A GLOW IN THE DARK:
SYNTHESIS AND ELECTROPOLYMERIZATION OF CHEMILUMINESCENT THIOPHENE DERIVATIVES

A THESIS SUBMITTED TO
THE GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES
OF
MIDDLE EAST TECHNICAL UNIVERSITY

BY

DEMET ASİL

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR
THE DEGREE OF MASTER OF SCIENCE
IN
CHEMISTRY

SEPTEMBER 2008

Approval of the thesis:

A GLOW IN THE DARK:
SYNTHESIS AND ELECTROPOLYMERIZATION OF CHEMILUMINESCENT THIOPHENE DERIVATIVES

submitted by **DEMET ASİL** in partial fulfillment of the requirements for the degree of **Master of Science in Chemistry Department, Middle East Technical University** by,

Prof. Dr. Canan Özgen ______________________
Dean, Graduate School of **Natural and Applied Sciences**

Prof. Dr. Ahmet M. Önal ______________________
Head of Department, **Chemistry**

Prof. Dr. Ahmet M. Önal ______________________
Supervisor, **Chemistry Department, METU**

Assist. Prof. Dr. Atilla Cihaner ______________________
Co-Supervisor, **Chemistry Group, Atılım University**

Examining Committee Members

Prof. Dr. Levent K. Toppare ______________________
Chemistry Dept., METU

Prof. Dr. Ahmet M. Önal ______________________
Chemistry Dept., METU

Prof. Dr. Engin U. Akkaya ______________________
Chemistry Dept., Bilkent University

Prof. Dr. Teoman Tinçer ______________________
Chemistry Dept., METU

Assist. Prof. Dr. Atilla Cihaner ______________________
Chemistry Group, Atılım University

Date: **September 4, 2008**

I hereby declare that all information in this document has been obtained and presented in accordance with academic rules and ethical conduct. I also declare that, as required by these rules and conduct, I have fully cited and referenced all material and results that are not original to this work.

Name, Last name : Demet Asil

Signature :

ABSTRACT

A GLOW IN THE DARK: SYNTHESIS AND ELECTROPOLYMERIZATION OF CHEMILUMINESCENT THIOPHENE DERIVATIVES

Asil, Demet

M.Sc., Department of Chemistry

Supervisor : Prof. Dr. Ahmet M. Önal

Co-Supervisor: Assist. Prof. Dr. Atilla Cihaner

September 2008, 63 Pages

Two novel chemiluminescent monomers, 2,3-dihydrothieno(3,4-d)pyridazine-1,4-dione (T-Lum) and 5,7-di-thiophen-2-yl-2,3-dihydro-thieno[3,4-d]pyridazine-1,4-dione (TTT-Lum), were synthesized. The reaction between T-Lum and TTT-Lum in alkaline solution with H_2O_2 gave chemiluminescence which can be catalyzed using Fe(III) ion. Owing to its sensitivitiy towards Fe(III) ion; T-Lum and TTT-Lum can be promising materials to detect bloodstains in the application of forensic science instead of luminol which gave response to a large family of metallic cations beside Fe(III). Also, TTT-Lum, which is based on a terthienyl system, was electropolymerized and its corresponding polymer (PTTT-Lum) was obtained via repetitive cycling or constant potential electrolysis in both 0.1 M $LiClO_4$ dissolved in acetonitrile containing 5% of BF_3-Et_2O by volume and neat BF_3- Et_2O solution. In addition, PTTT-Lum, soluble in alkaline water, was synthesized successfully without breaking the pyridazinedione unit (chemiluminescent unit), as proved by Scanning Electron Microscopy (SEM), Fourier Transform Infrared Spectroscopy (FTIR) and Electrochemical Luminescence (ECL) measurements. Thus, PTTT-Lum, bearing chemiluminescent unit, can be a good candidate to be used as a sensor in near future. Furthermore, the PTTT-Lum film has a very stable and well-defined reversible redox couple as well as electrochromic behavior during p-doping process. The polymer film has also a band gap of 1.74 eV with an absorption band in its neutral state at 536 nm. Finally, PTTT-Lum film was found to be electrochemiluminescence active, maintaining its activitiy over 1000 cycles.

