluoranthene with varying concentrations of titanium dioxide added

Table 9 and Figure 9 show a gradual decrease in the fluorescence intensity of fluoranthene in the presence of titanium dioxide. Again, in degassed solutions, the plot is mostly linear, while the plot for the data in the normal un-degassed solutions was not. This difference can only be surmised by noting that other quenching factors besides titanium dioxide had taken place. Fluoranthene seemed to resist concentration quenching even at high titanium dioxide concentrations, compared to pyrene and carbazole. Quenching by oxygen was estimated

to be about 29% at the lowest concentration of titanium dioxide and about 14% at the highest concentration. This is different from the previous results as apparent oxygen quenching was higher at the highest concentration of quenchers. Also two distinct slopes in the plot are seen in normal solutions of fluoranthene with added titanium dioxide. It seems that higher concentration of titanium dioxide nanoparticles may have mitigated the effect of oxygen quenching by having some interaction with oxygen.

Studies of Fluorescence of Carbazole in Mixture of Methyl Iodide and TiO_2 Nanoparticle Solutions

Carbazole is an aromatic heterocyclic organic compound. It has nitrogen atom in its dibenzopyrrole system. Carbazole and its derivatives are widely used as an intermediate in synthesis of pharmaceuticals. It is used in luminescence as a photosensitizing as an additional charge transport material. The structure of carbazole is given below:



Carbazole

Figure 10: Structure of Carbazole

Solutions of different concentrations of carbazole were prepared and its fluorescence measured in the presence of varying concentrations of methyl iodide and titanium dioxide.

Table 10 shows the results obtained and Figure 11 the corresponding plot of the fluorescence intensities when varied concentration of titanium dioxide and in the presence of a constant concentration of 2.0×10^{-3} M and 2.0×10^{-6} M methyl iodide were mixed with aliquots of carbazole solution.

Table 10: Fluorescent intensities of carbazole solutions with varying concentrations of titanium dioxide and with fixed concentrations of methyl iodide added

Varying Log Molar Concentration of TiO_2 Nanoparticles	Fluorescent Intensity of Carbazole + Methyl Iodide (2.0×10^{-3} M)	Fluorescent Intensity of Carbazole + Methyl Iodide (2.0×10^{-6} M)
-5.0	64.1	71.1
-4.0	52.4	65.8
-3.0	46.9	56.8
-2.0	37.2	13.2
-1.0	25.4	10.5

