DESIGN, SYNTHESIS AND ELECTROPOLYMERIZATION OF A NEW CHEMILUMINESCENT TERTHIENYL SYSTEM

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ABSTRACT

DESIGN, SYNTHESIS AND ELECTROPOLYMERIZATION OF A NEW CHEMILUMINESCENT TERTHIENYL SYSTEM

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A novel monomer, possessing chemiluminescence properties, 5,7-di-ethylenedioxythiophen-2-yl-2,3-dihydro-thieno[3,4-d]pyridazine-1,4-dione (ETE-Lum) was synthesized. Chemiluminescence properties of ETE-Lum were investigated in alkaline water solution in the presence of H_2O_2 and this reaction was catalyzed by Fe^{+3} ion and blood. This study submits a new opportunity to investigate forensic and analytical application instead of 5-amino-2,3-dihydro-1,4-phthalazine-dione (luminol). Response of other metalic cations was also investigated under the same reaction conditions. Electrochemical properties of ETE-Lum were studied in 0.1 M acetonitrile/tetrabutylammonium perchlorate solvent system containing BF_3-Et_2O and also in neat BF_3-Et_2O solution. In addition, the corresponding polymer film of ETE-Lum (PETE-Lum) was synthesized successfully via repetitive cycling by cyclic voltammetry and its electrochemical properties were investigated in a monomer-free electrolyte solution.

Spectroelectrochemical behavior of the polymer film on indium tin oxide working electrode was also investigated by recording the electronic absorption spectra, *in-situ*, in monomer-free electrolyte solution at different potentials. Furthermore, spectroelectrochemical studies revealed that PETE-Lum had an electronic band gap of 1.66 eV. The results of electrochemical and electroluminesence measurements indicated that chemiluminecent unit of monomer was protected during polymerization. In addition, PETE-Lum film was found to be electrochemiluminescence active, maintaining its activitiy over 1000 cycles.

Keywords: Forensic Science, Chemiluminescence, Luminol, Ethylene-dioxythiophene

ÖZ

KİMYASAL IŞIL-IŞIYAN YENİ BİR ÜÇLÜ TİYOFEN SİSTEMİNİN TASARIMI, SENTEZİ VE ELEKTROPOLİMERİZASYONU

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Kimyasal ışıl-ışıma özelliğine sahip yeni bir monomer, 5,7-di-etilendioksitiyofen-2-yl-2,3-dihidro-tiyeno[3,4-d]piridazin-1,4-dion (ETE-Lum) sentezlenmiştir. ETE-Lum' un kimyasal ışıl-ışıma özelliği bazik ortamda H_2O_2 varlığında incelenmiş ve bu reaksiyon Fe^{+3} iyonu veya kan kullanılarak katalizlenmiştir. Bu çalışma, 5-amino-2,3-dihidro-1,4-fitalazin-dion (luminol) yerine adli tıp ve analitik uygulamalardaki araştırmalar için alternatif bir olanak sunmuştur. Ayrıca ETE-Lum'un, aynı reaksiyon ortamında, diğer katyonlara verdiği cevaplarda araştırılmıştır. ETE-Lum' un elektrokimyasal özellikleri BF_3-Et_2O içeren 0.1 M asetonitril/tetrabutilamonyum perklorat ve yalnız BF_3-Et_2O ortamında çalışılmıştır. Döngülü voltametri kullanarak çoklu döngü aracılığıyla ETE-Lum monomerinin polimeri (PETE-Lum) sentezlenmiş ve elektrokimyasal özellikleri monomersiz ortamda çalışılmıştır. Polimerin spektroelektrokimyasal davranımı UV-vis ölçümü ile gerçekleştirilmiş ve nötr halde absorbsiyon band aralığı 1.66 eV olarak hesaplanmıştır. Elektrokimyasal çalışmalar sayesinde polimerizasyon sırasında elde edilen polimerin ışıl ışıma sağlayan birimlerinin korunduğu ve oldukça doğrusal bir

polimer zinciri elde edildiği görülmüştür. Ayrıca polimer elektrokimyasal aktivitesini 1000 kez anahtarlamadan sonra bile göstermiştir.

Anahtar Kelimeler: Adli Tıp Bilimi, Kimyasal Işıl-ışıma, Luminol, Etilen-dioksitiyofen

TO MY FAMILY

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ABBREVIATION

ACN	Acetonitrile
A.U.	Arbitrary Unit
BF_3-Et_2O	Borontrifluoro Diethylether
CL	Chemiluminescence
DMSO	Dimethylsulfoxide
E	Potential
ECL	Electrochemiluminescence
E_g	Band Gap
ETE-Lum	5,7-di-ethylenedioxythiophene-2-yl-2,3-dihydro-thieno[3,4-d]pyridazine-1,4-dione
FT-IR	Fourier Transform Infrared
HOMO	Highest Occupied Molecular Orbital
I	Current
ITO	Indium Tin Oxide
LUMO	Lowest Unoccupied Molecular Orbital
NMR	Nuclear Magnetic Resonance
PEDOT	Poly(3,4-ethylenedioxythiophene)
PMT	Photomultipler Tube
TBAP	Tetrabutylammonium Perchlorate
TTT-Lum	5,7-di-thiophen-2-yl-2,3-dihydro-thieno[3,4-d]pyridazine-1,4-dione
UV	Ultraviolet
vis	Visible
IR	Infrared

CHAPTER 1

INTRODUCTION

1.1. Chemiluminescence

Luminescence is defined as the emission of electromagnetic radiation in ultraviolet (UV), visible (vis) and infrared (IR) regions, which is produced by the transition of an electronic excited state to a lower state, i.e. to the ground state. When excitation is caused by means of a chemical reaction, the process is named as chemiluminescence (CL). During CL, chemical energy is transformed to light energy and the process is accompanied by the emission of electromagnetic radiation in UV, vis or IR region.

Since CL involves the oxidation of a proper molecule, the presence of a chemical oxidizing agent like hydrogen peroxide is necessary. The oxidation reaction produces an excited intermediate which relaxes to the ground state by several possible routes. These routes can be displayed diagrammatically by the Jablonski diagram (Figure 1.1).

If the singlet excited state is formed, the luminescence process is named as fluorescence; however, if the singlet excited forms an excited triplet state via intersystem crossing, at this time phosphorescent emission results [1]. Both kinds of emissions are called physical luminescence. Excited state can emit UV or IR radiation as well as vis light [1].

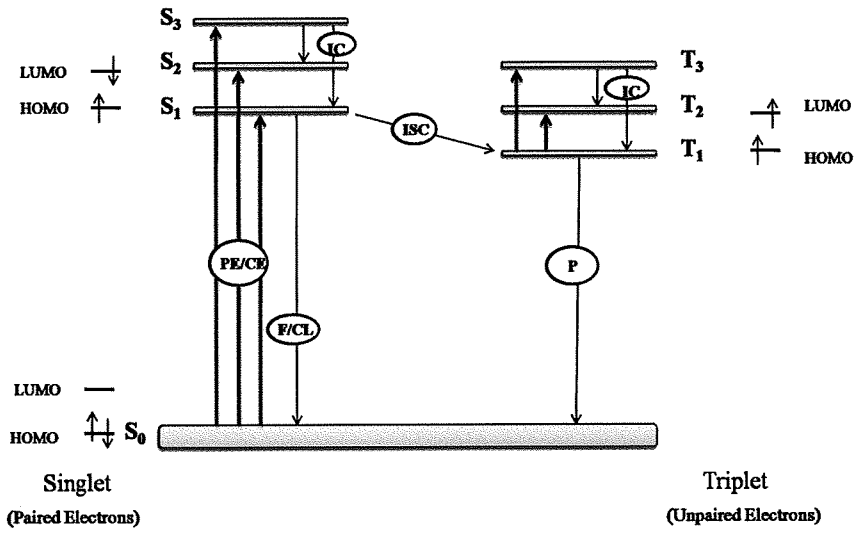


Figure 1.1 Jablonski energy diagram showing energy levels and transitions in a molecular compound: CL, chemiluminescence; F, fluorescence; P, phosphorescence; IC, internal conversion; ISC, intersystem crossing; PE, photoexcitation; CE, chemiexcitation; S_0, ground singlet state; S_1, S_2 and S_3 excited singlet states; T_1, T_2 and T_3 excited triplet states

Chemiluminescent reactions can be classified into three different groups depending on the origin of the process:

1. Chemiluminescent reactions: A chemical reaction, usually involving highly oxidized specie, is responsible for the formation of excited state.
2. Bioluminescent reactions: Living organisms, such as firefly or jellyfish, emit light due to a chemical reaction (i.e. oxidation of luciferin in the presence of an enzyme).
3. Electrochemiluminescent reactions: Light-emitting reaction is carried out by using electrical current.

1.1.1. Types of CL Reactions

The mechanism of CL reactions can be classified into two classes based on the continued studies in literature: direct and indirect CL [1].

Direct CL: Two reagents, a substrate and an oxidant interact to give a product (P) or intermediate in the presence of some co-factors. The CL precursor substrate is converted into the electronically excited intermediate [P]* which emits photons during relaxation to the ground state represented as below (1.1) [1-3].

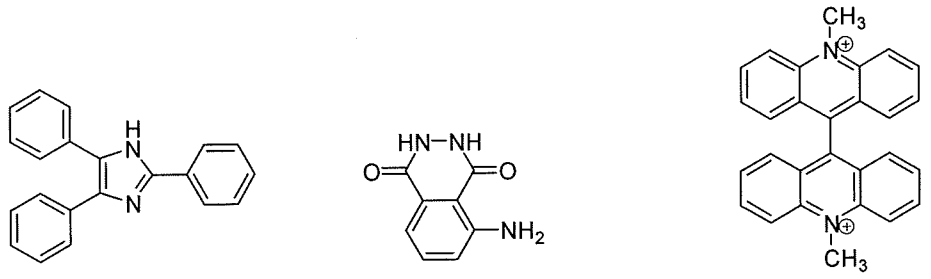
$$A + B \longrightarrow [P]^* \xrightarrow{h\nu\ (\text{light})} P \qquad (1.1)$$

Indirect CL: It is a different reaction mechanism in which energy of the excited specie [P]* is transferred to a fluorophore [F]* as shown below(1.2). This fluorophore with excess energy provides photon emission while returning back to the ground state, like peroxyoxalate reaction (light stick) [1-3].

$$A + B \longrightarrow [P]^* + F \longrightarrow P + [F]^* \xrightarrow{h\nu\ (\text{light})} P + F \qquad (1.2)$$

1.1.2. Historical Background of CL

Although it is now named as bioluminescence, earliest observations on CL was based on living organisms like firefly, luminescent bacteria and marine organisms which have luminescence property [4,5]. These earliest observations were followed by the discovery of several organic compounds having CL properties. In 1877, lophine (2,4,5-triphenylimidazole), the first synthetic chemiluminescent compound, was reported by Radziszewski, which is the pioneering work on the synthesis of chemiluminescent materials (Figure 1.2) [6].



2,4,5-Triphenylimidazol (Lophine) | 3-Aminophthalhydrazide (Luminol) | 10,10'-Dimethyl-9,9'-biscridinium (Lucigenin)

Figure 1.2 Chemical structures of some chemiluminescent compounds.

In the year 1888, the term "chemiluminescence" was introduced and luminescence phenomenon were classified by Wiedemann into six different types according to the excitation characteristics: *photoluminescence*, caused by the absorption of light, *electroluminescence*, produced by an electric discharge; *thermoluminescence*, produced by heating; *triboluminescence*, as a result of friction; *crystalloluminescence*, as a result of crystallization; and *chemiluminescence*, caused by a chemical reaction. [7]

In 1928, Albrecht discovered the chemiluminescent property of 3-aminophthalhydrazide (or 5-amino-2,3-dihydro-1,4-phthalazinedione), and reported that the luminescence intensity is related to its oxidation in alkaline solution [8] (Figure 1.2). The name "luminol" was given to this compound in the year 1934 [9] and it was reported that light intensity increased in the presence of hematin [10]. The first offer of using luminol as a probable test for blood detection in medicolegal investigations was reported by Specht in 1937 [11]. Also, in 1935, Gleu and Petsch reported that lucigenin (10,10′-dimethyl-9,9′- biscridinium nitrate) emits green light during its oxidation in alkaline medium (Figure 1.2) [12].

1.1.3. Applications of CL

In the early 1950s, the first application of CL was achieved with practicing analytical tool employing several substances such as luminol, lophine and lucigenin as volumetric indicators. According to Rauhut, chemically produced light is suitable for a variety of lighting applications such as emergency light for power failures in home, office buildings, theaters, factories, for disabled vehicles and aircrafts, for lifeboats, and lifejackets; as a marker light of pedestrians and bicycles on the dark street; as a portable light for hikers, campers and military units [13].

