

THE STUDY OF FLUORESCENCE ENHANCING AND QUENCHING EFFECT OF
LUMINESCENT MATERIALS IN SOL GEL FOR THE DETECTION OF GASES

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GASES

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*Here's a token of love and gratitude.
Especially for my family and friends,
As my sources of strength, comfort and patience,
Thank you all for having faith in me,
I'm truly blessed and inspired!*

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ABSTRACT

The control of CO₂, SO₂ and O₂ emission is important thus the determination of its concentration is important in many applications. This study is carried out to investigate and characterize the fluorescent properties of metal-chelate using fluorescent materials such as α -naphthoflavone (7, 8-benzoflavone), fluorescein and luminol. Initial investigation showed that α -naphthoflavone exhibit fluorescence at $\lambda_{em} = 426$ nm ($\lambda_{ex} = 343$ nm), fluorescein exhibit fluorescence at $\lambda_{em} = 510$ nm ($\lambda_{ex} = 483$ nm) and luminol at $\lambda_{em} = 421$ nm ($\lambda_{ex} = 386$ nm) at optimum pH 10.06, pH 8.90 and pH 8.62 respectively. The presence of CO₂, SO₂ and O₂ were detected by α -naphthoflavone, fluorescein and luminol. α -naphthoflavone, fluorescein and luminol giving a non-linear change in the emission intensity with increase volume of SO₂ and O₂ gas. Therefore, α -naphthoflavone, fluorescein and luminol are not suitable for detecting SO₂ and O₂ gas. When dissolving CO₂ gas into the complex, the emission wavelength ($\Delta\lambda_{em}$) for fluorescein was shifted by 6 nm and luminol was 9 nm. The fluorescence properties of α -naphthoflavone, fluorescein and luminol entrapped in sol gel were also investigated. Results indicated that the emission intensity of the entrapped fluorescent material in sol gel decreased as compared to fluorescence in solvent. For the detection of CO₂, the gas was bubbled onto the sol gel surface. It was observed that the emission intensity of α -naphthoflavone in sol gel increased linearly as the volume of CO₂ gases increased. Meanwhile, fluorescein and luminol shows a non linear change with the increased CO₂ amount in sol gel. Among the five metals ions that complex with α -naphthoflavone, fluorescein and luminol, it was found that the emission intensity of fluorescein-ferum complex decreases linearly after exposure to increasing amount of CO₂ in sol gel giving a linear relationship with an equation was $y = -7.2252 x + 271.01$ and a correlation of 0.9234.

ABSTRAK

Pengawalan perlepasan gas karbon dioksida, sulfur dioksida dan oksigen adalah sangat penting dalam pengesanan kepekatannya dalam banyak aplikasi. Dalam kajian ini, ciri-ciri bahan pendarfluor seperti α -naftoflavon (7,8-benzoflavon), fluoresin dan luminol dengan logam-kelat telah dikaji. Keputusan awal menunjukkan bahawa α -naftoflavon berpendarfluor pada puncak pemancaran λ_{em} 426 nm (λ_{ex} = 343 nm), fluoresin berpendarfluor pada puncak pemancaran λ_{em} 483 nm (λ_{ex} = 510 nm) manakala luminol pada λ_{em} 386 nm (λ_{ex} = 421 nm) masing-masing pada pH optimum 10.06, pH 8.90 dan pH 8.62. Kehadiran gas karbon dioksida telah diuji dengan menggunakan α -naftoflavon, fluoresin dan luminol dan hasil menunjukkan bahawa keamatan puncak pemancaran adalah berubah secara tidak linear dengan penambahan CO₂. Didapati juga perubahan kedudukan puncak pemancaran ($\Delta\lambda_{em}$) bagi fluoresin adalah 6 nm dan luminol adalah 9 nm. Bahan berpendarfluor α -naftoflavon, fluoresin dan luminol terperangkap dalam sol gel juga dikaji. Keputusan menunjukkan keamatan bahan berpendarfluor yang terperangkap dalam sol gel berkurang. Gas CO₂ dialirkan di permukaan sol gel. Keamatan puncak pemancaran bagi α -naftoflavon dalam sol gel bertambah secara linear dengan kehadiran gas CO₂. Sementara itu, fluoresin dan luminol menunjukkan perubahan yang tidak linear dengan penambahan CO₂ dalam sol gel. Dalam lima ion logam yang dipilih untuk membentuk kompleks dengan α -naftoflavon, fluoresin dan luminol, didapati bahawa keamatan puncak pemancaran bagi kompleks fluoresin-ferum berkurang secara linear setelah gas CO₂ dialirkan di permukaan sol gel menghasilkan satu persamaan linear dengan $y = -7.2252 x + 271.01$ dan dengan korelasi 0.9234.