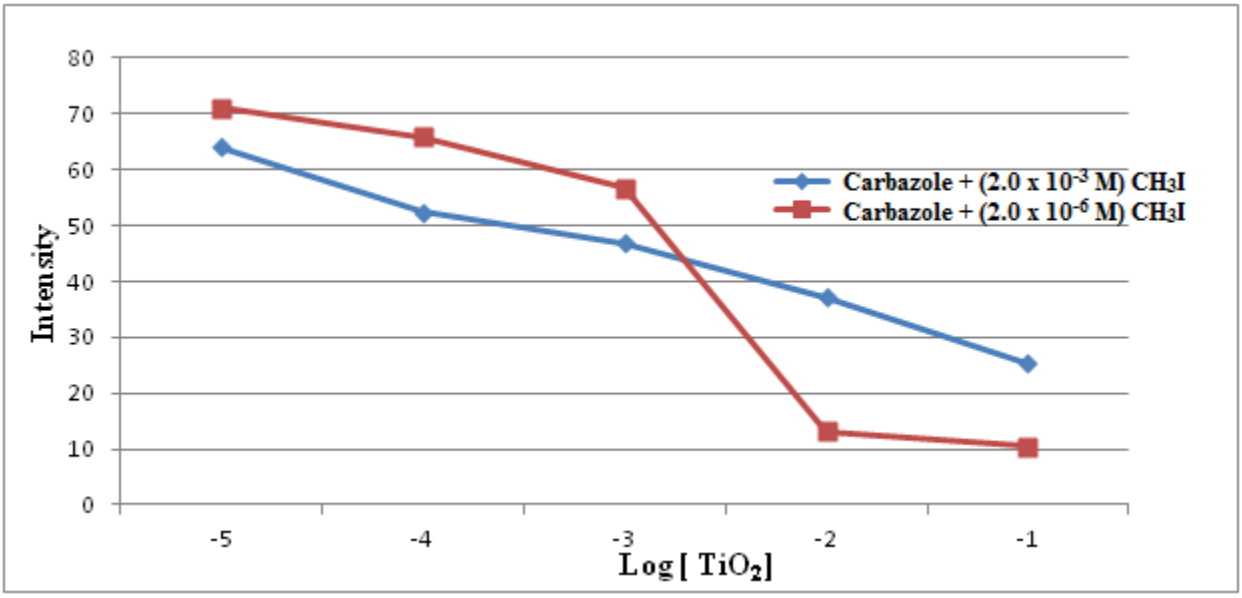


Figure 11: Plots of fluorescence intensities obtained from carbazole solutions with varying concentrations of titanium dioxide and with 2.0×10^{-3} M (blue) and 2.0×10^{-6} M (red) methyl iodide added

The results show a moderate gradual quenching of carbazole for both constant concentration of methyl dioxide added. For the carbazole solutions with 2.0×10^{-3} M CH_3I added, varying the concentration of TiO_2 nanoparticles showed that quenching was rather linear as was observed in the case of carbazole solutions without added CH_3I. However, for the

solutions in which 2.0 x 10^{-6} M of methyl dioxide was present, when the titanium dioxide concentration reached 1.0 x 10^{-3} M, carbazole was abruptly and substantially quenched. This anomaly cannot be readily explained and may have to await further research.

Table 11 shows the results obtained and Figure 12 the corresponding plot of the fluorescence intensities when varied concentration of methyl iodide and in the presence of a constant concentration of 1.0 x 10^{-4} M and 1.0 x 10^{-7} M titanium dioxide nanoparticles were mixed with aliquots of carbazole solution.

Table 11: Fluorescence intensities of carbazole solutions with varying concentrations of methyl iodide containing added fixed concentrations of titanium dioxide nanoparticles

Log Molar Concentration of Methyl Iodide	Fluorescent Intensity of Carbazole + 2.0 x 10^{-4} M TiO_2 Nanoparticles	Fluorescent Intensity of Carbazole + 2.0 x 10^{-7} M TiO_2 Nanoparticles
-5.0	71.2	76.4
-4.0	75.6	32.5
-3.0	81.4	28.6
-2.0	48.8	15.7
-1.0	35.1	11.8