CL has also an important and powerful place as a tool for drug analysis because newly discovered CL systems related to direct oxidation of molecules with different common oxidants in acid or alkaline media. Metal ions, e.g. Cr^{3+}, Cu^{2+}, Co^{2+}, Cd^{2+}, Fe^{2+}, Ni^{2+} and Mn^{2+} can be analyzed. Vitamin B_{12}, cyanide, α-aminoacids, phenols and heme containing compounds such as ferritin hemoglobin, myoglobin, hematin and catalase or

complexed forms of the analytes (e.g. with ethylenediaminetetraacetic acid) can be reduced during the oxidation of luminol, which allows the detection of above mentioned species [14]. Also, hydrogen peroxides can be detected easily during oxidation process of luminol, oxalate ester or lophine compounds. By this way compounds such as glucose (in a combination with glucose oxidase, produces H_2O_2) and nicotinamide adenine dinucleotid (NADH, which converts O_2 to H_2O_2 in the presence of methylene blue) were quantified at 10^{-9} M and 10^{-7} M, respectively. [15]

In addition, direct CL procedures have been developed for air pollutants e.g. NO/NO_2, O_2, O_3, hydrocarbons, for bacteria and biomass determination, carbohydrates for nucleotides other than adenosinetriposphate (ATP) [16]. These assays are based on the influence of the analytes on the CL reaction, release of a reactant or a chemiluminescent compound.

1.2. Luminol (Phthalhydrazide)

1.2.1. CL of Luminol

Although the CL feature of luminol was firstly reported by Albrecht in 1928 [8], it became a widely used CL reagent over the last 20 years because of its sensitivity and selectivity, especially in the application of forensic and analytical sciences [2].

The light-producing pathway for the oxidation reaction of luminol is a multi-step and complex process which depends on several factors like pH, temperature, ionic strength of the reaction medium and reactive species that can be present in solution which interact with luminol, metal catalyst or hydroxide ions [17].

White et al. observed that an intermediate molecule 3-aminophthalate was formed during luminol oxidation process and its fluorescence spectrum perfectly matched the CL spectrum of luminol, thus, excited intermediate could be considered as the source of light emission while returning to the ground state as shown in Figure 1.3 [18-20]. This was proved in 1965 by Gundermann [21].

The most plausible CL reaction mechanism for luminol under basic conditions is given in Figure 1.3 [1]. According to this mechanism, luminol in basic solution reacts with the hydroxide ion and then a dianion is formed. The oxygen produced from the hydrogen peroxide reacts with the luminol dianion to form organic peroxide. This peroxide is very unstable and immediately decomposes with the loss of nitrogen to produce 3-aminophthalic acid. As the excited state relaxes to the ground state, the excess energy is liberated as a photon, visible as blue light [22]. The oxidation of luminol (usually by H_2O_2) initiates the chemiluminescent reaction which usually requires a catalyst (or co-oxidant) such as $[Fe(CN)_6]^{-3}$, Cu^{+2} or Co^{+2}. The light emission, which is blue in water and yellow-green in DMSO, is identical with the fluorescence of the 3-aminophthalate oxidation product [20].

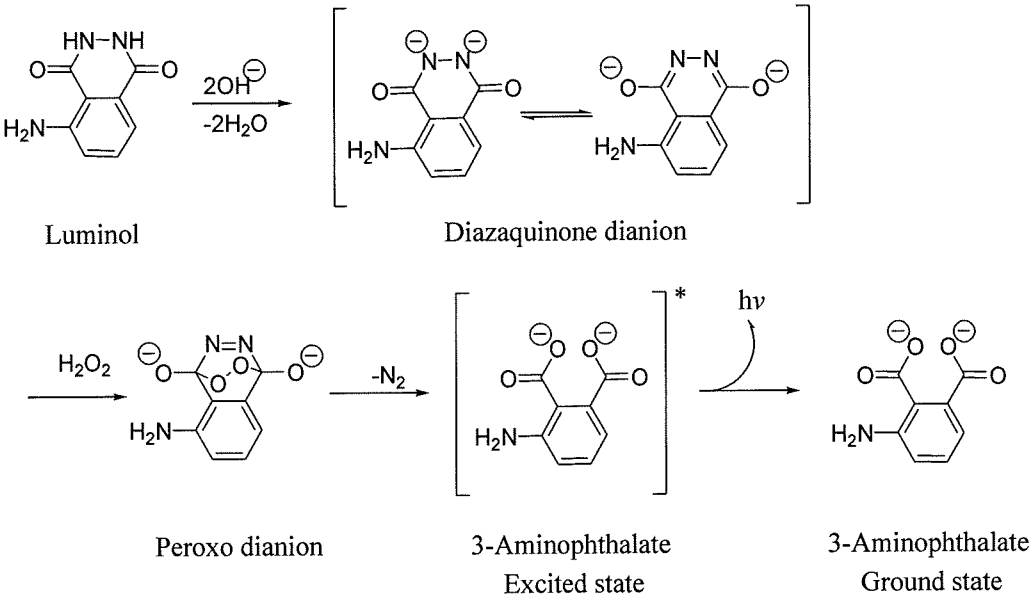


Figure 1.3. Proposed CL reaction of luminol [23].

1.2.2. Electrogenerated CL of Luminol

Electrogenerated CL is a kind of luminescence produced by electrode reactions and this process is commonly known as electrochemiluminescence (ECL) or electroluminescence [3]. A classical reaction system includes a solution containing reactants A and D in the presence of a supporting electrolyte, for example, tetrabutylammonium perchlorate in acetonitrile. The reaction sequence (1.3-1.6) to generate an excited state and light emission is given below [24]:

$A + e- \rightarrow A^{-\bullet}$	(reduction at the electrode)	(1.3)
$D - e^{-} \rightarrow D^{+\bullet}$	(oxidation at the electrode)	(1.4)
$A^{-\bullet} + D^{+\bullet} \rightarrow {}^{1}A^{*} + D$	(excited state formation)	(1.5)
${}^{1}A^{*} \rightarrow A + h\nu$	(light emission)	(1.6)

Actually, ECL is a sort of CL and the way to control luminescence is the most important difference between them. In CL, light emission is produced by mixing the emitter with necessary reagents in a reaction vessel while light emission is controlled by the electrode potential in ECL where the energetic electron transfer reaction is the source of light emission. ECL has found wide applications in clinical and biomedical diagnostics [25-28].

In ECL reaction of luminol (Figure 1.4), it is oxidized and goes to the diazaquinone form, and in the presence of H_2O_2 light emission is observed. In literature, many researchers extensively investigated the ECL reaction pathway of luminol to be amenable in H_2O_2 sensors [29].

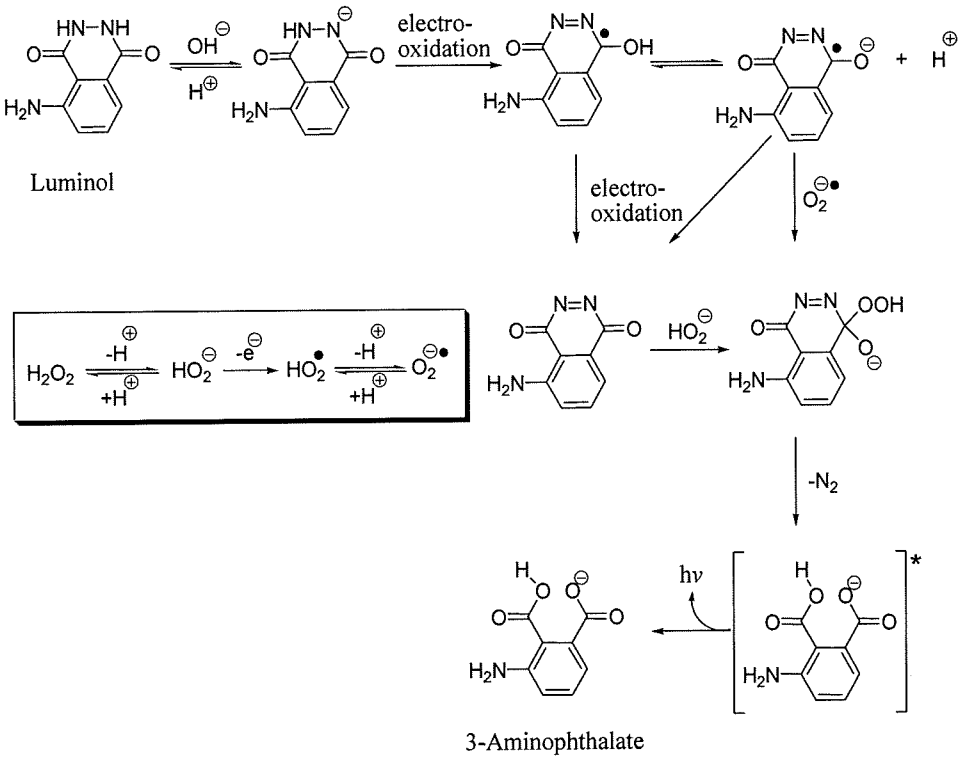


Figure 1.4 A proposed mechanism for ECL reaction of luminol [30].

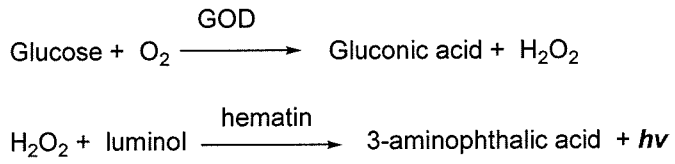
1.2.3. Application Areas of Luminol

The luminol reaction is important because of its use in detecting oxidizing agents such as hydrogen peroxide, for enzymes such as peroxidase and xanthine oxidase, and for metal ions such as iron or copper that catalyze this CL reaction [22]. Also, luminol was being used for more than 40 years to detect bloodstains which are hidden from the naked eye at crime scenes. Use of luminol makes it possible to detect the presence of trace

amount of bloodstains diluted down to a level of $1:10^{-6}$ as well. Other major examples of metal containing biomolecules in living organisms include the enzyme co-factors cobalamin or vitamin B12 (containing Co^{2+}) [31], and plants and bacterial pigments containing Mg^{2+} or Mn^{2+} [32, 33].

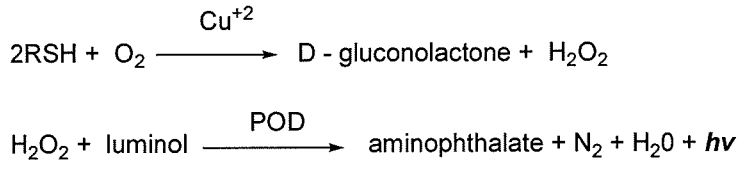
The luminol reaction is suitable for identifying contamination of the hospital environment in infection control audits. Some methods were also introduced for use in the haemodialysis unit, and also for other units at risk for contamination of the environment with blood, e.g. delivery rooms and operating theatres [34]. Also, ECL was used to detect hydrogen peroxide in aqueous solutions in the presence of electrolytically oxidized luminol. [35]

A flow injection analysis detection method for glucose is presented which is based on the oxidation of glucose by glucose oxidase , generated H_2O_2 can be detected by using luminol CL reaction [36]. Therefore, glucose, for example, can be detected by the ECL-based biosensors (1.7) [37].



(1.7)

Another application of luminol is the determination of glutathione (GSH). GSH was indirectly determined by measuring the amount of H_2O_2 formed during the Cu(II)-catalyse oxidation of GSH with oxygen (1.8) (also GSH reduces Fe^{3+} to Fe^{2+}) [2,38]



(1.8)

1.2.4. Luminol in the Forensic Science

CL, produced via oxidation of luminol, has been used for forensic science to detect latent blood stain which is not seen by naked eyes. The hemoglobin in blood produces blue-green color light (455nm) in the presence of luminol in the dark room [39]. The luminol test for blood was first described by Sprech in 1937 [11] and since then its forensic use has greatly expanded [39, 40].

Today, all over the world, luminol test is used in crime scene investigations to detect trace amounts of blood because of its great sensitivity. As investigations of violent crimes, blood is one of the most common and important physical evidence and founded blood provide valuable information that can be decided to solve crime events at a crime scene. A fresh bloodstain have a characteristic red color due to the erythrocytes, but as time passes its color turns to brown due to changes in the hemoglobin [41]. Thus, blood stains can not be seen very well on different substrates and difficult to separate from other materials [41]. Blue-green CL is produced by luminol reaction with blood and needs no light source and test is carried out in darkness (Figure 1.5). Although cleaning attempts have been made to hide the blood stain, this procedure has great sensitivity to detect blood patterns or drag marks [42].

Figure 1.5 (a) Apparence of the crime scene in the day light, no bloodstains can be seen by naked eye and **(b)** apparence after the luminol solution is applied, the intense blue color luminescence indicates the presence of bloodstains which are invisible to the naked eye in the day light.

Hemoglobin which contains oxygen molecule found in the erythrocytes of all vertebrates and some invertebrates and is responsible for the red color of blood [39]. Prosthetic heme group contains a Fe^{+2} ion in the presence of globin protein which constitutes tetrameric hemoprotein structure [39,43]. Outside of the organism Fe^{+2} ion in the heme groups goes to the ferric state (Fe^{+3} in hematin) and the color turns to brown (Figure 1.6). The Fe^{3+} facilitates the reactions that result in a strong CL, but the reaction mechanisms is still not well-known.