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LIST OF SYMBOLS

ANF	-	α -naphthoflavone
CO ₂	-	carbon dioxide
NaOH	-	sodium hydroxide
NH ₄ OH	-	ammonium hydroxide
λ_{em}	-	emission wavelength
λ_{ex}	-	excitation wavelength
nm	-	nanometer
pH	-	$-\log [H^+]$
°C	-	degrees Celsius
mL	-	millilitres
μ L	-	micro litres
g	-	gram
R ²	-	correlation coefficient
DMF	-	N,N-dimethylformamide
mol L ⁻¹	-	mole per litres
La	-	lanthanum
Mg	-	magnesium
Mn	-	manganese
Cd	-	cadmium
Fe	-	ferum / iron

CHAPTER 1

INTRODUCTION

1.1 Principle of Fluorescence

All chemical compounds absorb energy which causes excitation of electrons in the molecule, resulting in the transitions between discrete electronic energy states. For a transition to occur, the absorbed energy must be equivalent to the difference between the initial electronic state and a high-energy state. This value is constant and is characteristic of the molecular structure. This is termed as the excitation wavelength. If conditions permit, an excited molecule will return to the ground state by emission of energy through heat and/or emission of energy quanta such as photons. The emission energy or wavelength of these quanta are also equivalent to the difference between the two discrete energy states and are characteristic of the molecular structure [1-3].

Fluorescence occurs when a molecule absorbs photons from the UV-visible light spectrum (200-900 nm), causing transition to a high-energy electronic state and then emits photons as it returns to its initial state, in less than 10^{-9} sec. Some energy, within the molecule, is lost through heat or vibration so that emitted energy is less than the excited energy; i.e., the emission wavelength is always longer than the excitation wavelength. The difference between the excitation and emission wavelengths is called the Stokes shift [2, 5].

Fluorescent compounds or fluorophors can be identified and quantified on the basis of their excitation and emission properties. The excitation spectra is determined by measuring the emission intensity at a fixed wavelength, while varying the excitation wavelength. The emission spectra are determined by measuring the variation in emission intensity wavelength for a fixed excitation wavelength. The excitation and emission properties of a compound are fixed, for a given instrument and environmental condition, and can be used for identification and quantification [1-3].

The principal advantage of fluorescence over radioactivity and absorption spectroscopy is the ability to separate compounds on the basis of either their excitation or emission spectra, as opposed to a single spectra. This advantage is further enhanced by commercial fluorescent dyes that have narrow and distinctly separated excitation and emission spectra.

Although maximum emission occurs only for specific excitation and emission wavelength pairs, the magnitude of fluorescent intensity is dependent on both intrinsic properties of the compound and on readily controlled experimental parameters, including intensity of the absorbed light and concentration of the fluorophor in solution. The intensity of emitted light, F , is described by the relationship

$$F = \phi I_0 (1 - e^{-\epsilon bc})$$

where ϕ is the quantum efficiency, I_0 is the incident radiant power, ϵ is the molar absorptivity, b is the path length of the cell, and c is the molar concentration of the fluorescent dye [1, 5].

The quantum efficiency is the percentage of molecules in an excited electronic state that decay to ground state by fluorescent emission; i.e., rapid emission of a light photon in the range of 200-900 nm. This value is always less than or equal to unity and is characteristic of the molecular structure. A high efficiency is

desirable to produce a relative higher emission intensity. All non-fluorescent compounds have a quantum efficiency of zero.

The intensity of the excitation light, which impinges on the sample, depends on the source type, wavelength and other instrument factors. The light source, usually mercury or xenon, has a characteristic spectrum for emission intensity relative to wavelength.

At high dye concentrations or short path lengths, fluorescence intensity relative to dye concentration decreases as a result of "quenching". As the concentration of molecules in a solution increases, the probability that excited molecules will interact with each other and lose energy through processes other than fluorescent emission increases. Any process that reduces the probability of fluorescent emission is known as quenching. Other parameters that can cause quenching include presence of impurities, increased temperature, or reduced viscosity of the solution media [2-5].

1.1.1 Factors Affecting Quantitative Accuracy of Fluorescent Measurement

1.1.1.1 Temperature Effect

Temperature usually changes the fluorescence of a solution only a few percent per degree. Changes in temperature affect the viscosity of the medium and hence the number of collisions of the molecules of the fluorophors with solvent molecules. Fluorescence intensity is sensitive to such changes and the fluorescence of many certain fluorophors shows temperature dependence [1, 4, 5].

At low temperature the fluorescence reaches a limiting maximum, and at high temperatures tends to zero. Examples are the enhancement of fluorescence by low temperatures, by embedding molecules in rigid or highly viscosity glasses and

plastics by structural effects inhibiting free internal movements such as the oxygen and nitrogen bridges in xanthanene [5].

1.1.1.2 pH Effect

Relatively small changes in pH will sometimes radically affect the intensity and spectral characteristics of fluorescence. Accurate pH control is essential and when particular buffer solutions are recommended in an assay procedure, they should not be changed without investigation [1, 5]. Quinine or β -naphthol may be used as fluorescence indicator for titration purpose of coloured solutions because of their marked changes of fluorescence between acid and alkaline condition [1].

Most phenols are fluorescent in neutral or acidic media, but the presence of a base leads to the formation of non-fluorescent phenate ions [1]. The ionization of a weak acid or base produces a considerable change in the electronic structure of the molecule and this affect both the light-absorption curve and the power of fluorescence.