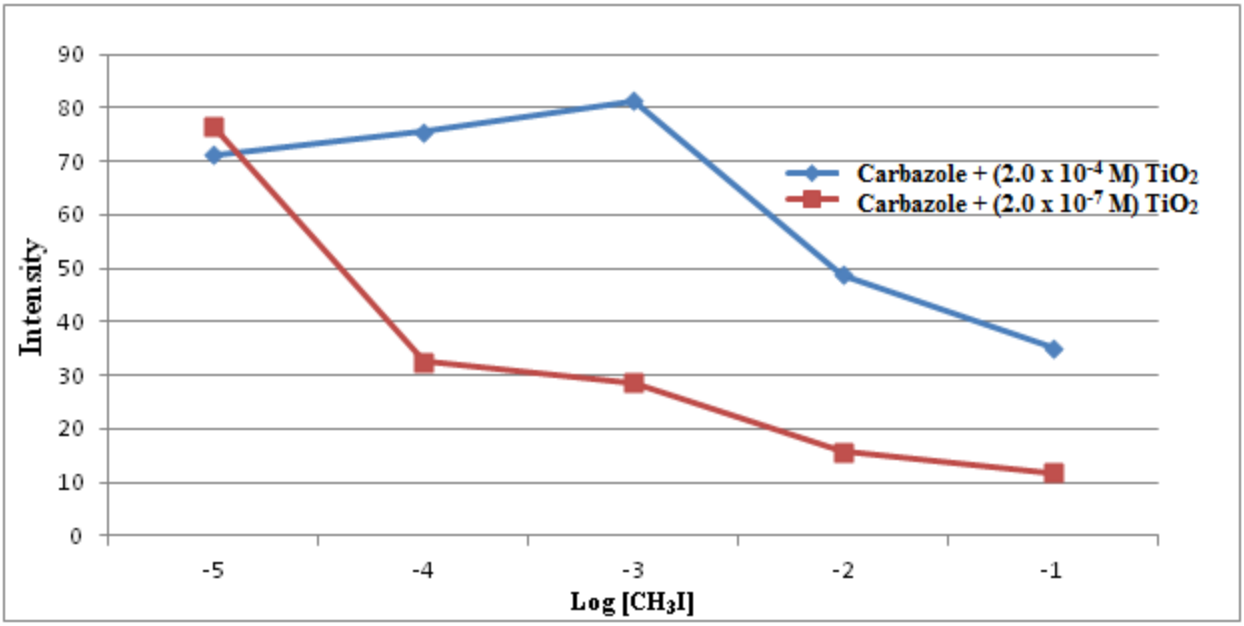


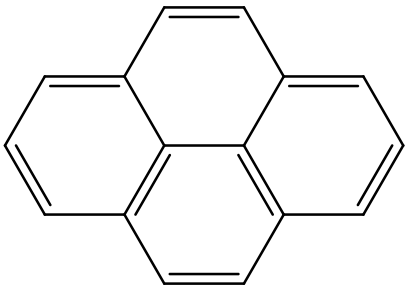
Figure 12: Fluorescent intensities of carbazole solutions with varying concentrations of methyl iodide containing fixed concentrations of 2.0 x 10^{-7} M (red) and 2.0 x 10^{-4} M TiO2 (blue) added

The results from this set of experiments are puzzling, especially in the case where the relatively high concentration of 2.0 x 10^{-4} M of TiO_2 nanoparticles were added to the aliquots of carbazole. An increase in the fluorescence intensities actually occurred instead of been quenched in the range of 10^{-5} M to 10^{-3} M TiO_2 nanoparticles and carbazole solutions. The possible explanation for this initial increase could be that at low concentrations of methyl iodide the amount of titanium dioxide nanoparticles present interacts with methyl iodide and inhibit its ability to quench carbazole. The presence of the titanium dioxide is known to lessen the effect of methyl iodide quenching so far with pyrene and carbazole.

At low concentration of titanium dioxide of 2.0 x 10^{-7} M added to the carbazole, varying concentrations of methyl iodide did not affect the quenching ability of methyl iodide on carbazole and the fluorescence intensities of carbazole was quenched in proportional to concentrations. The nonlinearity may be due to additional factors like differing degree of oxygen quenching on top of quenching by methyl iodide.

Studies on Fluorescence of Pyrene in Mixtures of Methyl Iodide and TiO_2 Nanoparticles

Pyrene, a fluorescent compound, and its derivatives are commercially used for dyes and dye precursors. Some of its derivatives are also important molecular proves through fluorescence spectroscopy. The chemical structure of pyrene is shown below:



Pyrene

Figure 13: Structure of Pyrene

Table 12 shows the fluorescence intensities of pyrene with varying concentrations of TiO_2 nanoparticles with 2.0 x 10^{-3} M and 2.0 x 10^{-5} M CH_3I added and Figure 14 the corresponding plots of the results.

Table 12: Fluorescent intensities of pyrene solutions with varying concentrations of titanium dioxide nanoparticles containing fixed concentrations of methyl iodide added

Varying Log Molar Concentration of TiO_2 Nanoparticles	Fluorescence Intensity of Pyrene+ 2.0 x 10^{-3} M CH_3I	Fluorescence Intensity of Pyrene + 2.0 x 10^{-5} M CH_3I
-5.0	49.6	61.5
-4.0	52.0	55.0
-3.0	47.5	50.4
-2.0	41.3	44.8
-1.0	34.9	37.7