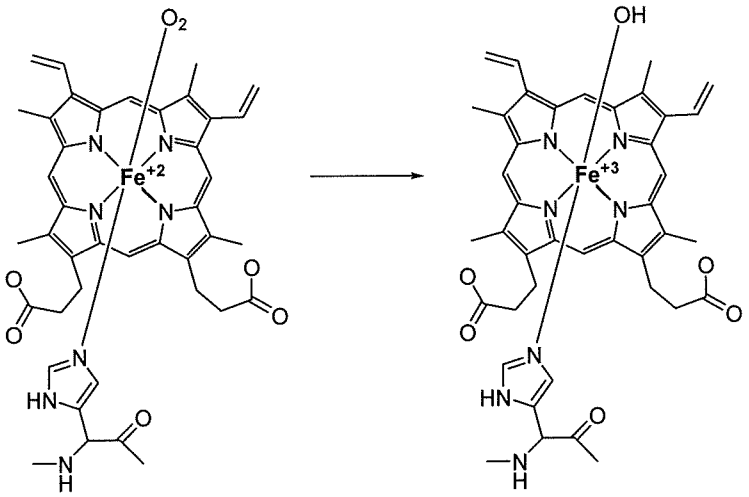


Figure 1.6 Structure of hemoglobin.

However, the tests for blood detection in forensic investigations have some limitations like selectivity for blood. It can produce false positive results when it is used in the presence of strong oxidants, some metal ions and peroxidases. This means that luminescence, although less pronounced, can be seen when luminol is sprayed on copper (or any alloy), bleaches, and horseradish. Luminol also has few detrimental effects on the subsequent DNA recovery and typing [39,44].

Many fields of biochemistry are interested in the chemistry of CL of luminol. "The chemical reactions that lead to the CL of luminol are complex and they are in many cases probably not known in detail yet" [39,45,46].

1.3. Conducting Polymers

Since their dicovery in the 1970s, conducting polymers bearing unique electronic and optical properties have been an extensively studied research area for scientists and for industrial applications: electrochromics, display technologies, energy storage and conversion, electrochemical switching, polymer photovoltaics, electromechanical actuators, separation technologies, chemical and physical sensors, corrosion protection, etc [47,50].

Chemically obtained black polyacetylene film is an insulator and its conductivity can be increased about one billion times by iodine doping and it looks like a golden metallic sheets. This pionnering work was first reported by A.G. MacDiarmid, A. Heeger and H. Shirakawa and the Chemistry Nobel Prize in the year 2000 was awarded to these scientists for their contribution to the field.

Although, polyacetylene is the simplest conducting polymer, unfortunately, it has some limitations due to its solubility and processing properties. To overcome these disadvantages, new organic electroactive polymers were designed and synthesized as alternative conducting polymers like polypyrrole [49-51], polythiophene [52-53], poly(3,4-ethylenedioxythiophene) (PEDOT) [54-56], polyfuran [57], and other aromatic polymers such as polycarbazole [58], polyfluorene [59], polyaniline [60], poly(*p*-phenylene vinylene) [61], and poly(*p*-phenylene) [62] (Figure 1.7).

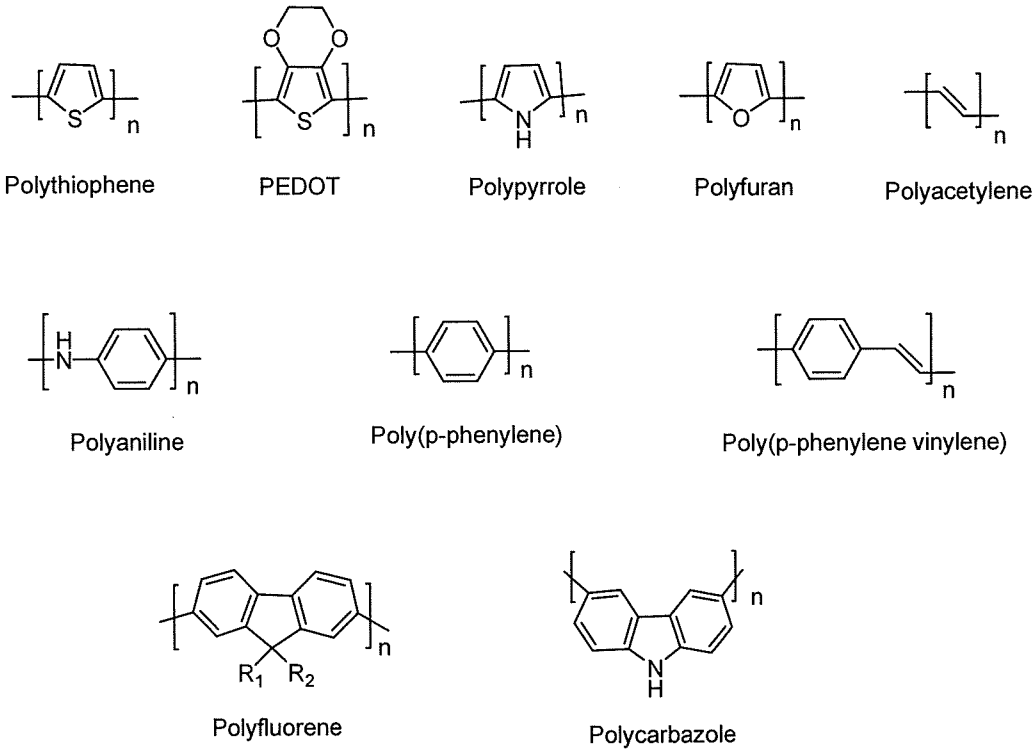


Figure 1.7 Structure of some conducting polymers.

1.3.1. Polyaniline

Polyaniline have been known as the oldest conducting material. Letheby firstly synthesized polyaniline via electrochemical polymerization of aniline in sulphuric acid solution and the generated material was named "aniline black" [63]. Then, in 1980s, A.G. MacDiarmid et al. reported the various form of polyaniline (Figure 1.8) and this discovery attracted the attention of scientists to the conducting polymers [64, 65].

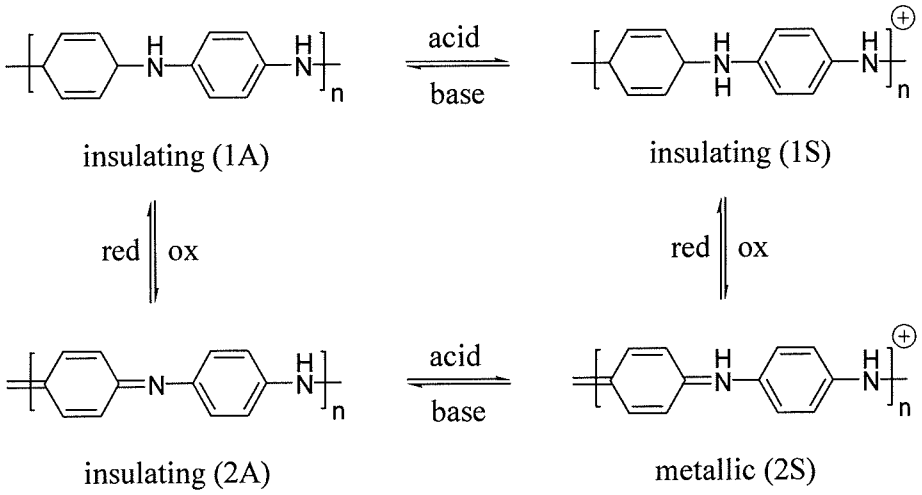


Figure 1.8. Various forms of polyaniline (1S and 2S are derived from the ammonium salts; 1A and 2A are derived from the amine forms).

Polyaniline films can be cycled reversibly by potential scanning between its neutral and oxidized states. During this process, the polymer film undergoes color changes like yellow ↔ green ↔ blue-violet. Polyaniline can be found mostly in three different oxidation states: leucomeraldine, emeraldine and pernigraniline. These states are determined by the quinone/benzene units ratio in the polymer backbone shown as in Figure 1.9.

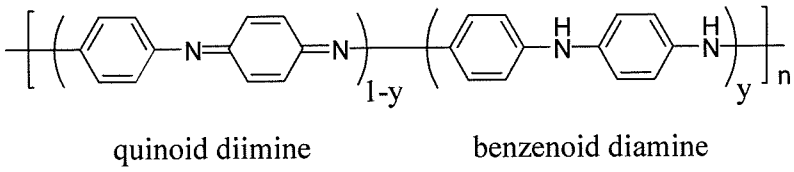


Figure 1.9. Different oxidation states of polyaniline: leucoemeraldine (y=1), emeraldine (y=0.5) and pernigraniline (y=0).

Polyaniline have some promising properties when compared to other conducting polymers like polypyrrole and polythiophene. For example, N heteroatom in polyaniline participates directly in the conjugation, which differs from polypyrrole and polythiophene. Also, polyaniline has a unique feature due to its reversible redox and fast switching between base and salt forms by treatment with acid and bases (pH switching property) [66]. In addition, polyaniline continue to become one of the most extensively studied conducting polymers due to its electrical conductivity, cheapness of synthesis and its good environmental stability.

1.3.2. Polyluminol

In order to obtain processible and functional polyaniline, a tremendous range of aniline derivatives has been investigated [67]. Luminol is also a derivative of aniline and as expected, its electrooxidation generated an electroactive polymer film called polyluminol, like polyaniline, on the electrode surface. Electrochemically polymerized

luminol film have been studied on various electrode [68] and widely used in the field of chemical [69] and electroanalysis and electrocatalysis [70-76].

Electrochemical oxidation of luminol in basic solution was first observed by Harvey [77]. Early studies on the electrochemical behavior of luminol were mostly achieved in alkaline solution and ignored in acidic solution. Epstein et al. [78] studied the electrochemical oxidation of luminol in alkaline solution and they concluded that hydrazide did not adsorb on Pt electrode. On the other hand, Sakura [79] reported that luminol could adsorb on the platinum in phosphate buffer solution (pH 7.4).Then, depositon of polyluminol was achieved successfully in strongly acidic solution (0.1 M H_2SO_4 aqueous solution) by cyclic voltammetry on glassy carbon, platinum and gold electrodes [67].

However, due to the solubility problem, which prevents sufficient mass transfer, only very thin polymer films were deposited on the electrode surface. From the results of electrochemical measurements it is found that luminol oxidized on the phthalimide-group site under the low electrode potentials KIO_3 (E_{ox}= 1.085 V) and on the amino group site at high potentials $(NH_4)_2S_2O_8$ (E_{ox}= 2.05 V) in water–organic solvent mixture [80].

As a result, still many questions have not found answer about reaction mechanism of luminol. One possible reaction mechanism is shown in Figure 1.10

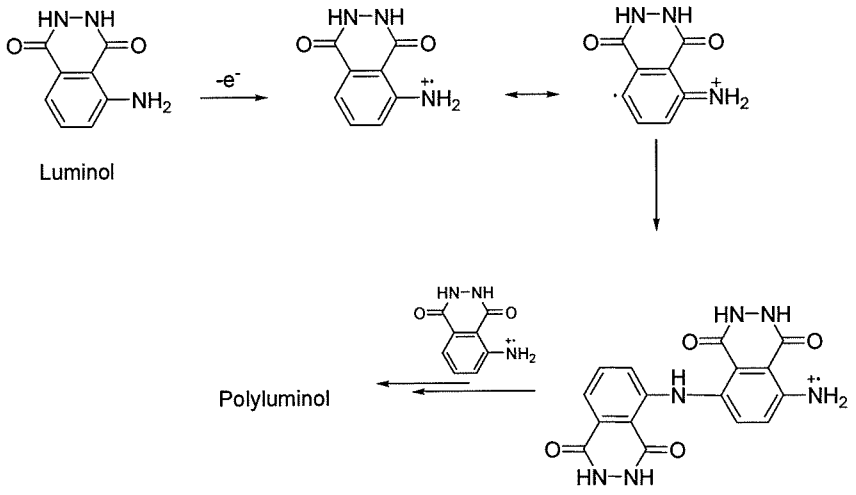


Figure 1.10 Proposed electropolymerization of luminol [81].

1.4. Aim of this work

Based on the foregoing results, it can be easily concluded that polyluminol can be a good candidate for use in electronic and biotechnological applications as well as forensic science. In order to make it amenable for use in these areas, some drawbacks (solubility, reaction mechanism, destruction of chemiluminescent units) of luminol during polymerization must be overcome. Recently, bearing these facts in mind, we have initiated a program aimed at the design and synthesize of new electropolymerizable material by the combination of a chemiluminescent unit in a trimeric thiophene rings (TTT-Lum) (Fig. 1.11). The electrogenerated polymer film bearing chemiluminescent units has robust and electroactive processable properties as well as good redox couple and switching stability. Shortly after this initial work, inspired by the encouraging results, we designed a new material by the assembly of 3,4-ethylenedioxythiophene

(EDOT) as external units and 3,4-substituted thiophene ring bearing chemiluminescent unit as central unit, namely 5,7-di-ethylenedioxythiophen-2-yl-2,3-dihydro-thieno [3,4-d] pyridazine-1,4-dione (ETE-Lum). It is expected that EDOT will be a good candidate as external rings yield an electron rich and low oxidation potential system by avoiding the destruction of chemiluminescent units, overoxidation and side reactions during polymerization.