1.1.1.3 Solvent Effect

The intensity of fluorescence of a substance may vary considerably with change of solvent, but although the difference can be expressed formally as the influence of the solvent in facilitating non radiational inter- or intra-molecular deactivation processes. Gross interaction with the solvent, as the formation of oxonium ion compounds in strong sulphuric acid solutions or changes in the degree of ionization or of hydrogen bond effects are bound to affect the fluorescence intensity and colour through the changes of molecular structure involved.

In some instances, as for dimethyl-naptheurhodine, the fluorescence band moves to longer wavelength as the solvent changes from liquids of low to high

dielectric constant [1]. This could be due to the greater interaction of the solvent molecules with the excited than with the ground fluorescent molecules states, depending on the polarizabilities, and altering the positions of both the excitation and emission bands [1, 5].

1.1.1.4 Inner filter

The inner filter effect also occurs whenever there is a compound present in the sample with an absorption band and the fluorescence intensity will be reduced which overlaps either the excitation or emission band of the fluorescent analyte. It becomes a problem only when the absorption is high or when the concentration of the absorbing species varies from sample to sample. At high concentrations this is caused by absorption due to the fluorophor itself.

1.1.1.5 Quenching

Although the inner filter effect has the results of reducing the intensity of the radiation detection, it is not quenching. True quenching involves the removal of the energy from an excited molecule by another molecule, usually as the results of a collision. The decrease in the fluorescence intensity by the interaction of excited state of the fluorophor with the surroundings is known as quenching and is fortunately relatively rare. Quenching is not random [1, 2, 5]. Each example is indicative of a specific chemical interaction, and the common instances are well known.

Compounds containing unpaired electrons can also act as efficient quenching agents. The most important compound of this type is molecular oxygen. Moreover, quinine fluorescence is quenched by the presence of halide ion despite the fact that the absorption spectrum and extinction coefficient is identical in 0.5 M sulphuric acid (H_2SO_4) and 0.5 M hydrochloric acid [5].

1.2 Research Background

Synthesis and characterization of fluorescent particles is currently an important area of research. The synthesis of these fluorescent particles has attracted a great deal of attention for their interesting chemical and physical properties and potential technological applications [6-8].

In recent years, developments of high sensitive and selective sensory materials based on fluorescent materials have been carried out by several researchers. Some recent developments have used the idea of trapping fluorescence materials in matrices [8]: fluorescence material trapped in matrices can be more stable [9]. Han and co-workers [10] have doped the fluorescent material Rhodamine B (Rh B) into organic-inorganic silica films and their patterning were fabricated by sol-gel process combined with a soft lithography. Besides that, the fluorescence materials were incorporated into porous glasses by diffusion [11]. A thin film dissolved oxygen sensor fabricated by trapping fluorescent material in sol-gel matrix was studied by Bailey and co-workers [12].

Fluorescence detection offers several advantages over several other methods in terms of its sensitivity and specificity. The integration of fluorescence detection systems has received particular attention due to the large expense and size of current bio-fluorescence detection systems [13].

The possibility of obtaining new particles with enhanced fluorescent properties will be of great interest in the micro-analytical sciences. The new fluorophors will also be widely applicable in the gas sensing applications for industrial use as well as in bioassays. There has been much interest in the use of carbon dioxide and oxygen sensors based on luminescence quenching of organic fluorophors due to their fast response, high sensitivity and specificity [12-15].

1.3 Fluorescence Probe

Fluorescent and phosphorescent probes are widely used in applications for detecting biological events. Sensors based on luminescence detection usually result in higher sensitivity than those based on absorption or reflectance. The association with intensity-based systems such as drift in optoelectronic detection of luminescence lifetime, rather than intensity, can overcome many of the problem components.

1.3.1 pH Indicator

New fluorescence optical sensing phases for pH measurements have been developed, based on the use of 2', 7'-dibromo-5-(hydroxymercury) fluorescein (mercurochrome) as fluorescent pH indicator. Fluorescence emission of mercurochrome changes reversibly with the pH in a relatively wide range of pH. The pH sensing material has been prepared by trapping this fluorescent dye in a rigid inorganic matrix prepared following the sol-gel technology. The resulting sensing phase showed a strong pH dependence ($\lambda_{\text{ex}} = 528 \text{ nm}$, $\lambda_{\text{em}} = 549 \text{ nm}$) over 6 pH units, with reversible fluorescent changes [16].

The fluorescence property of fluorescein isothiocyanate (FITC) in acid-alkaline medium was studied by spectrofluorimetry. A novel pH chemical sensor was prepared based on the relationship between the relative fluorescence intensity of FITC and pH [17].

1.3.2 Fluorimetric Determination of Gases

In the past, a variety of O₂ gas sensor based on fluorescence quenching have been reported. The quenching of fluorescence of naphthalene in polymethyl methacrylate (PMMA) was studied by oxygen in thin films after displacement of

nitrogen atmosphere over the sample by oxygen [18]. A simple fluorescence technique was proposed for the measurement of the diffusion coefficient of oxygen into latex. These latex films were prepared by annealing pyrene (P) labelled polymethyl methacrylate (PMMA) particles above the glass transition temperature. Diffusion coefficients of oxygen were determined using fluorescence quenching [18].