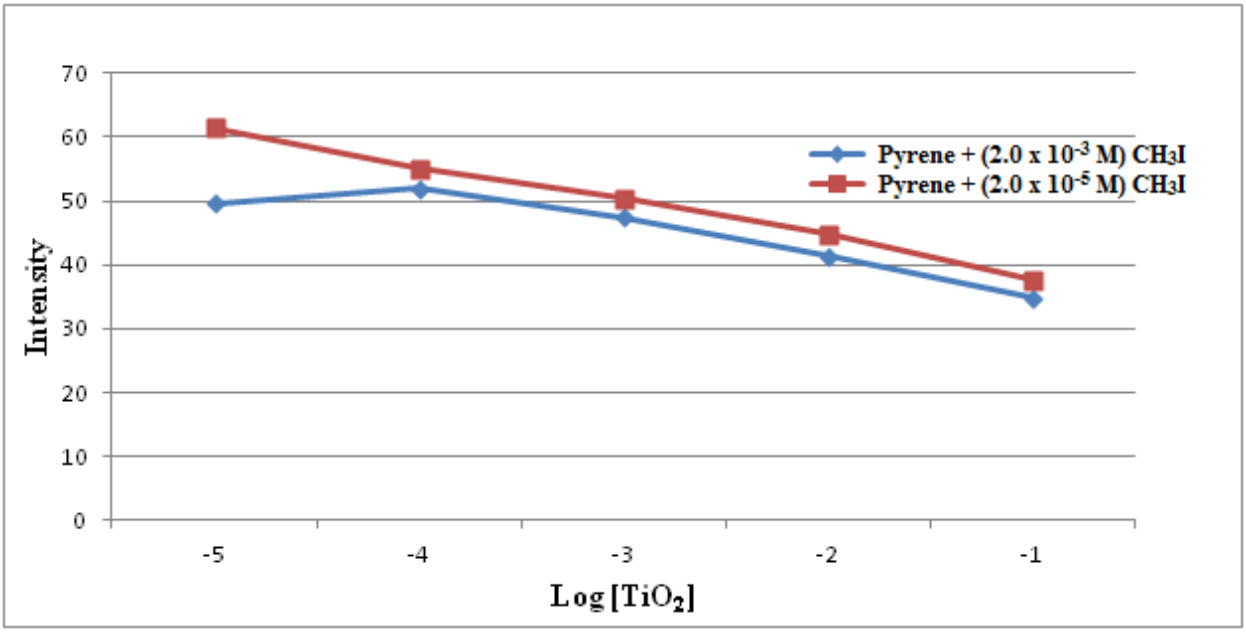


Figure 14: Plots of the fluorescence intensities of solutions of pyrene with varying concentrations of titanium dioxide with 2.0 x 10^{-5} M (red) and 2.0 x10^{-3} M (blue) of methyl iodide added

At a high methyl iodide concentration of 2.0 x 10^{-3} M added to pyrene solutions, pyrene showed, a gradual decrease in intensity except for the anomalous pyrene solution at 1.0 x 10^{-5} M TiO_2 nanoparticles concentration, the quenching of pyrene was quite linear with varying concentrations of titanium dioxide nanoparticles. In the case with 2.0 x 10^{-5} M CH_3I present, the quenching by TiO_2 nanoparticles was practically linear with concentrations, with a very small different in degree of oxygen quenching taking place as the two plots are almost parallel and close to one another.

Table 13 shows the fluorescence intensities of pyrene with varying concentrations of CH_3I with 2.0 x 10^{-4} M and 2.0 x 10^{-7} M TiO_2 nanoparticles added and Figure 15 is the corresponding plots of the results.

The results showed a similar quenching trend as before. It seems that the addition of either methyl iodide or titanium dioxide nanoparticles to the fluorophore solutions did not affect how much quenching occurs. Thus the fluorescence intensities of the pyrene solutions decreased with increasing concentrations of quenchers practically linearly.

Table 13: Fluorescent intensities of pyrene solutions with varying concentrations of methyl iodide containing fixed concentrations of titanium dioxide nanoparticles added

Varying Log Molar Concentration of Methyl Iodide	Fluorescent Intensity of Pyrene + 2.0 x 10^{-4} M TiO_2 Nanoparticles	Fluorescent Intensity of Pyrene + 2.0 x 10^{-7} M TiO_2 Nanoparticles
-5.0	62.1	75.7
-4.0	53.4	70.8
-3.0	47.5	68.4
-2.0	45.7	63.6
-1.0	42.6	59.5