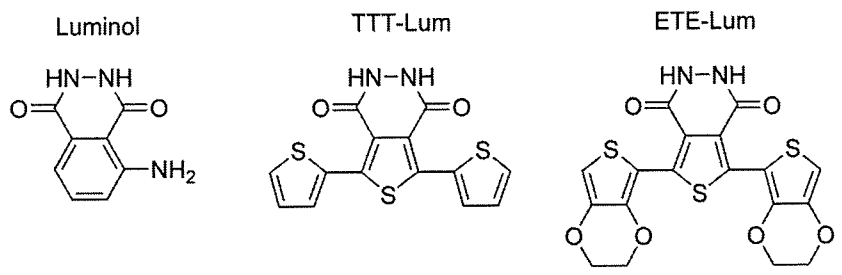


Figure 1.11 Structure of luminol, TTT-Lum and ETE-Lum.

Herein, we report our results concerning the synthesis, electropolymerization and characterization of a novel chemiluminescent material, ETE-Lum, and its corresponding polymer, PETE-Lum, as well as its application in forensic science to detect bloodstains. Furthermore, for the sake of comparison, chemiluminescent, electrochemical and optical properties of ETE-Lum and its corresponding polymer film were elaborated in comparison with luminol, TTT-Lum and their polymers.

CHAPTER 2

EXPERIMENTAL

2.1. Materials

All chemicals were purchased from Aldrich Chemical. For electrochemical studies, acetonitrile (ACN) was freshly distilled over CaH_2 and stored under N_2 atmosphere. Tetrabutylammonium perchlorate (TBAP) and neat borontrifluoro diethylether (BF_3-Et_2O) solutions were also used as electrolyte solution. An indium-tin oxide (ITO, Delta Tech. 8–12 Ω, 0.7 cm x 5 cm) and a platinum wire were used as working and counter electrodes, respectively, as well as a Ag wire as a reference.

For CL and ECL measurements, all the working solutions were freshly prepared. Dilutions were done by using doubly distilled water.

Freshly distilled solvents were used during the synthesis of monomers and all monomers were kept under N_2 and stored in refrigerator.

2.2. Instrumentation

2.2.1. Cyclic Voltammetry

Electrochemical synthesis and cyclic voltammetry measurements were performed using Gamry PCI4/300 potentiostat–galvanostat. Cyclic voltammetry studies were carried out utilizing a three-electrode system namely working, counter and reference electrodes.

An ITO as working electrode, a platinum wire as counter electrode and a Ag wire as reference electrode. The cell had a total volume of about 3 mL.

2.2.2. Electrochemical Polymerization

ETE-Lum was polymerized in ACN containing 10^{-4} M monomer, 0.1 M TBAP and 20 μL BF_3-Et_2O. Polymerization was achieved either via repetitive cycling at a scan rate of 100 mV/s between 0.0 and 0.85 V or constant potential electrolysis at 0.9 V vs Ag wire. After polymerization, the films were washed with ACN and transferred into monomer-free solution containing 0.1 M TBAP in ACN.

2.2.3. Spectroelectrochemistry

Spectroelectrochemical studies were performed using Hewlett–Packard 8453A diode array spectrometer. A Pt wire was used as a counter electrode and a Ag wire as a pseudo-reference electrode which was calibrated externally using 5 mM solution of ferrocene/ferrocenium couple in the electrolyte solution. An ITO electrode coated with the polymer film was used as working electrode. In order to equilibrate the redox behavior of the polymer films and to obtain reproducible results, the polymer films were switched between neutral and doped states several times in monomer-free electrolyte solution. The transmittance and absorption spectra of the polymer film were recorded *in-situ* under various applied potentials.

2.2.4. Spectroscopic Measurements

Nuclear Magnetic Resonance (NMR) spectra were recorded on a Bruker NMR Spectrometer (DPX-400) in $CDCl_3$ and DMSO. Fourier Transform Infrared (FT-IR) spectra were performed with a Bruker Vertex 70 Spectrophotometer.

2.2.5. CL Measurements

The light intensity of ETE-Lum was measured utilizing Pro-K.2000 Rapid Kinetic System combined with photomultiplier tube (PMT, 9131/350B/Electrontubes (9 mm effective diameter, low dark count and after pulse rate/11 LF Number&type of dynodes with EM6 Application software) (Figure 2.1). The monomer dissolved in 0.1 M NaOH aqueous solution containing H_2O_2 and the catalyst (metal cation, or blood) were injected towards CL cell and efficiently mixed together. The signal is monitored immediately after mixing utilizing a computer program.

2.2.6. ECL Measurements

ECL was achieved by electron transfer reactions of electro-generated species with applying potential at electrode. Light emission from monomer solution and polymer film (in monomer-free solution) were measured by a homemade system combining cyclic voltammetry with PMT as shown in Figure 2.2 The working electrode is an ITO fitting a UV cuvette. A Ag-wire is used as a reference and a Pt wire is used as a counter electrode. For potential control, all three electrodes were connected to a Gamry PCI4/300 potentiostat-galvanostat.

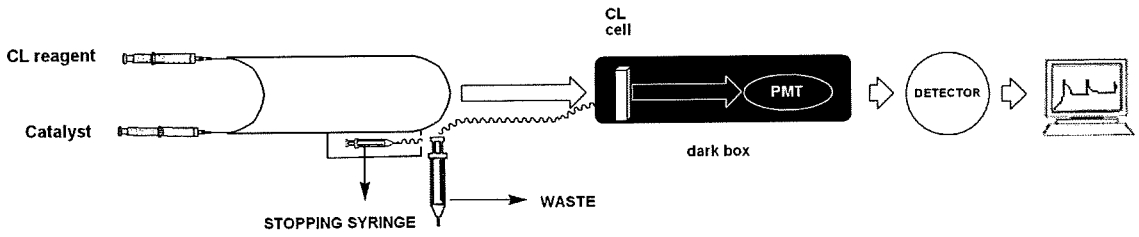


Figure 2.1 Schematic diagram for stopped-flow CL measurements

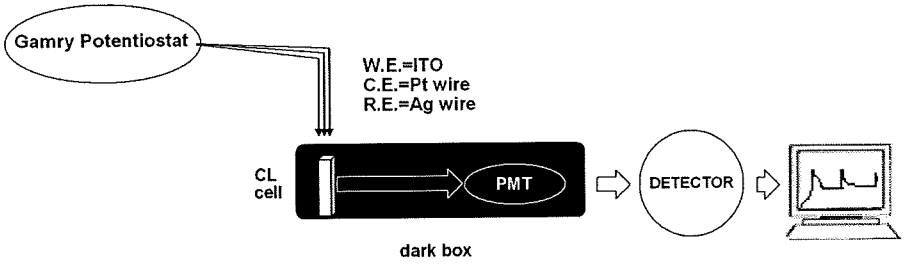


Figure 2.2 Schematic represantation of homemade ECL system

2.3. Synthesis of Monomer

Reaction pathways for the synthesis of ETE-Lum are given in Figure 2.3 Also, a detailed experimental procedure is given for all products

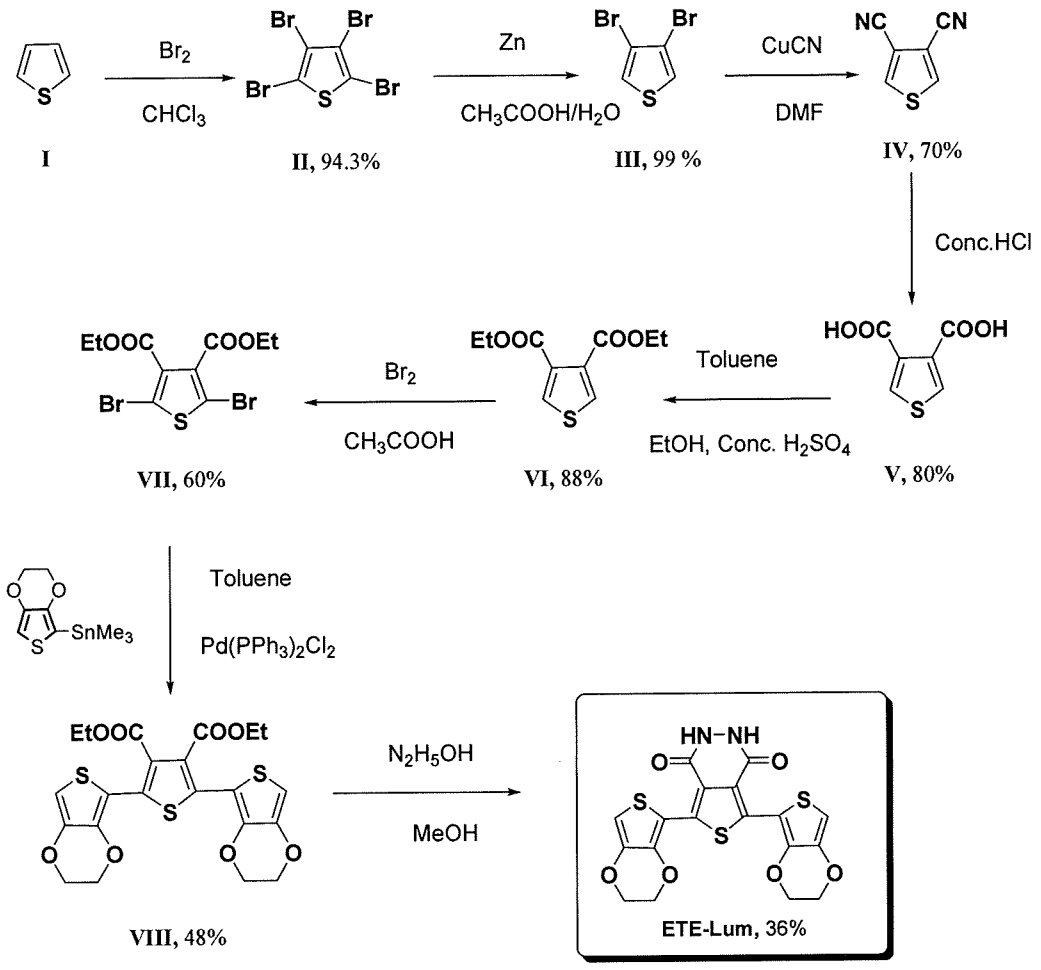
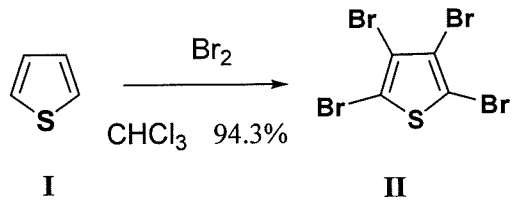


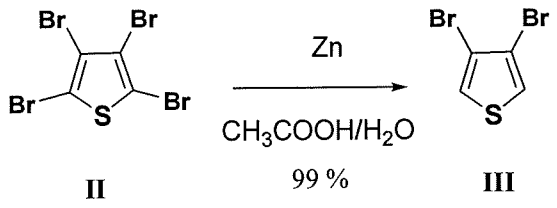
Figure 2.3 A synthetic route for the synthesis of ETE-Lum.

2.3.1. Synthesis of tetrabromothiophene (II):



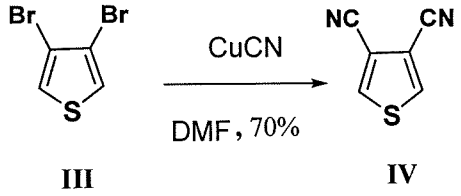
33.75 g (0.4 mol) thiophene and 14 mL $CHCl_3$ were placed in reaction balloon and kept in cold water bath at 0°C. 324 g (4 mol) bromine was added drop wise to this mixture and then the mixture was stirred for about 16 h at 110-115 °C. After that, the mixture was cooled to room temperature and 250 mL methanol was added. The solid phase was removed via filtration. The yellow solid product was recrystallized using methanol. (151.07 g, % 94.3 yield) [82]. ^{13}C NMR (100 MHz, $CHCl_3$): δ/ppm: 116.48, 109.80; IR (KBr, cm^{-1}): 1480, 1270, 1008, 854, 732.