Poly (1,4-phenylene diphenylvinylene), p-PDV, a photoluminescent conjugated polymer was synthesized by Mehamod *et al.* [19] for the detection of oxygen based on the occurrences of the quenching phenomena in the presence of oxygen.

1.3.3 Application of Fluorescence in Polymer

Sorption and drying processes were monitored in situ in polymer by fluorescence rotor probe, 4 tricyanovinyl-[N-(2-hydroxyethyl)-N-ethyl]aniline (TCl), a solvachromatic fluorescence probe, 4-(N,N-dimethylamino) 4'-nitrostibene (DANS) and pyrene by Ellison and co workers [20]. The probes showed sensitivity to desorption or drying of both water and organic sorbates.

In addition, a method for encapsulation of a fluorescent molecule into silica “nanobubbles” was reported [21]. Fluorescein isothiocyanate (FITC) dye molecules were coadsorbed onto the surface of gold nanoparticles with 3-aminopropyltrimethoxysilane.

1.3.4 Determination of Proteins

Protein analysis continues to be an important area of investigation in the fields of chemical and biochemical analysis. Fukada and co-workers [22, 23] have established a series of new systems for protein determination using

chemiluminescence, which detect as little as nanogram amounts of protein. Erythrosin B (EB) binding to proteins causes a decrease in the fluorescence maximum of EB at 550 nm [24]. Its measurable range was from 1.95 to 1000 ng/ml.

1.3.5 Determination of Metals and Ions

A highly selective and sensitive fluorometric method for the determination of bisphenol has been developed by Yoshida and co-workers [25]. This method is based on an intramolecular excimer-forming fluorescence derivatization with pyrene reagent. There were also many workers [26, 27] using the fluorescence quenching to determine the concentration of heavy metals such as copper (II), ferum, and zinc in industrial waste.

1.4 α -naphthoflavone (7, 8-benzoflavone)

α -naphthoflavone or 7,8-benzoflavone (Figure 1.1) is one of the fluorescent material that has found many uses in analytical chemistry. It is also a natural product although it has been used in a number of biological studies [28-31].

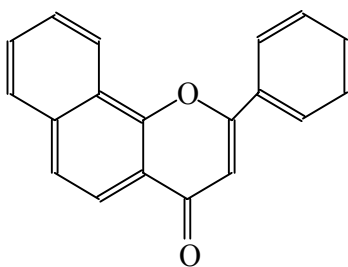


Figure 1.1: The molecular structure of 7, 8-benzoflavone.

For example, it is an activator of protein as investigated by Stermitz *et al* [29]. Besides that, α -naphthoflavone also is an inhibitor of benzopyrene-caused DNA damage [30] and Zangar and co-workers [31] found that α -naphthoflavone

binds to adenosine receptors. α -naphthoflavone has also been used to examine the mechanism of flavone action and further suggested as selective inhibitor to discriminate between human enzymes [32].

1.5 Fluorescein

Fluorescein was the first fluorescent dye used for water tracing work [33] and is still used for qualitative (visual) studies of underground contamination of wells. In recent years, Rhodamine WT has almost completely replaced fluorescein for flow measurements and circulation, dispersion, and plume studies [34]. Nonetheless, fluorescein has a role in such studies, and can be used for masking, hydraulic model studies, and underground studies. The molecular structure is as shown in Figure 1.2.

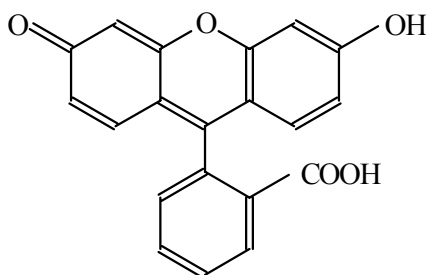


Figure 1.2: The molecular structure of fluorescein.

Fluorescein is also used widely in the fluorescence tracing of antibody and rapid diagnosis of some diseases in medical fields [35, 36]. The chemical sensor for pH measurement had been investigated based on the fluorescence property of FITC [17]. A sensitive pH sensor using phospholipids coating the particles labelled with fluorescein was used for intra-cellular pH measurement in murine macrophages [17].

Fluorescein can be used quantitatively for underground tests, subject to limitations imposed by the higher background of naturally occurring fluorescent materials. An advantage of fluorescein in underground studies is its light sensitivity. Should it reach an open receiving body of water, the colour will be less of a problem because it will disappear rapidly in the sunlight.

1.6 Luminol

Luminol (3-aminophthalhydrazide or 5-amino-2, 3-dihydro- 1,4-phthalazinedione) is an organic compound which, when oxidized, emits light and this phenomenon is known as *chemiluminescence*. This is similar to the reactions that a firefly uses to emit light, and to those used in "glow-sticks" and some roadside emergency lights [37, 38]. The chemiluminescence reaction of luminol with oxidizing agents was first reported in 1928 by Albrecht [38]. Since then, the reaction has been mainly used to determine hydrogen peroxide, other oxidants and metal ions [39 - 41].