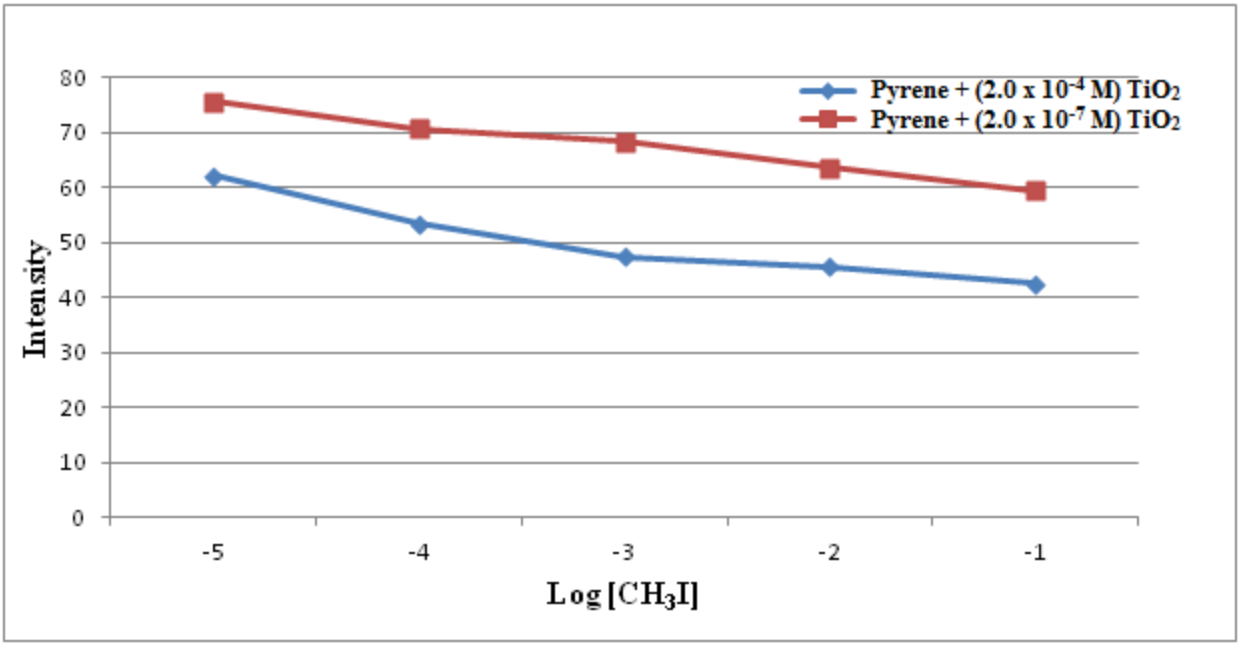


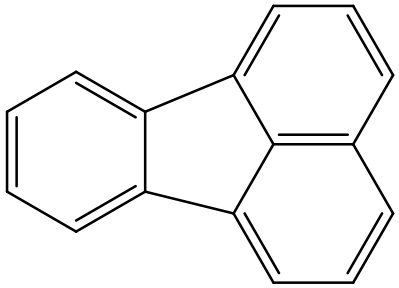
Figure 15: Fluorescent intensities of pyrene solutions with varying concentrations of methyl iodide containing fixed concentrations of 2.0 x 10^{-7} M (red) and 2.0 x 10^{-4} M TiO_2 nanoparticles (blue) added

The plots are not as linear as they should be, indicating that some other quenching factors might be present causing variations. Pyrene solutions containing a higher concentration of TiO_2 nanoparticles of 2.0 x 10^{-4} M however do have lower measured fluorescence intensities. This

greater quenching could be attributed to some inner-filter effect due to the presence of lots of molecules in the solution. But this should only occur more at combined higher methyl iodide concentration, not throughout the whole series. Also compared to the previous case where the concentrations of the TiO_2 nanoparticles are varied instead of CH_3I, the lines are further apart, that is, the difference in quenching was greater. Thus, the simultaneous presence of a quencher containing a heavy atom like iodide and titanium dioxide nanoparticles must have led to some charge transfer interactions that cause differences in the degree of quenching by either of them alone by themselves.

Studies of the Fluorescence of Fluoranthene in Mixture of Methyl Iodide and TiO_2 Nanoparticles

Fluoranthene, a polycyclic aromatic hydrocarbon, consists of a benzene and naphthalene unit connected by a five membered ring. It is a nonalternate conjugated hydrocarbon that has been of lots of interest in theoretical and experimental studies (66). It is not thermodynamically stable as compared to pyrene. It is highly fluorescent and its structure is shown below:



Fluoranthene

Figure 16: Structure of Fluoranthene

Table 14 shows the fluorescence intensities of fluoranthene with varying concentrations of CH_3I with 2.0×10^{-4} M and 2.0×10^{-7} M TiO_2 nanoparticles added and Figure 17 is the corresponding plots of the results.