2.3.2. Synthesis of 3,4-dibromothiophene (III):



120 mL AcOH, 40 mL H_2O and 50 g (0.77 mol) Zn power were placed in one necked reaction balloon and refluxed for 1 h. At the end of 1 h, temperature was slightly lowered and 100 g (0.25 mol) tetrabromothiophene was added. The mixture was stirred for about 5 h at at 110-120 °C. By using separatory funnel bottom phase was removed and saturated $NaHCO_3$ was added over this phase in order to remove AcOH from this phase. The organic phase was washed with water three times, dried over $MgSO_4$ and solvent was evaporated under vacuum (59.8 g, 99% yield). Since the product contained both 3,4-dibromothiophene and 3-bromothiophene, by using hexane solvent system and silica gel, product was separated [82] ^{1}H NMR (400 MHz, $CDCl_3$): δ/ppm: 7.3 (s, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): δ/ppm: 133.9, 114.1; IR (KBr, cm^{-1}) : 3115, 1472, 1399, 1325, 1310, 1115

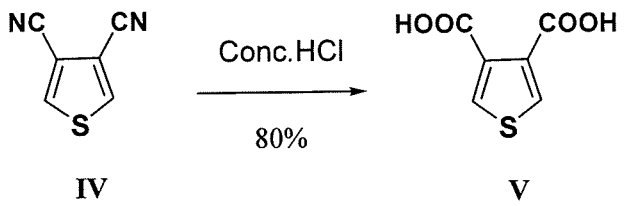
2.3.3. Synthesis of 3,4-dicyanothiophene (IV):



A solution of 3,4-dibromothiophene (3 g, 12 mmol) and cuprous cyanide (3.12 g, 34.8 mmol) in dry DMF (3mL) was refluxed at 110-120 °C for 4 hr under Ar(g). Then, the mixture was cooled to room temperature and a solution of hydrated $FeCl_3$ (12 g,74 mmol) in HCl (21 mL, 1.7 M).

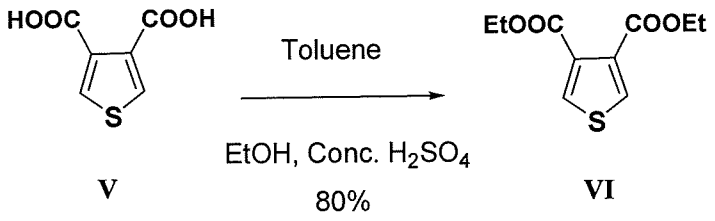
The mixture was kept at 60-70 ^{0}C for 30 min. After cooling the solution to room temperature, the raw product was taken into CH_2Cl_2 (15 mL) phase. Then, the organic phase was washed with 1.2 mL portions of HCl (6 M), water, saturated $NaHCO_3$ solution, and again with water. Final solution was dried over $MgSO_4$ and concentrated under vacuum to give a slightly yellow product. Then, further purification was done by recrystallizing from acetonitrile which yielded a white crystalline product. (1.16 g, 70% yield) [83]. ^{1}H NMR (400 MHz, DMSO): δ/ppm: 8.15 (s, 2H); ^{13}C NMR (100 MHz, DMSO): δ/ppm: 133.5, 133.6, 164.4; IR (KBr, cm^{-1}): 3189, 3108, 2231, 1738, 1509, 1299, 1163, 1108.

2.3.4. Synthesis of 3,4 thiophenedicarboxylic acid (V):



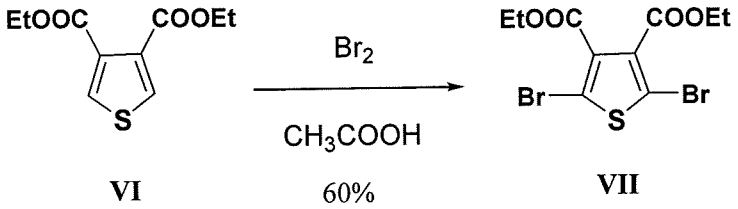
3,4-dicyanothiophene (0.7 g, 5.2 mmol) was refluxed in concentrated HCl (13 mL) for 6 h at 100 °C. After the completion of reaction, the mixture was cooled and the precipitated product was collected and recrystallized from water-ethanol (2:1) which yielded a white solid product (0.72 g, 80% yield). [84]. ^{1}H NMR (400 MHz, DMSO): δ/ppm: 8.16 (s, 2H); ^{13}C NMR (100 MHz, DMSO): δ/ppm: 133.5, 133.6, 164.4; IR (KBr, cm^{-1}): 3101, 3050, 1690, 1461, 1292, 1237, 1181, 949.

2.3.5. Synthesis of 3,4- thiophenedicarboxylic acid ester (VI):



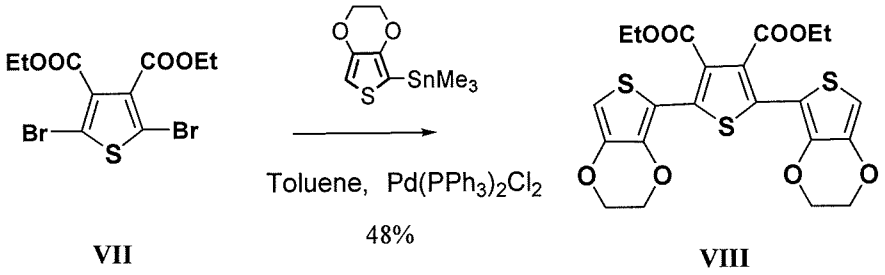
3,4-Thiophenecarboxylic acid (0.2 g, 1.16 mmol) was refluxed (5h) in ethanol (8.2 mL), dry toluene (4.1 mL) and concentrated HCl (40 μL, catalyts). A Dean-stark system was attached to the system containing ethanol -toluene (1:1) mixture. This mixture was refreshed every 1 h. After the completion of esterification reaction (checked by TLC) the reaction mixture was cooled to room temperature and concentrated under vacuum. Then, cold saturated $NaHCO_3$ solution was added and the product was extracted into benzene (4x 2 mL). Afterwards, organic phase was dried using $MgSO_4$ and the solvent was removed under reduced pressure to give a light yellow oily product (0.23 g 88 % yield). [85]. ^{1}H NMR (400 MHz, $CDCl_3$): δ/ppm: 1.34 (t, 6H, J= 7.1 Hz), 4.33 (q, 4H, J=6.9 Hz), 7.84 (s, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): δ/ppm: 14.1, 60.8, 131.4, 133.6, 163.02; IR (KBr,cm^{-1}): 3112, 2983, 1716, 1450, 1237, 1119, 1045.

2.3.6. Synthesis of diethyl 2,5-dibromothiophene-3,4-dicarboxylate (VII):



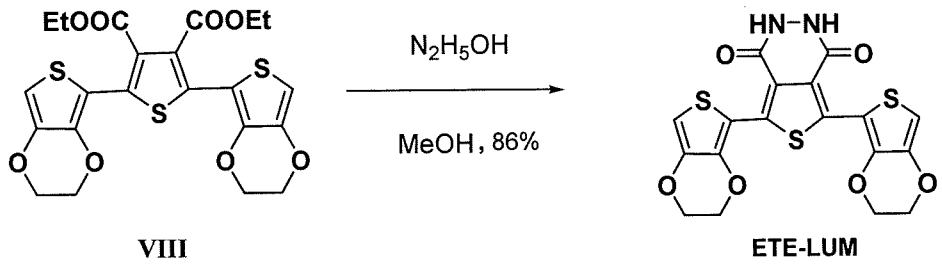
0.064 mL (1.25 mmol) bromine was added dropwise to a mixture of diethyl thiophene-3,4-dicarboxylate (58 mg, 0.25 mmol) in acetic acid (1 mL). Then, the mixture was refluxed at 70 °C until all the starting materials were consumed (TLC). After the reaction was complete, the flask was cooled and saturated $NaHCO_3$ solution was added to remove acetic acid and then the solution was extracted with dichloromethane. Organic phase was dried (MgSO4) and solvent was removed under reduced pressure to give a red liquid (59 mg, 0.15 mmol) in a yield of 60% [86]. ^{1}H NMR (400 MHz, $CDCl_3$): δ/ppm: 1.36 (t, J = 7.1 Hz, 6H), 4.35 (q, J = 7.1 Hz, 4H); ^{13}C NMR (100 MHz, $CDCl_3$): δ/ppm: 14.0, 61.9, 115.6, 133.8, 161.4.

2.3.7. Synthesis of Diethyl 2,5-di(3,4ethylenedioxythiophene-2-yl)thiophene-3,4-dicarboxylate (VIII):



Diethyl 2,5-dibromothiophene-3,4-dicarboxylate (62 mg, 0.18 mmol) was treated with $Pd(PPh_3)Cl_2$ catalyst (35 mg, 0.05 mmol) in the presence of 2-(trimethylstannyl)-3,4-ethylenedioxythiophene (23 mg, 1.15 mmol) and dry toluene (50 ml) as solvent under inert atmosphere and the solution was heated under reflux until all the starting materials were consumed (TLC). The flask was cooled and the solvent was removed under reduced pressure. The residue was filtered through a short pad of silica gel by eluting with a mixture of hexane and ethyl acetate (4:1) to give a yellow solid product (44 mg 48% yield) [87]. ^{1}H NMR (400 MHz, $CDCl_3$): δ/ppm: 1.3 (t, J=7.0, 3H) 4.29 (q, J=7.1, 2H) 4.36 (t, J=4.0, 2H) 5.9 (s, J=5.4 1H); ^{13}C NMR (100MHz $CDCl_3$,): δ/ppm: 14.1, 60.9, 65, 65.3, 94,9, 114.3, 131.6, 147.7, 150.3, 153.4, 160

2.3.8. Synthesis of 5,7-di-ethylenedioxythiophen-2-yl-2,3-dihydro-thieno[3,4-d]pyridazine-1,4-dione (ETE-Lum):



$N_2H_4.H_2O$ (6 mmol) was added to a solution containing diethyl 2,5-di(3,4ethylenedioxythiophene-2-yl)thiophene-3,4-dicarboxylate (54 mg, 0.19 mmol) in methanol (1 mL). Mixture was stirred approximately 1-2 h at room temperature, and then kept in a water bath for 3 days at 70°C. After the reaction was completed the reaction mixture was concentrated under reduced pressure. The crude solid product was dissolved in minimum amount of hot water containing 1% NH_4OH (by volume) and precipitated by adding concentrated HCl to this solution. The solid product was filtred and wash with cold water, and finaly dried under vacuum at 70 °C. (24 mg 36%) [88]. ^1H NMR (400MHz DMSO,) : δ/ppm: 4,3 (t, 2H) 5,9 (s, 1H) 8.0 (s, 1H); ^{13}C NMR (100MHz DMSO,): δ/ppm: 65-65.3-94.9-114.3-136.9-147.7-152.4-156.1-167.3

CHAPTER 3

RESULTS AND DISCUSSION

3.1. CL Properties of ETE-Lum

Since monomer ETE-Lum possesses the same chemiluminescent group as luminol, it is expected to undergo chemiluminescent reaction under proper conditions (i.e., in alkaline medium in the presence of H_2O_2 and catalyst). That is why, initially, the CL properties of ETE-Lum was investigated. ETE-Lum was dissolved in alkaline water (0.1 M NaOH) which contained an oxidant (H_2O_2 or $KMnO_4$) and a catalyst (Fe^{+3} or blood). As expected, a green bright light was observed in the dark by the naked eyes due to the CL reaction. In order to find the λ_{max} of emitted light, emission spectrum of ETE-Lum in DMSO was measured after excitation at 390 nm. The resulting absorption/emission spectrum recorded at room temperature is depicted in Figure 3.1. As seen from the figure, ETE-Lum exhibits an absorption band at about 400 nm due to $\pi \rightarrow \pi^*$ transition and an emission band at λ_{max}=500 nm corresponding to green light emission. A comparision of emission λ_{max} of ETE-Lum with TTT-Lum (λ_{max} =495 nm) and luminol (λ_{max}=425 nm) reveals that ETE-Lum emits visible light at higher wavelenghts than those of two other compounds (Figure.3.2). It is important to note that this light emission at higher wavelenghts is less energetic; thus, it is expected to be less dangerous for biological materials like DNA.