Luminol is prepared by reduction of the nitro derivative (3) formed on thermal dehydration of a mixture of 3-nitrophthalic acid (1) and hydrazine (2). High-boiling triethylene glycol with a boiling point of 290 °C is added to an aqueous solution of the hydrazine salt. The excess water is distilled, and the temperature is raised to a point where dehydration to (3) is completed within a few minutes. Nitrophthalhydrazide (3) is insoluble in dilute acid but soluble in alkali, by virtue of enolization. It is reduced to luminol by sodium hydrosulfite (sodium dithionite) in alkaline solution [1]. The synthesis scheme is shown Figure 1.3.

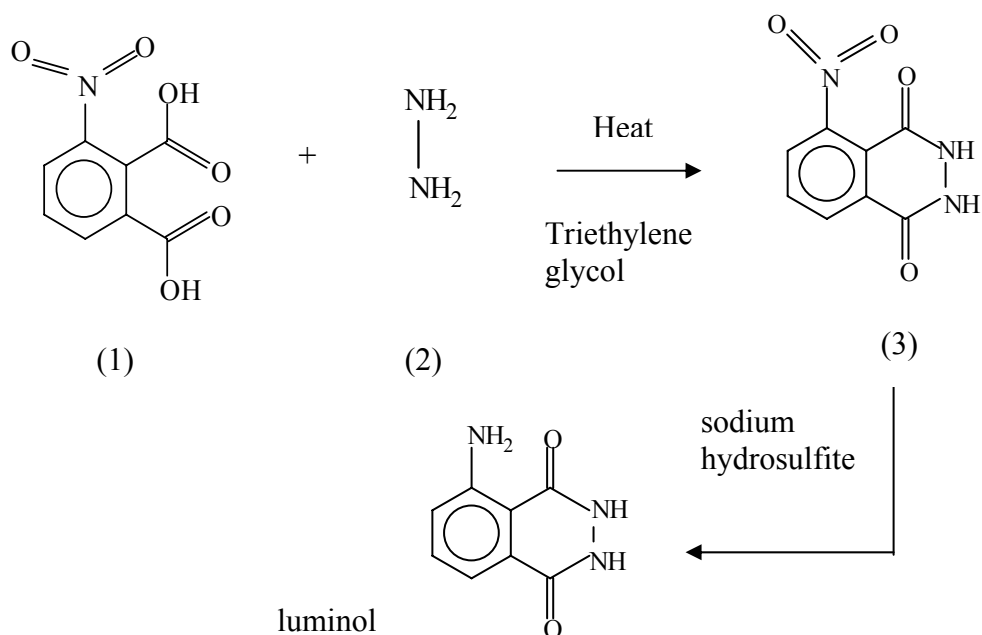


Figure 1.3: The synthesis scheme of luminol.

The luminol is converted by the basic solution into the resonance-stabilized dianion (1), (Figure 1.4) which is oxidized by hydrogen peroxide into the dicarboxylate ion (2), accompanied by the loss of molecular nitrogen, N_2 . When the molecule (2) is formed, it is in an excited (higher energy) electronic state, and sheds its "extra" energy by emitting a photon of light ($h\nu$), allowing the molecule to go to its ground state to form (3).

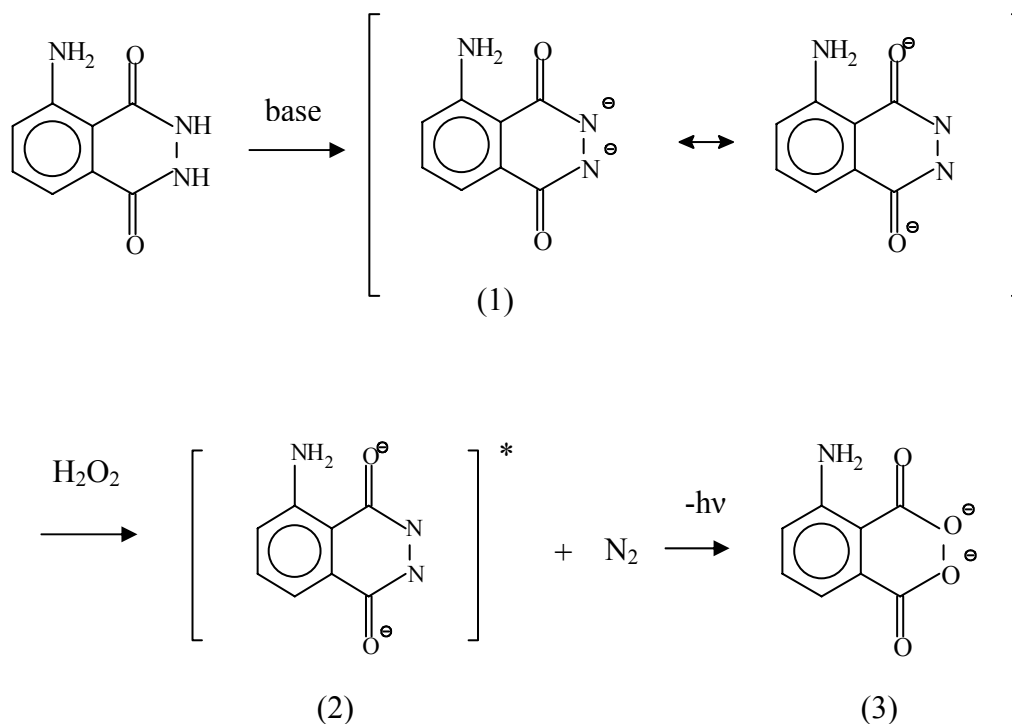


Figure 1.4: The reaction of luminol in basic condition.