Keywords: Forensic Science, Chemiluminescence, Luminol, Polythiophene

ÖZ

KARANLIKTA BİR IŞIK:
KİMYASAL IŞIL-IŞIMA ÖZELLİĞİNE SAHİP TİYOFEN TÜREVLERİNİN SENTEZİ VE ELEKTROKİMYASAL POLİMERİZASYONU

Asil, Demet,

Yüksek Lisans, Kimya Bölümü

Tez Yöneticisi : Prof. Dr. Ahmet M. Önal

Tez Yöneticisi Yardımcısı : Yrd. Doç. Dr. Atilla Cihaner

Eylül 2008, 63 Sayfa

Bu çalışmada kimyasal ışıl-ışıma yapan iki yeni monomer; 2,3-dihidrotiyeno(3,4-d)piridazin-1,4-dion (T-Lum) ve 5,7-di-tiyofen-2-yl-2,3-dihidro-tiyeno[3,4-d]piridazin-1,4-dion (TTT-Lum) sentezlenmiştir. Alkali ortamda ve H_2O_2 varlığında çözünmüş olan T-Lum ve TTT-Lum çözeltileri sadece Fe^{+3} iyonuyla katalizlenebilen kimyasal ışıl-ışıma reaksiyonu vermektedir. Bu reaksiyonun kan varlığında da meydana geldiği gözlenmiştir. Bunun sonucu olarak T-Lum ve TTT-Lum adli tıp biliminin kan bulgularının tespiti alanında, Fe^{+3}'ün yanısıra bir çok metal iyonuna cevap veren luminol maddesinin yerine kullanılmak üzere umut verici maddeler olabilirler. TTT-Lum, elektrokimyasal olarak polimerleştirilmiş ve ilgili polimeri (PTTT-Lum) hacimce %5 BF_3-Et_2O içeren 0.1 M $LiClO_4$/asetonitril ve sadece BF_3-Et_2O ortamlarında döngülü voltametre veya sabit potansiyel kullanılarak elde edilmiştir. Ayrıca, alkali ortamda çözünebilen PTTT-Lum, kimyasal ışıl-ışıma yapan birimleri içermektedir; polimerleştirme sırasında söz konusu birimler herhangi bir deformasyona uğramamıştır. Bu bulgu Taramalı Elektron Mikroskopu (SEM), Fourier Dönüşüm Kızılötesi Spektroskopisi (FTIR) ve Elektrokimyasal Işıma (ECL) teknikleri kullanılarak kanıtlanmıştır. Sonuç olarak, kimyasal ışıl-ışıma birimleri içeren PTTT-Lum gelecekte sensör olarak kullanılabilmeye adaydır. Bunlara ek olarak, PTTT-Lum filmi tanımlı ve tersinir bir redoks davranımına sahiptir. PTTT-Lum ayrıca, pozitif katkılandırma sırasında elektrokromik davranım göstermektedir. Sentezlenen polimer filmi 1.74 eV değerinde bir bant aralığına sahiptir ve nötr halde 536 nm de soğurma bandı vardır. Son olarak, PTTT-Lum filminin elektrokimyasal ışıma yapabilme özelliğine sahip olduğu kanıtlanmıştır. Elde edilen film aktivitesini 1000 anahtarlama sırasında koruyabilmiştir, bu da bu elektrotun binlerce kez kullanımına olanak sağlamıştır.

Anahtar Kelimeler: Adli Tıp Bilimi, Kimyasal Işıl-Işıma, Luminol, Politiyofen

TO MY PARENTS

ACKNOWLEDGMENTS

Throughout the journey of life, I have had the honor of being sorrounded by exceptional people. Without their guidance and friendship, the completion of this stage in my journey would not have been possible. First, I would like to express my eternal gratitude to my parents, Akkız and Kemal Asil and to my brother, Ümit Asil for allowing me to pursue my goals and for remaining supportive of the decisions I made to reach those goals.

I give special thanks to my supervisor Prof. Dr. Ahmet M. Önal for his patience and guidance throughout this work. I wholeheartedly thank my co-supervisor Assist. Prof. Dr. Atilla Cihaner for encouraging me to work in this project and special thanks for his endless patience and jokes.

Friendship is a powerful bond that I do not take lightly so those that I consider friends truly hold a special place in my heart; Cem Alptekin, Filiz Erdal, Hülya Esmer and Zeynep Koyuncu are such friends with whom I have shared valuable times that will not be forgotten.

I extend thanks to the members of Prof. Dr. Ahmet M. Önal Research Group, Nurdan Atılgan, Buket Bezgin, Merve Özçelik and Seha Tirkeş for their help, patience and friendship.

Finally, I want to thank the Scientific and Technical Research Council of Turkey (TUBITAK-TBAG106T355) and METU-Chemistry Department for financial and technical support of this work, and Dr. Fatih Algı for his help and valuable discussions.