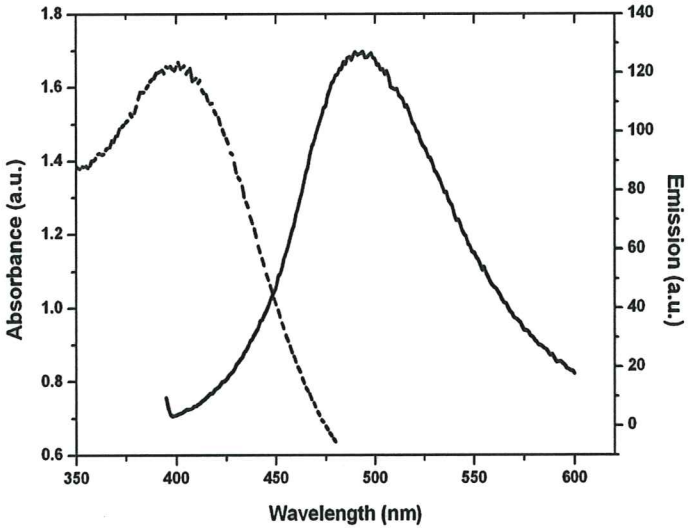


Figure 3.1 Absorption and emission (excited at 390 nm) spectrum of ETE-Lum in DMSO

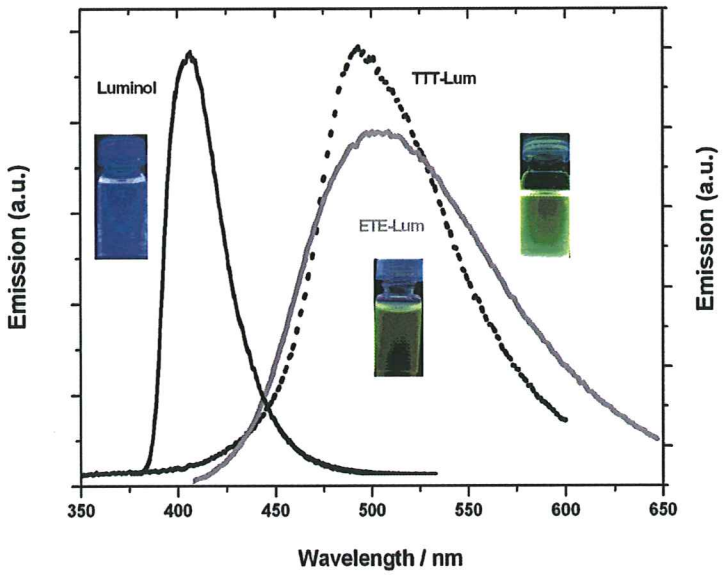


Figure 3.2 Emission spectra of ETE-Lum, TTT-Lum and luminol in DMSO.

Although, H_2O_2 is an indispensable strong oxidant for CL reaction which occurs in one step (Figure 3.3), $KMnO_4$, on the other hand, is another strong oxidant capable of giving CL reaction. However, the reaction mechanism is slightly different from that of H_2O_2. When luminol derivatives meet the oxidant, CL reaction occur for the second time. Initially, some of luminol derivatives oxidized then rest of them oxidized together with catalyst (Figure 3.4) Thus, higher intensity of emitted light was expected when $KMnO_4$ is used as oxidant. This was also tested by using 10^{-4} M $KMnO_4$ solution containing 10^{-5} M ETE-Lum in 0.1 NaOH solution and the result was compared with the one obtained using the same concentration of H_2O_2 as oxidant. Almost 200 fold increase in the light intensity was observed when $KMnO_4$ is used as oxidant (Figure 3.5). Besides the large increase in the light intensity, another difference, worth to mention that ETE-Lum is only highly sensitive to Fe^{+3} (Figure 3.6).

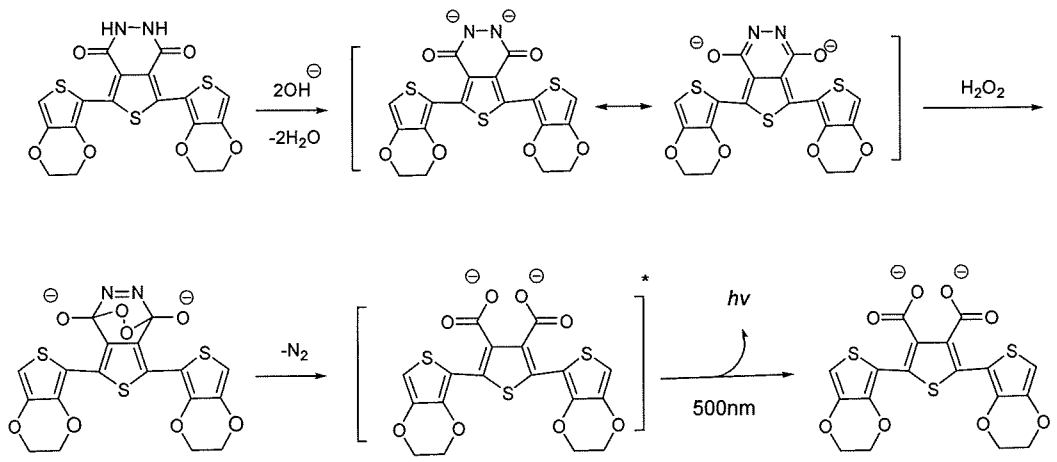


Figure 3.3 Reaction mechanism between ETE-Lum derivatives with H_2O_2.

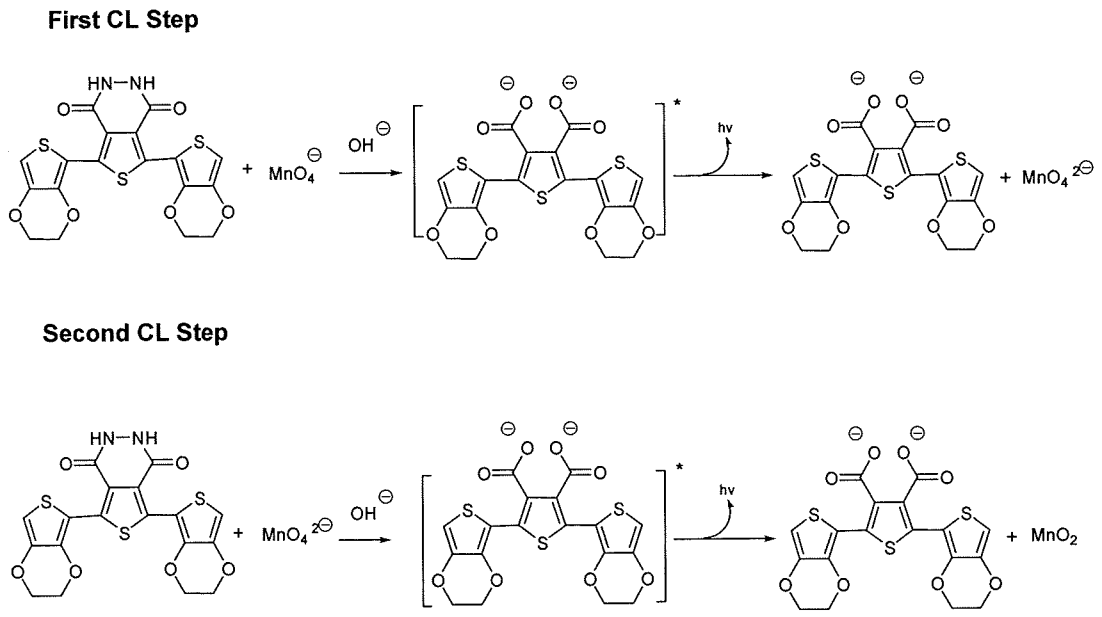


Figure 3.4 A Proposed CL reaction mechanism of ETE-Lum with $KMnO_4$ [89].

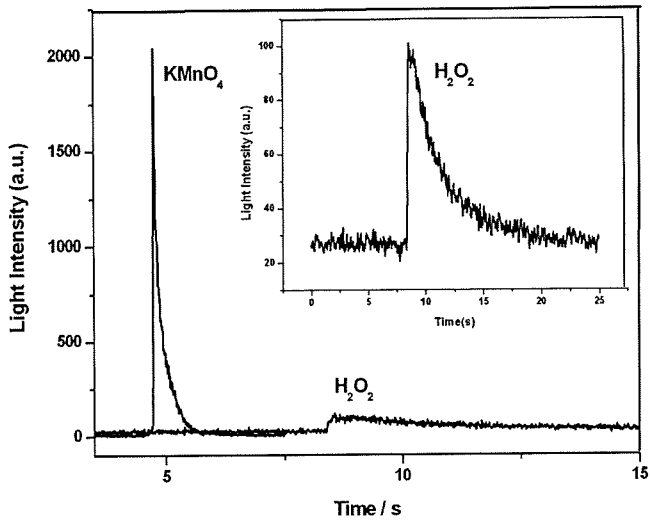


Figure 3.5 The intensity of the light obtained from the CL reaction of ETE-Lum (10^{-5} M) in 0.1 M NaOH solution with H_2O_2 (10^{-4} M), and $KMnO_4$ (10^{-4} M).

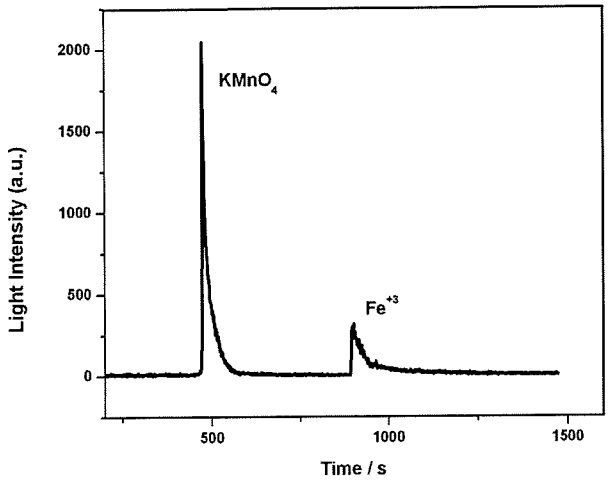


Figure 3.6 The intensity of the light obtained from the CL reaction between ETE-Lum (10^{-5} M) in 0.1 M NaOH solution with $KMnO_4$ (10^{-4} M) in presence Fe^{+3}

Generally, the chemical reaction between luminol and H_2O_2 is very slow which can be speeded up by adding a proper catalyst. This will not only increase the reaction rate but also provides enough light photon to see glowing light. It is well known that CL reaction of luminol can be catalyzed by various metal ions, especially Fe^{3+}. Thus, in order to find proper metal ion to catalyze CL reaction of ETE-Lum, light emission was monitored in the presence of some metal ions. Fe^{3+}, Co^{2+}, Cr^{3+}, Cu^{2+}, Ni^{2+}, Ag^{+}, Cd^{2+} and Zn^{2+} were tested for this purpose utilizing a flow injection system having a PMT. As seen from Figure 3.7, ETE-Lum is very sensitive to Fe^{+3} and green light emission still can be detected in the presence of 10^{-7} M Fe^{3+}. It is noteworthy to mention that ETE-Lum exhibited responce selectively only towards Fe^{+3} ion (Figure 3.8)

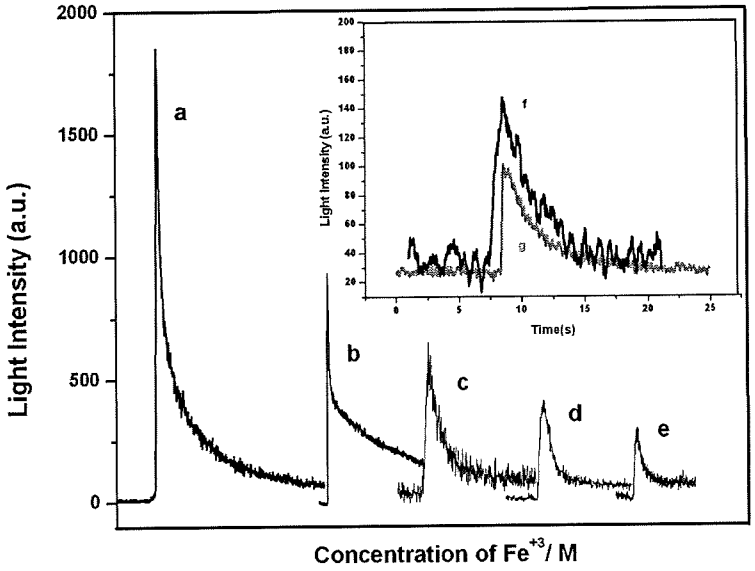


Figure 3.7 The intensity of the light obtained from the CL reaction between ETE-Lum (10^{-5} M) in 0.1 M NaOH solution and H_2O_2 (10^{-4} M) in presence different Fe^{+3} concentration: (a) 10^{-2} M, (b) 10^{-3} M (c) 10^{-4} M (d) 10^{-5} M (e) 10^{-6} M (f) 10^{-7} M (g) background (10^{-4} M H_2O_2).

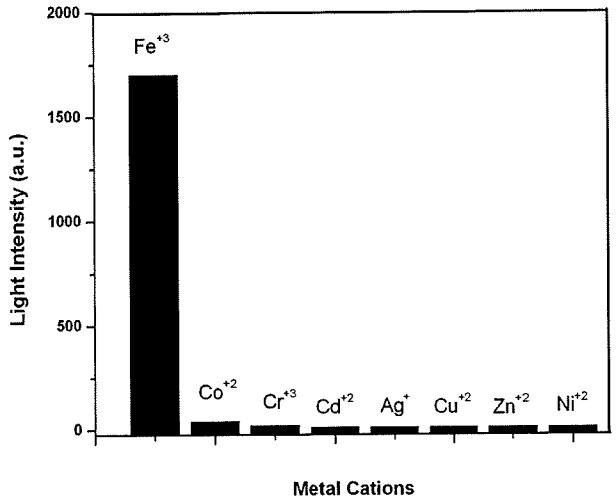


Figure 3.8 The intensity of the light obtained from the CL reaction between ETE-Lum (10^{-5} M) in 0.1 M NaOH solution and H_2O_2 (10^{-4} M) in the presence of different metal cations (10^{-2} M).