In aqueous solutions, the luminol oxidation is catalyzed by the presence of a metal ion, such as iron (II) or copper (II). For this reason, luminol can be used in the detection of blood, since it can be activated by the iron in hemoglobin.

Most recently, Yuan and Shiller [42] report a subnanomolar detection limit for H_2O_2 using luminol chemiluminescence. This method was used to determine hydrogen peroxide content in sea water, based on the cobalt (II) catalytic oxidation of luminol. While cobalt is the most sensitive luminol metal catalyst; it is also present in sea water at very low concentrations. Moreover, luminol was also

observed to determine bromide ion in seawater with chemiluminescence reaction [40].

1.7 Carbon Dioxide (CO₂)

Carbon dioxide is an essential constituent of tissue fluids and as such should be maintained at an optimum level in the blood. The gas therefore is needed to supplement various anaesthetic and oxygenation mixtures under special circumstances such as cardiac pulmonary by-pass surgery and the management of renal dialysis. It also has a limited place as a respiratory stimulant and is used in the investigation and assessment of chronic respiratory disease.

The carbon dioxide present in the atmosphere is produced by respiration and by combustion. However, it has a short residence time in this phase as it is both consumed by plants during photosynthesis.

Carbon dioxide is a colourless odourless gas and is soluble in water, ethanol and acetone. It has a melting point of -55.6 °C, boiling point at -78.5 °C and density is 1.977 g cm⁻³ [43].

The CO₂ molecule has a linear shape. This means that the atoms in carbon dioxide are arranged as in Figure 1.5. The green circle represents one atom of carbon and the two grey circles represent oxygen atoms.

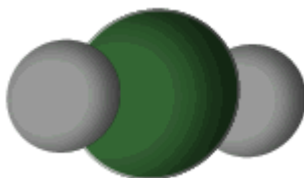
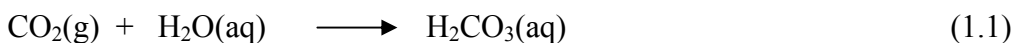


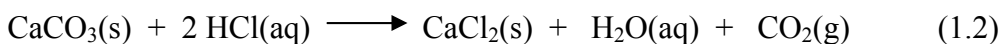
Figure 1.5: The structural formula of carbon dioxide.

Carbon dioxide is an acidic oxide and reacts with water to give carbonic acid as equation 1.1.

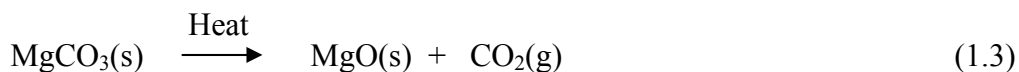


1.7.1 Preparation of Carbon Dioxide

Carbon dioxide is prepared by treating any metallic carbonate with dilute mineral acids as in equation 1.2



or by heating carbonates of metals other than alkali metals as in equation 1.3.



1.7.2 Uses of Carbon Dioxide

Large quantities of solid carbon dioxide (i.e. in the form of dry ice) are used in processes requiring large scale refrigeration. Carbon dioxide is also used in fire extinguishers as a desirable alternative to water for most fires. It is a constituent of medical gases as it promotes exhalation. It is also used in carbonated drinks [43].

1.8 The Determination of Carbon Dioxide

Recently, carbon dioxide level in air has increased considerably due to continuous damage to the environment and the increased use of fossil fuels. Accordingly, it has become an important task to monitor and control the carbon dioxide which causes both green house effect and possibility of respiratory organ disease. Until now, many works have been carried out to develop solid-state carbon dioxide gas sensors [44, 45].

An optical sensor for the measurement of high levels of carbon dioxide in gas phase has been developed [46]. It is based on fluorescence resonance energy transfer (FRET) between a long-lifetime ruthenium polypyridyl complex and the pH-active disazo dye Sudan III. The donor luminophore and the acceptor dye are both immobilized in a hydrophobic silica sol–gel/ethyl cellulose hybrid matrix material.

Pt/Na⁺ ion conductive ceramic thin film/Pt/carbonate (Na₂CO₃:BaCO₃ = 1:1.7 mol) system CO₂ micro gas sensor was fabricated and the sensing properties were investigated [44].

Fiber-optic carbon dioxide sensors with a dip-coated sol–gel film containing indicator dye of thymol blue were prepared and characterized. The sensitive film has both organic and inorganic parts with good gas permeability. The difference between attenuations in N₂ gas and in CO₂/N₂ mixture gas increases with the increase in the CO₂ concentration in the 0.55 – 0.7 M range [45].