Table 14: Fluorescent intensities of fluoranthene solutions with varying concentrations of methyl iodide containing fixed concentrations of titanium dioxide nanoparticles added

Varying Log Molar Concentration of Methyl Iodide	Fluorescent Intensity of Fluoranthene + 2.0 x 10^{-4} M TiO_2 Nanoparticles	Fluorescent Intensity of Fluoranthene + 2.0 x 10^{-7} M TiO_2 Nanoparticles
-5.0	74.6	97.3
-4.0	70.7	84.0
-3.0	67.6	77.5
-2.0	66.1	73.8
-1.0	61.2	68.0

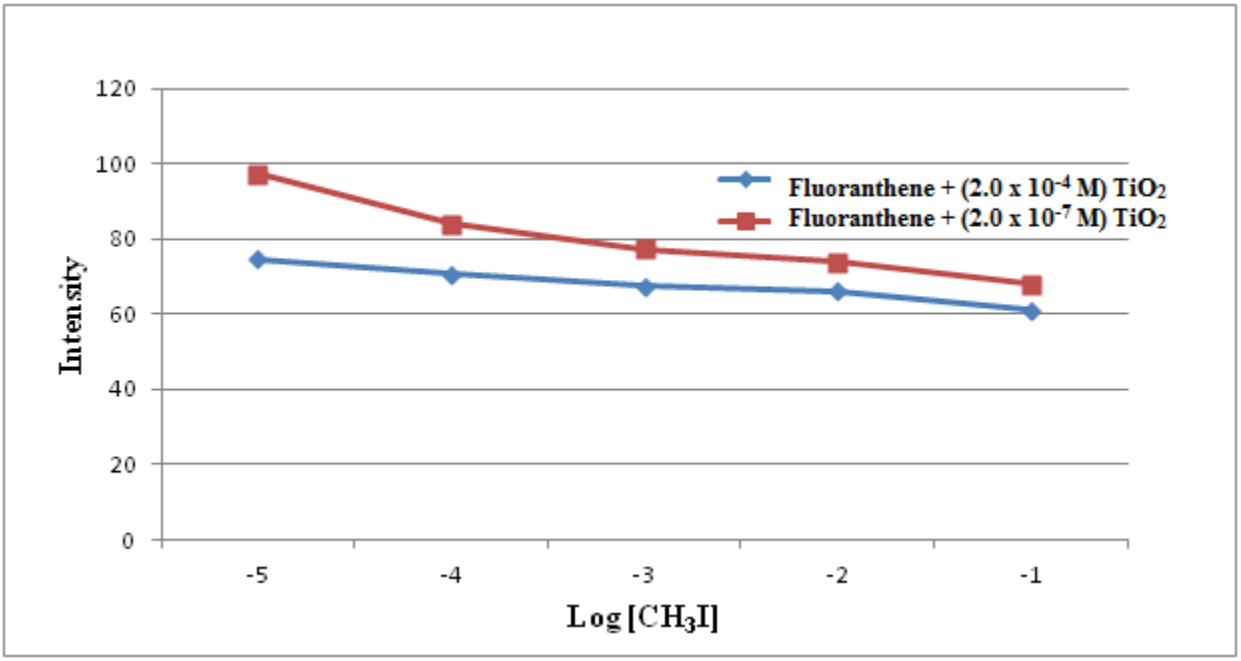


Figure 17: Fluorescent intensities of fluoranthene solutions with varying concentrations of methyl iodide containing fixed concentrations of 2.0 x 10^{-7} M (red) and 2.0 x 10^{-4} M TiO_2 nanoparticles (blue) added

From the results shown in the table and plots in the figure, methyl iodide seemed not to quench fluoranthene much when the added concentration of TiO_2 nanoparticles was high, while at the lower added TiO_2 nanoparticles concentration, quenching by varying concentrations of

methyl iodide appeared to be concentration dependent. Quenching also may be caused by other factors to a small extent in the fluoranthene solutions with a high concentration of TiO_2. In fluoranthene solutions with a high concentration of titanium dioxide nanoparticles, some interactions took place that inhibited quenching by methyl iodide. It would be interesting to explore this further.