On the basis of above findings, one might think that ETE-Lum can be used for detection of trace amount of hidden bloodstain since it contains Fe^{3+}. Hemoglobin in red blood cell contains Fe^{+2} ion which is oxidized to Fe^{+3} out of organism. Thus, old blood can be detected when ETE-Lum alkaline solution containing H_2O_2 was sprayed on bloodstains in the dark. As expected, the emission of green color was observed which unambigiously confirmed the presence of bloodstains (Figure 3.9). It is also found that ETE-Lum is very sensitive to blood samples even at very low concentrations (1ppm).

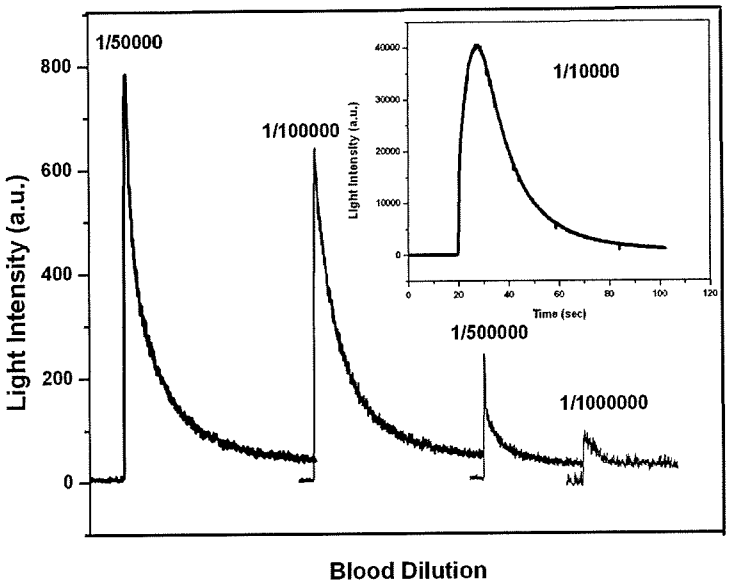


Figure 3.9 The intensity of the light obtained from the CL reaction between ETE-Lum (10^{-5} M) in 0.1 M NaOH solution and H_2O_2 (10^{-4} M) in the presence of different dilution ratio of blood with water.

3.2. Electrochemical Behavior of ETE-Lum and its polymer, PETE-Lum

Since a new molecule, TTT-Lum, possessing CL property was synthesized in our group, it would be interesting to compare its electrochemical behavior with ETE-Lum. Figure 3.10 shows the cyclic voltammogram of both monomers recorded under the same conditions. A close inspection of Figure 3.10 reveals that the first oxidation peak of ETE-Lum (0.88 V vs Ag/AgCl) is slightly lower than that of TTT-Lum (1.1 V vs Ag/AgCl). This difference can be explained in terms of electron donating effect of ethylenedioxide groups attached to the thiophene ring.

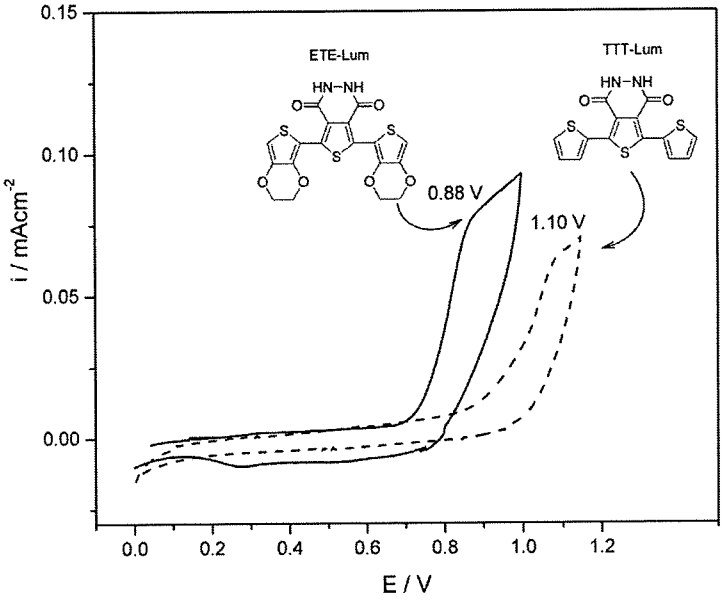


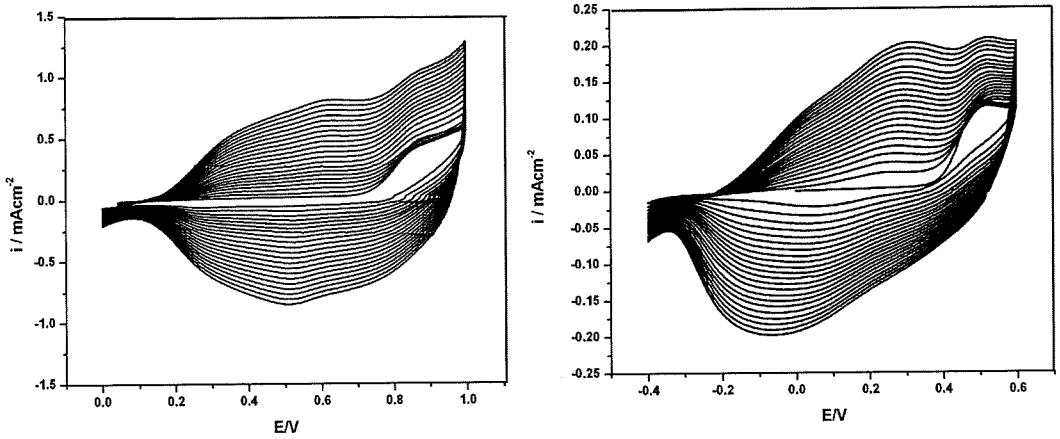
Figure 3.10 Electrochemical behaviors of ETE-Lum in 0.1 M TBAP/ACN containing BF_3- Et_2O and TTT-Lum in 0.1 M $LiClO_4$/ACN containing BF_3- Et_2O vs Ag/AgCl.

Besides lower oxidation potential, another advantage of ETE-Lum over TTT-Lum is that formation of linear polymer chains is guaranteed. The reason for this is that there exists only 2 and 5 positions of the thiophene rings which are left available for the growth of polymer chain. This linear structure is further protected by intermolecular interaction between sulfide and oxygen atoms. In contrast, polymerization of TTT-Lum takes place at the 2-position of thiophene side groups. However, polymer growth is also possible via 3-position which might prevent linear polymer chain formation.

Although there appears an irreversible peak at 0.88 V during the first anodic scan, a new reversible peak at about 0.55 V also starts to intensify during repetitive scans (Figure 3.11 a). The appearance of this new reversible peak and the increase in its current

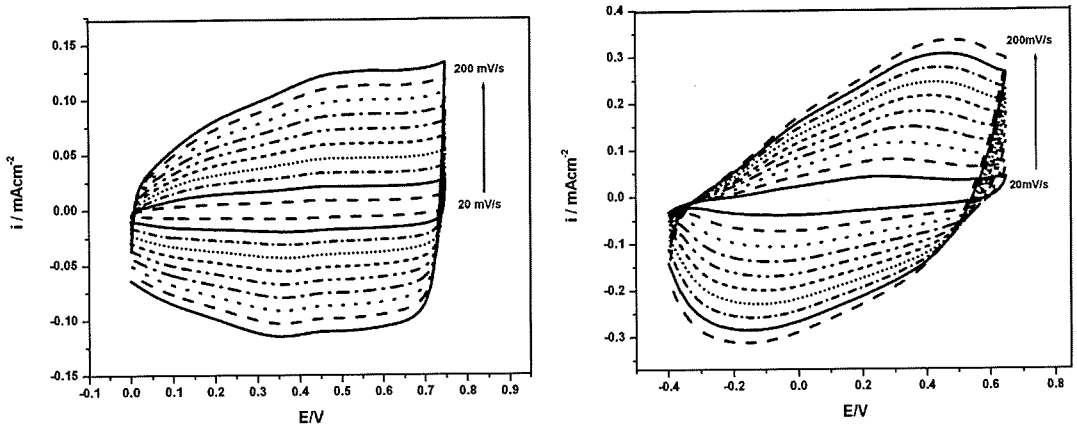
intensity with successive scans indicates the formation of a conducting polymer film on the electrode surface (Figure 3.12 a).

Electropolymerization of ETE-Lum was also studied in neat BF_3-Et_2O solution . When compared to ACN/BF_3-Et_2O mixture, neat BF_3-Et_2O solution caused a decrease in the oxidation potential of the monomer (0.5 V) and the electropolymerization can be achieved at lower potential by repeating potential scanning (Figure 3.11 b). The polymer film obtained via potensiodynamic method was removed from the cell and then washed with ACN to remove the traces of monomer or electrolyte on the polymer film surface. Then, redox behaviour of polymer film was monitored by recording the cyclic voltammogram in monomer-free electrolyte solution at different scan rates. The polymer film obtained either in ACN/BF_3-Et_2O mixture or neat BF_3-Et_2O showed a well-defined reversible redox couple (0.4 V) in monomer-free electrolyte solution. (Figure 3.12 b). It is also found that the anodic and cathodic peak currents increase linearly with increasing voltage scan rate (Figure 3.13), indicating a well-adhered polymer film and a non-diffusional redox process.



(a) (b)

Figure 3.11 Cyclic voltammogram of 10^{-4} M ETE-Lum on an ITO electrode in 0.1 M TBAP/ACN containing BF_3- Et_2O and neat BF_3- Et_2O at a scan rate of 100 mV/s vs Ag wire.



(a) (b)

Figure 3.12 .Redox behaviour of PETE-Lum film in 0.1 M TBAP/ACN and neat BF_3-Et_2O at different scan rate: 20 mV/s-200 mV/s

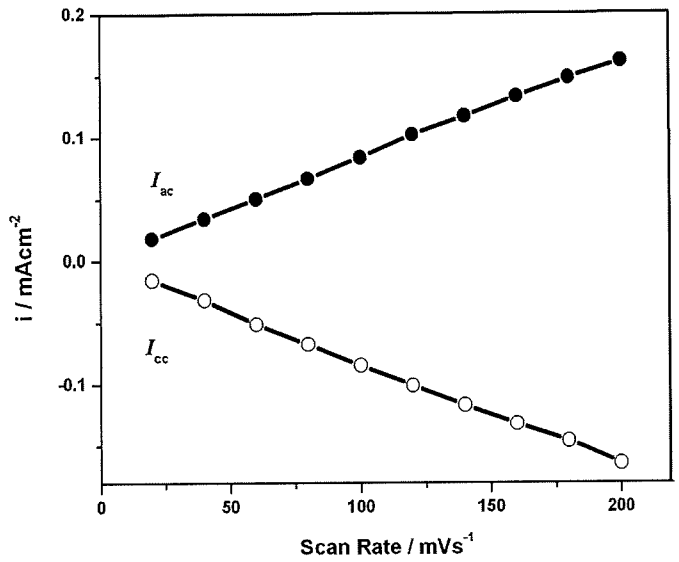


Figure 3.13 Relationship of anodic and cathodic current peaks as a function of scan rate for neutral and oxidized PETE-Lum in 0.1 M TBAP/ACN

As seen from Figure 3.12 a, the shape of cyclic voltammogram look likes a rectangle, indicating that film has capacitance property even at high scan rates. Furthermore, stability of PETE-Lum film was also tested by cycling 500 times at a scan rate of 200 mV/s between 0.0 and 0.75 V. The result is depicted in Figure 3.14 and a close inspection of figure indicates that film lost only 8% of its activity after 500th cycle.

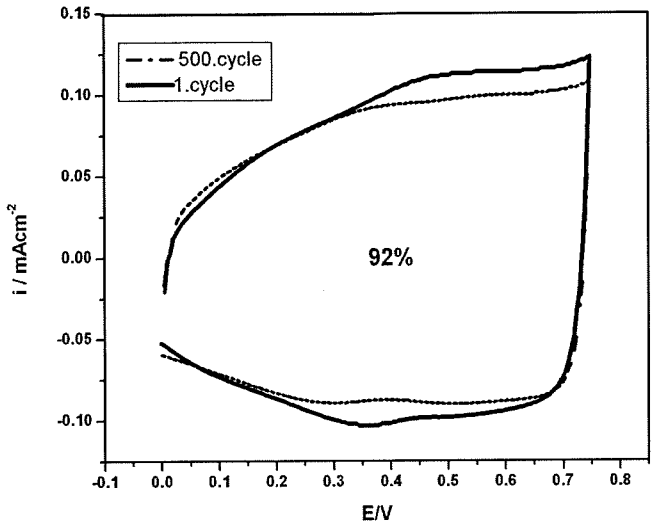


Figure 3.14 Stability of PETE-Lum film in 0.1 M TBAP when switched between 0.0 V and 0.75 V at a scan rate of 200 mV/s vs Ag wire.