A thin film sensor for detection of carbon dioxide dissolved in liquids with attention focused on its use for clinical blood gas analysis. Carbon dioxide from the analyte penetrated into a hydrogel electrolyte through a gas permeable membrane and is chemisorbed on a rhodium working electrode. The concentration of CO₂ collected by this way and determined by an amperometric measurement technique based on the inverse voltammetry [47, 48].

The detection of CO₂ is usually based on infrared detection [49] and a Severinghaus electrode with bulky and expensive devices used. There have been

many publications on single optical fibre O₂ sensor based on fluorescent quenching of a dye by molecular O₂ and CO₂ sensors based on the pH modulation accomplishing with the colour change of a dye [50].

1.9 Sol Gel Glass and Sol Gel Process

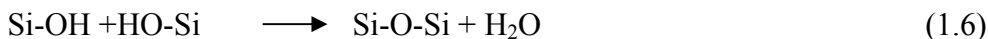
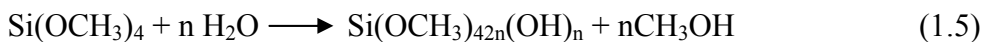
The sol–gel technique is a low-temperature route widely employed to prepare thin films for use in the different fields, because it can offer homogeneous thin films at molecular scale and control of chemical purity. In recent years, great interest was devoted to the preparation of thin films for optical applications.

Avnir and co-workers [51] demonstrated the possibility and applications of doping a gel with an organic dye. Recent work with silica gel has attracted a great deal of attention because of its potential utility indicated higher stability and better lasting properties than those based on polymer [52-54]. The sol-gel reaction occurs at room temperature, therefore, organic molecules can be incorporated in the gel network with no risk of thermal degradation.

In the sol-gel process, hydrolysis and condensation reaction are highly affected by water content and solution pH [52]. Under acidic conditions, most commonly used for tetraalkyloxysilane (TAOS) sol-gel, hydrolysis is fast relative to condensation and the polymers formed are more open, three-dimensional structures. Under basic condition, hydrolysis is the rate-limiting step [52-56]. Repulsion between negatively charged particles prevents chain-like linkages and promotes denser, more colloidal gels [52, 57].

Moreover, sol gel technology provides a relatively straightforward way to fabricate glasslike or ceramic material via the hydrolysis and condensation of suitable metal alkoxides. The most popular starting precursors for the fabrication of silica based materials are tetramethoxysilane (TMOS) and tetraethoxysilane (TEOS). These reagents can be hydrolyzed (equation 1.4) and condensed (equation 1.5 and/or

1.6) under relatively mild conditions (Room temperature and pressure) as illustrated in the following simplified reaction sequence for TMOS (1.5).



In a typical procedure, TMOS is mixed with water in a mutual solvent (methanol) and catalysts [acid (HCl), base (NH₃) or nucleophile (F⁻)] is added. During sol-gel formation, the viscosity of the solution gradually increases when the sol (colloidal suspension of small particles) becomes interconnected to form rigid, porous structure gel [57]. Gelation can take place on a timescale ranging from the seconds to months depending on the proceeding conditions. (Si:H₂O ratio, type and concentration of catalyst, alkoxide precursors, etc).

During drying, alcohol and water evaporate from the pores, causing the matrix to shrink. Xerogels or fully dried gels are significantly less porous than their hydrated counterparts. To maintain porosity and pore structure, the gel can be supercritically dried to form aerogel. The surface area of these materials often exceed 1000 m²/g. The physical properties of the resultants structure, such as average, pore size distribution, pore shape and surface area, strongly depends on the sol-gel process parameters and the method at which the material is prepared and dried [57-60].

The sol-gel process provides a relatively simple way to encapsulate reagents in a stable host matrix. Moreover, sol-gel derived glasses used as host materials provide better optical transparency, stability and permeability than many organic polymers. Protein and enzymes entrapped in silica gel have been used in numerous biological-sensing applications, and sol-gel materials doped with organic and organometallic compounds have been utilized as sensors for gases, metals, ions and pH [52].

Today the use of sol-gel to prepare inorganic and organic-inorganic composite materials is blossoming. Compared with commonly used organic polymers, the sol-gel process affords enormous flexibility in terms of the types of materials that can be prepared, the surface can be formed on, and their ion-exchange properties.

1.9.1 Application of Sol Gel in Analytical Chemistry

Nowadays, there are many researchers using the sol gel as a probe for the detection of gases such as carbon dioxide, oxygen and others. Tetraethylorthosilicate (TEOS) and tetramethylorthosilicate (TMOS) are the common chemicals that are used in the preparation of sol gel [60-65].

1.9.1.1 Sol Gel Encapsulated Fluorescent Materials as Gas Sensors

The sensing of molecular oxygen based on luminescence quenching is regarded as one of the most typical and widespread optosensing application.

As for the matrix, materials such as polymer films, sol gel phases, zeolites and siloxanes have been tested. A good matrix for inclusion of the sensing molecules is chemically inert and optically transparent, possesses photochemical and thermal stability and shows negligible intrinsic fluorescence. Many polymer and sol gel phases largely fulfil these requirements [57, 63]. The porosity of the sensing phase is also important since the quencher should be able to interact with the immobilized luminophore [63, 64].