In the next study, the concentrations of titanium dioxide nanoparticles were varied. Table 15 shows the fluorescence intensities of fluoranthene with varying concentrations of TiO_2 nanoparticles with 2.0×10^{-3} M and 2.0×10^{-5} M CH_3I added and Figure 18 is the corresponding plots of the results.

Table 15: Fluorescent intensities of fluoranthene solutions with varying concentrations of titanium dioxide nanoparticles containing fixed concentrations of 2.0×10^{-3} M and 2.0×10^{-5}M methyl iodide added

Varying Log Molar Concentration of TiO_2 Nanoparticles	Fluorescent Intensity of Fluoranthene + 2.0×10^{-3} M CH_3I	Fluorescent Intensity of Fluoranthene + 2.0×10^{-5} M CH_3I
-5.0	50.0	30.2
-4.0	45.5	28.4
-3.0	40.9	24.2
-2.0	35.6	20.6
-1.0	26.0	16.0

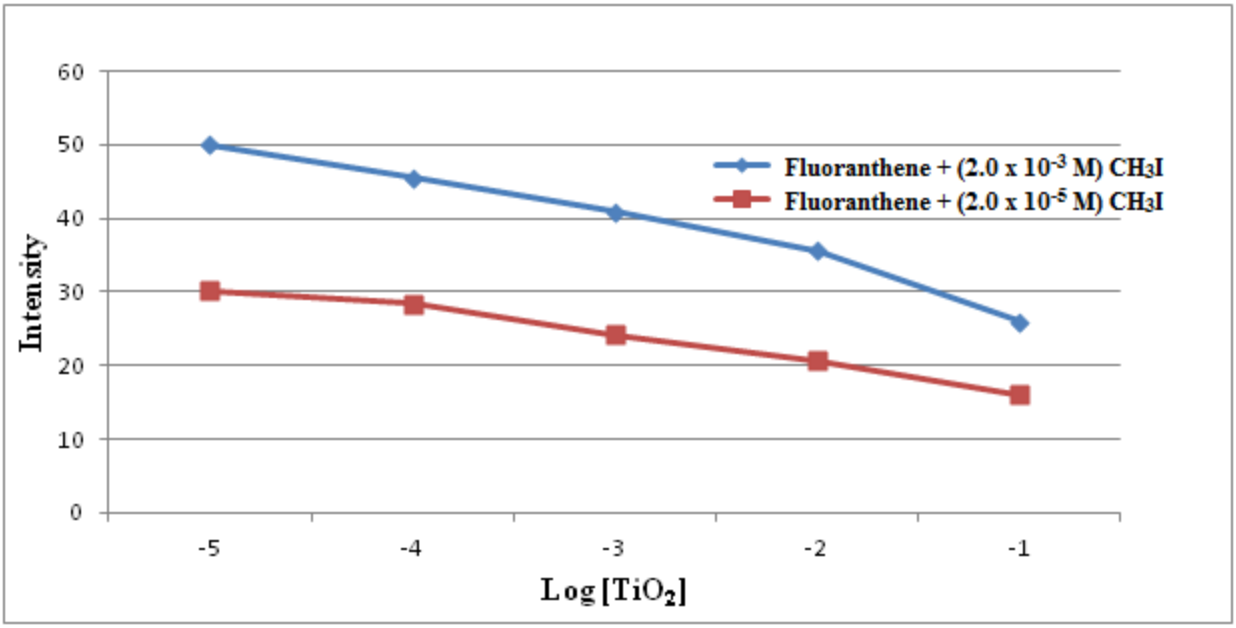


Figure 18: Plots of the fluorescence intensity of solutions of fluoranthene with varying concentrations of titanium dioxide nanoparticles with 2.0 x 10^{-5} M (red) and 2.0 x 10^{-3} M (blue) of methyl iodide added

The results and plots show that quenching by varying concentrations of the titanium nanoparticles were quite linear with other quenching factors superimposed upon it. Also the fluorescent intensities started at a much lower values indicating that methyl iodide present in all the fluoranthene solutions exerted its quenching effect quite strongly especially at its concentration of 2.0 x 10^{-3} M. However, interestingly at the low 2.0 x 10^{-5} M methyl iodide quenching by varying concentrations of titanium dioxide was at a steeper rate than in the case with methyl iodide at 2.0 x 10^{-3} M. Why the high concentration of methyl iodide with increasing concentration of titanium dioxide did not quench as much is not fully understood. These observations were peculiar to fluoranthene. This could once again be attributed to the tendency of fluoranthene to be less susceptible to quenching.