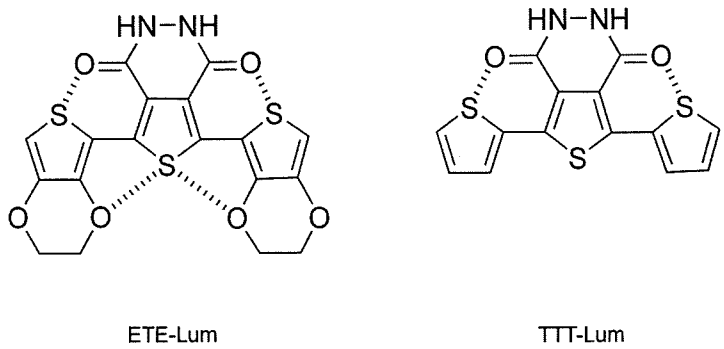


Figure 3.15 A proposed intermolecular interaction of ETE-Lum and TTT-Lum

3.3. Spectroelectrochemical Properties of PETE-Lum

The spectroelectrochemical properties of ETE-Lum film deposited onto ITO coated glass were investigated by monitoring the changes in electronic absorption spectra during doping process. The polymer film exhibits a well-defined absorption band at 550 nm in its neutral state due to $\pi \rightarrow \pi^*$ transition. The electronic band gap is defined as the onset energy for the $\pi \rightarrow \pi^*$ transition and it was determined as 1.66 eV (Figure 3.16). This value was found to be slightly lower than that of TTT-Lum (E_g=1.74 eV). Upon oxidation, the intensity of the π-π^* transition band of PETE-Lum starts to decrease and a new absorption band intensifies at 860 nm due to the polaron formation. Further oxidation caused the disappearance of 550 nm band, which is accompained by the appearence of a new band at about 1080 nm due to bipolaron formation.

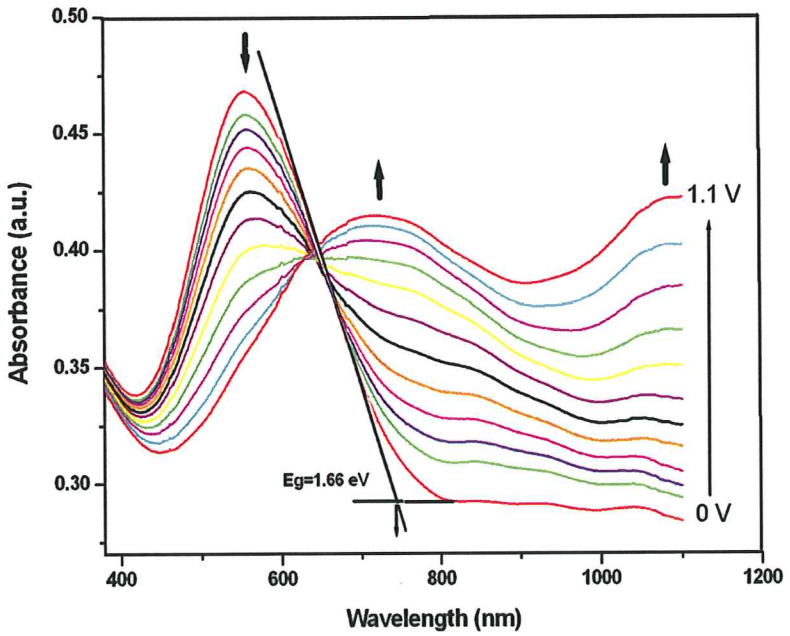


Figure 3.16 Spectroelectrochemistry of PETE-Lum on ITO in ACN containing 0.1 M TBAP at various applied potentials between 0.0 V and 1.1 V.

3.4. ECL Properties of PETE-Lum

Although ETE-Lum exhibits one irreversible oxidation peak at 0.88 V, there appears two more irreversible oxidation peaks when the potential window was increased beyond 1.5 V vs Ag wire (Figure 3.17). The first oxidation peak at 0.88 V was attributed to the oxidation of EDOT side groups, while the other peaks were attributed to the fragment of the chemiluminescent group and the overoxidation of the oligomers and/or the polymer film, respectively. In order to support these findings, we combined cyclic voltammetry with PMT. All attempts to observe light emission due to ECL reaction of ETE-lum was unsuccesful even at potentials as high as 2.0 V in organic solvent. This observation is contrary to the results obtained by using TTT–Lum, and it might be due to steric hindrance caused from EDOT side groups.

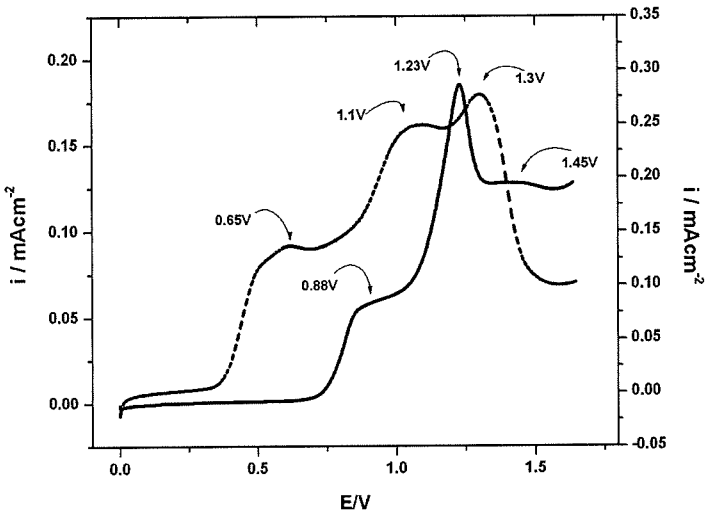


Figure 3.17 First anodic scan of PETE-Lum in 0.1 M TBAP/ACN (—) and neat BF_3-Et_2O (---) at a scan rate of 100 mV/s between 0 V and 1.7 V.

Since PETE-Lum also exhibits three oxidation peaks due to polymer film oxidation (doping), oxidation of chemiluminescent group and overoxidation, respectively (see Figure 3.18), we have repeated ECL measurements utilizing PETE-Lum film on ITO working electrode. Fortunately, light emission was detected by PMT when PETE-Lum coated electrode potential was adjusted to 0.9 V vs Ag wire. It is worth to mention that this light intensity was found to increase with increasing working electrode potential.

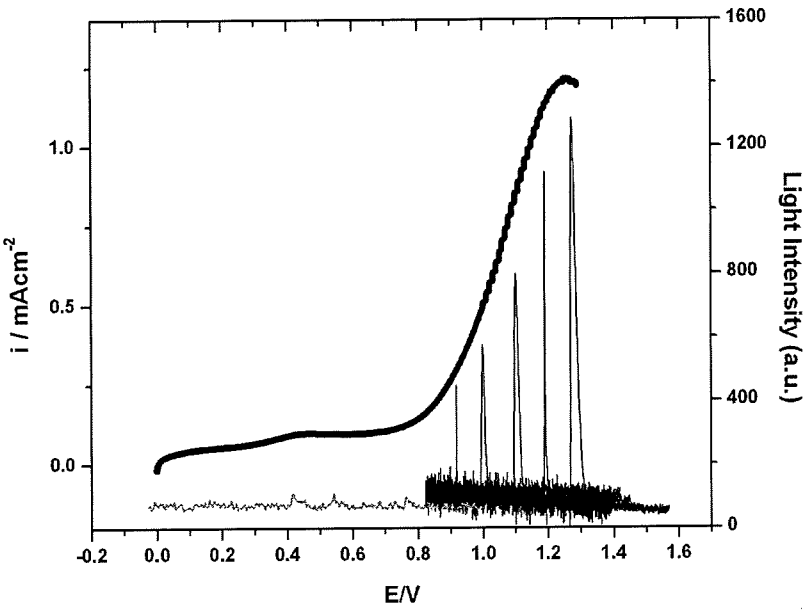
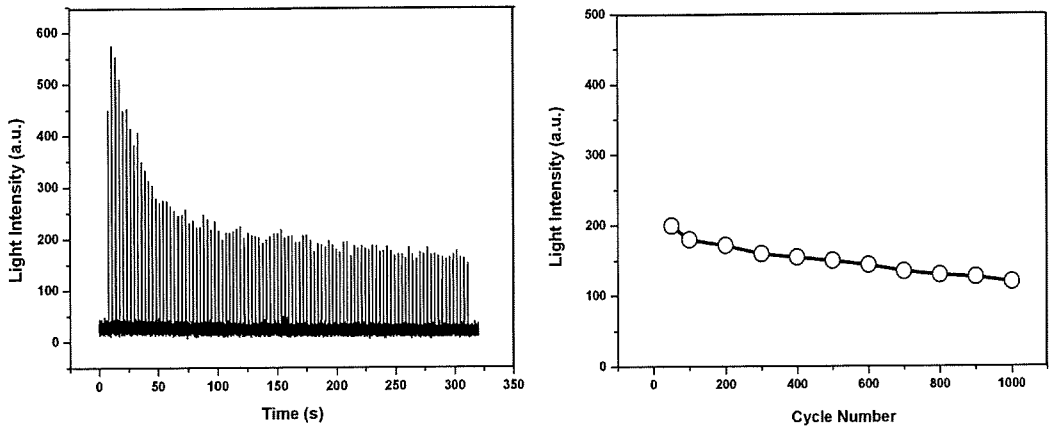


Figure 3.18 Cyclic voltammogram of PETE-Lum on an ITO electrode in 0.1 M TBAP/ACN containing BF_3- Et_2O at a scan rate of 100 mV/s while the generated light emission during potential scanning.

Furthermore, we have also tested repetitive usage of PETE-Lum coated ITO electrode by applying potential pulses for different time intervals(Fig. 3.19 a).. It is found that PETE-Lum coated (3.2 mC/cm^2) ITO working electrode can be switched thousand times between 1.1 V (0.1 s) and 0.0 V (0.3 s) without an appreciable loss in the light intensity in spite of 1000 times switching (Fig. 3.19 b).



(a) (b)

Figure 3.19 First 100 and 1000 switching behavior of PETE-Lum between at 1.1 V (0.1 s) and 0.0 V (3.0 s) in 0.1 M TBAP/ACN vs Ag wire.

These observations revealed that chemiluminescent groups in the middle of the ETE-Lum structure was protected upon polymerization. In other words, chemiluminescent groups did not undergo ring opening until 0.95 V. These observations also proved that ETE-Lum polymerized via 2 positions of the EDOTs without any other possibility.

CHAPTER 4

CONCLUSION

In this work, a monomer possessing CL properties was synthesized successfully and Fe^{3+} and blood detection limit were determined in 0.1 M NaOH solution in the presence of an oxidant (10^{-4} M H_2O_2) via PMT. ETE-Lum produces green color light in the presence of Fe^{3+} and blood in the dark room. Catalytical effect of other metal ions, e.g. Cr^{3+}, Cu^{2+}, Co^{2+}, Cd^{2+}, Ni^{2+}, Ag^{+}, Zn^{2+} were also analyzed concentration and it is found that ETE-Lum shows selectivity towards Fe^{3+} ion. The lowest detection limit for Fe^{3+} ion was found to be 10^{-7} M at 10^{-4}M H_2O_2. This makes ETE-Lum an alternative candidate for blood detection instead of luminol. It is also found that ETE-Lum can be used easily for detection of hidden blood stain in forensic science application. Electroactive polymer films were obtained by electrochemical oxidation of ETE-Lum in ACN containing BF_3-Et_2O, TBAP electrolyte solution as well as in neat BF_3-Et_2O. Well-adhered and very stable polymer films, especially in neat BF_3-Et_2O, were prepared on ITO working electrode surface for spectroelectrochemical investigations. The band gap of the polymer was found to be 1.66 eV from the absorption spectrum of the neutral form of the film. ECL properties of PETE-Lum was also invetigated using PMT combined with cyclic voltammetry in ACN/TBAP solvent system. Generated light was observed by oxidized chemiluminescent part of PETE-Lum with the applied potential at 0.9 V. It was proved that chemiluminescent part of monomer was protected while electrochemical polymerization went on. Also, coated polymer on electrode (only 3.2 mC/cm^2) gave response even after 1000 swichting at 1.1 V.

In conclusion, both ETE-Lum and TTT-Lum have some advantages over luminol which is a well-known chemiluminescent agent in literature. The most important one is that luminol gives only very thin films on electrode surface. Free standing polyluminol film can not be obtained by electrochemical techniques and also polymerization mechanism is not well known for luminol (both amine and hydrazine sites are suitable for polymerization). Solubility is another important problem for luminol because it dissolves in only alkaline water while ETE-Lum and TTT-Lum dissolve in some organic solvents like ACN, methanol, dimethyl formamide, DMSO. As a result, a new novel monomer having CL properties was synthesized and its electroactive, well-adhered polymer giving ECL response was obtained. All these properties were gained new point of view for forensic and analitical applications.

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