Oxygen sensors based on this principle have also been extensively studied [59, 61]. The most common sensor elements studied are those based on an organic or inorganic compound suspended in a thin silicone membrane. Advantages of using an aerogel-based sensor element over other systems include a rapid response time (due

to rapid diffusion of gases through the aerogel pore network), and improved resistance to photo-bleaching (as the photoluminescence is caused by stable defect centers in SiO₂).

The sol-gel coating processes of the Rhodamine B-doped SiO₂-TiO₂ system, and the influence of the compositions of the SiO₂-TiO₂ system on the fluorescence properties of Rhodamine B-doped films have been investigated by Hao and co-workers [60].

1.9.1.2 Detection of Proteins

The entrapment of biomolecules in a silica sol gel matrix and their use in chemical sensing applications have blossomed during the past decade. It has been that proteins and enzymes can be entrapped in a random orientation in sol gel derived glasses while maintaining their native properties and relativities [65, 66].

Work by Jordan and co-workers [67] has demonstrated the potential for sol-gel entrapment of active proteins in an array format. They have optimized alkoxy silane-based sol-gel formulations for protein (keratinocyte growth factor and glucose oxidase) stability and antibody (anti-fluorescein) activity, and has recently demonstrated fluorescence based glucose biosensing using a glucose oxidase-based microarray deposited onto a tris(diphenylphenanthroline) ruthenium (II) chloride doped sol-gel film.

Silica xerogel membranes are well suited for encapsulating biomolecules and biosensors have been described using encapsulated enzymes and proteins [16-18, 68, 69]. The glass polymer provides a rigid structure that prevents protein movement and intramolecular interaction, while allowing the biomolecules to retain their activity [16].

Jordan and co-workers [67] used sol gel glass for encapsulated of anti-fluorescein antibodies, an artificial receptor element, and ribonuclease inhibitor.

Many workers [67, 70] used absorption and fluorescence spectroscopies to characterize the properties of bovine serum albumin (BSA) and horse heart myoglobin (Mb) entrapped in sol gel. They observed that a large fraction of BSA entrapped in the sol gel glass was in a native conformation but the reversible conformational transitions were sterically restricted.

1.9.1.3 The Other Uses of Sol gel

Great interest was devoted to the preparation of thin films for optical applications especially elaboration of planar waveguides doped with the active elements such as rare-earth ions. Among the rare-earth ion-doped planar waveguides, Er^{3+} doping in different matrix materials prepared by the sol-gel method has attracted much attention [71].

Metal adsorption using porous films can be of great interest for the development of fuel cells or microbatteries. The cluster formation necessary for fuel cells requests the presence of small structures, easy to manipulate, such as microchannels. It has already been shown in the literature that copper can be adsorbed by SiO_2 films obtained by sol-gel process [1].

Conventional methods of enzyme immobilization include physical or chemical adsorption at a solid surface; covalent binding or cross-linking to a matrix, and entrapment within a membrane, surfactant matrix, polymer or microcapsule [1]. Previous work [8, 9, 15] has demonstrated that sol-gel method could be a promising alternative method for the enzyme immobilization. With the combination of the unique features of sol-gel process including high purity and uniformity, low process temperature and easy control on the reaction degree, the sol-gel encapsulation method is supposed to offer several advantages over conventional entrapment method.

1.10 Statement of the Problem and the Needs of the Study

The industrial and automobile exhausts are identified as one of the major sources of air pollution [29]. Due to incomplete combustion of fuel, automobiles emit toxic gases such as carbon monoxide (CO), carbon dioxide (CO₂), and sulphur dioxides (SO₂) to the environment. Therefore, a study on the method of detection of toxic gases released to the air is needed.

Since many workers used luminescence materials to detect the toxic gases, therefore this study will be carried out to investigate the possible use of fluorescent metal-chelate based luminol, fluorescein and α -naphthoflavone in sol gel matrices for the detection of carbon dioxide, sulphur dioxide and oxygen gases.

α -naphthoflavone was chosen due to lack of previous study on the detection gases. Luminol has been mainly used to determine hydrogen peroxide and metal ions [40, 41] and fluorescein has been used for the detection of carbon dioxide and oxygen [49]. However, the study of fluorescein complexes and luminol complexes for the detection of carbon dioxide, oxygen and sulphur dioxide have not been previously reported.

1.11 Objectives of Research

The research focuses on the study of the effect of carbon dioxide, oxygen and sulphur dioxide on the fluorescence intensity of α -naphthoflavone, fluorescein and luminol.

In this study, the fluorescent properties of metal-chelate based on fluorescent particles in matrices such as sol gel will be investigated using fluorescence spectrophotometer. The physical and chemical properties of these fluorophors also will be observed, at the same time the excitation and emission characteristic and the reaction of gases on the fluorescents materials would be carried out.

1.12 The Detection of Gases Using Fluorescent Materials

Initial works were carried out to study the optimum conditions for the fluorescent materials α -naphthoflavone, fluorescein and luminol. Five parameters pH, temperature, effect of solvent, metal-chelate and concentration were firstly optimised. The detection of CO₂, O₂ and SO₂ were carried out by observing the effect of these gases on the changes in the fluorescent intensity of the fluorescent materials.

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