CHAPTER 5

CONCLUSION

The fluorescence intensities of fluorescent compounds are susceptible to quenching by diverse compounds and environmental factors. In this study, the fluorophores; carbazole, pyrene, and fluoranthene were used to study the effects of oxygen, a well know quencher methyl iodide and, finally, titanium dioxide nanoparticles. The findings from the experiments fulfilled most of the expectations of the research. Some results however, were not obvious and need further investigation.

Methyl Iodide the quencher used for this research was proven to be an efficient quencher of all three compounds and quenched via dynamic quenching. Methyl iodide contains a heavy atom giving rise to the well-known heavy atom effects that quench by promoting inter system crossing. Quenching by molecular oxygen was also evident with all three compounds, as can be seen with considerable increase in the fluorescent intensities after the removal of oxygen from the solutions by degassing with helium gas. Titanium dioxide was also found to quench dynamically and found in most cases that quenching to be linear with increasing concentrations.

Carbazole was quenched by both methyl iodide and titanium dioxide, to a lesser degree by titanium dioxide. Carbazole was also quenched by molecular oxygen because a considerable increase in the signal intensities was observed after the solution was degassed. The effects on quenching by varying concentration of methyl iodide in the presence of 2.0×10^{-4}M TiO_2 nanoparticles gave anomalous results as compared to when 2.0×10^{-7} M of it was added to the solutions of carbazole, and the cause is not clear and need further work to confirm them. In the case when the concentration of titanium dioxide nanoparticles was varied with a given concentration of methyl iodide added to the solution of carbazole, the results obtained were close to what was expected.

The fluorescence of pyrene was quenched dynamically by methyl iodide, titanium dioxide nanoparticles, and oxygen. Quenching of pyrene by titanium dioxide nanoparticles was again to a lesser degree by methyl iodide as in the case of carbazole. Oxygen quenching of pyrene is well known and pyrene was quenched to a larger extent by oxygen than was carbazole.

Interestingly, the quenching by varying concentrations of titanium dioxide nanoparticles with 2.0 x 10^{-3} M and 2.0 x 10^{-5} M methyl iodide added to the solutions did not show much difference. However quenching by varying concentrations of methyl iodide with the addition titanium dioxide nanoparticles to the pyrene solutions were quite different and to a larger extent at higher concentration of 2.0 x 10^{-4} M than 2.0 x 10^{-7} M. This might be attributed to the tendency of titanium dioxide to form a complex with pyrene, based on a charge transfer interaction. High concentrations of both titanium dioxide nanoparticles thus might exacerbate quenching by methyl iodide.

Fluoranthene, a highly fluorescent fluorophore, was slightly quenched by both methyl iodide and titanium dioxide. Quenching by molecular oxygen was also observed. Quenching by varying concentrations of methyl iodide, in the case of fluoranthene, was quite different and to a much lower degree by the presence of added titanium dioxide nanoparticles at both higher concentration of 2.0 x 10^{-4} M or the lower concentration of 2.0 x 10^{-7} M. Also the quenching did not change greatly with changes in the methyl iodide concentration. However, when the concentration of titanium dioxide nanoparticles was varied while the added concentration of methyl iodide was kept at 2.0 x 10^{-3} M or 2.0 x 10^{-5} M, the fluorescence intensities varied much more greatly and the overall intensities were much lower. It was found, in the case of fluoranthene, titanium dioxide nanoparticles quench more strongly in the presence of methyl iodide. This was not studied or reported before, so should be explored further.

From the results obtained for all compounds, it can be concluded that methyl iodide is an efficient dynamic quencher for all three compounds studied. Oxygen also quenched all three fluorophores but to a greater extent for pyrene. Also all fluorophores were quenched to a lesser degree by titanium dioxide as compared to quenching by methyl iodide. Finally, varying concentrations of titanium dioxide nanoparticles in the presence of methyl iodide, and vice versa, had different effects on the quenching of these fluorophores. Fluoranthene, however, could be an interesting focus of further research as some of its fluorescence characteristics in the presence of both methyl iodide and titanium dioxide were not expected.

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