TABLE OF CONTENTS

CHAPTER

LIST OF FIGURES

FIGURES

ABBREVIATIONS

ACN	Acetonitrile
E_g	Band Gap
BF_3-Et_2O	Borontrifluoro diethylether
CL	Chemiluminescence
DMSO	Dimethylsulfoxide
ECL	Electrochemiluminescence
FTIR	Fourier Transform Infrared Spectroscopy
ITO	Indium-tin oxide
NMR	Nuclear Magnetic Resonance
ir	Infrared
PMT	Photomultiplier Tube
PTTT-Lum	Poly(5,7-di-thiophen-2-yl-2,3-dihydro-thieno[3,4-d]pyridazine-1,4-dione)
RGB	Red-Green-Blue
SEM	Scanning Electron Microscopy
TLC	Thin Layer Chromatography
T-Lum	2,3-dihydrothieno(3,4-d)pyridazine-1,4-dione
TTT-Lum	5,7-di-thiophen-2-yl-2,3-dihydro-thieno[3,4-d]pyridazine-1,4-dione
UV	Ultraviolet
vis	Visible

CHAPTER 1

INTRODUCTION

1.1 Chemiluminescence (CL)

Luminescence is defined as the emission of electromagnetic radiation in the ultraviolet (UV, 100 nm- 400 nm), visible (vis, 400 nm- 760 nm) and infrared (ir, 760 nm- 1000 μm) regions, which is produced by the transition of an electronic excited state to a lower state, usually ground state. All types of luminescence processes have been distinguished by a prefix indicating the energy source responsible for the initiation of emission: the absorption of light (photoluminescence), heating (thermoluminescence), an electric current (electroluminescence), a chemical reaction (chemiluminescence, CL) and so on. In CL, for example, the process of light emission is initiated by a chemical reaction and then one of the reaction products in the excited state emits light on falling to the ground state. On the other hand, instead of the chemical reaction, UV or vis light can be used to initiate luminescence phenomena. If the electronically excited state returns to the ground state (S_0) from the lowest singlet excited state (S_1), it is called fluorescence or if it returns from the triplet excited state (T_1), it is called phosphorescence. These possible transitions are shown by Jablonski energy diagram in Fig. 1.1. During the decay of the electronically excited state to the ground state in CL, the light emitted may be in different degrees of intensity, lifetime and wavelength; that is, the light emitted may be in UV and ir regions as well as vis region [1]. Thus, CL emission is not restricted to vis light only.

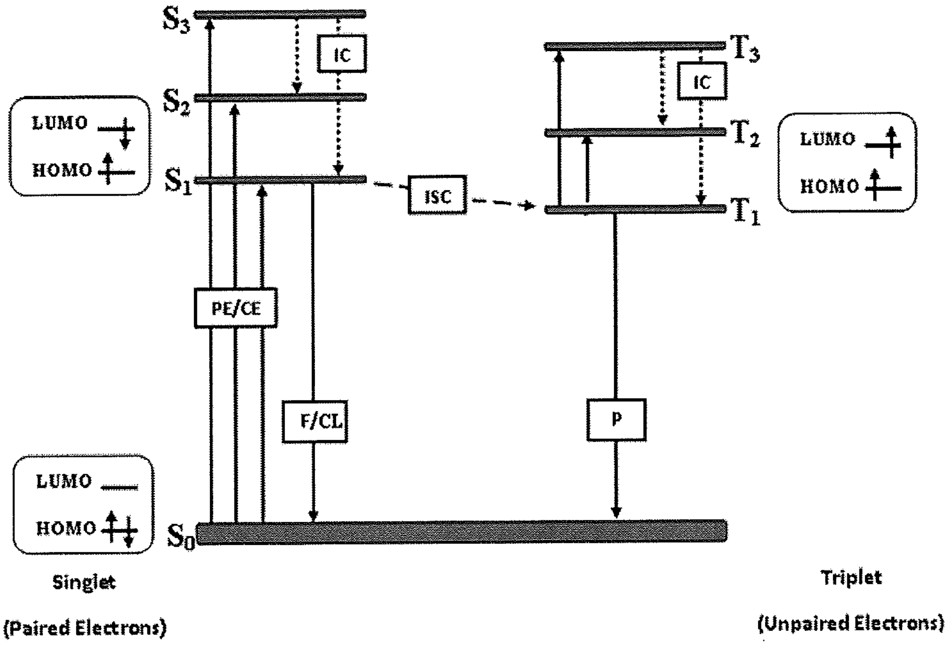


Figure 1.1 Jablonski energetic diagram: PE, Photoexcitation; CE, Chemiexcitation; CL, chemiluminescence; F, fluorescence; P, phosphorescence; IC, internal conversion; ISC, intersystem crossing; S_0, ground singlet state; S_1, S_2, S_3 excited singlet states; T_1, T_2, T_3 excited triplet states; ⟶, radiative transition; — — ▸, ·········▸, non-radiative transitions.

Actually CL reactions are uncommon, not every molecule can make CL, there are three energetic requirements for a molecule to emit light from a chemical reaction;

a) Reaction pathway to produce an electronically excited state should be favorable. If most of the chemical energy is lost as heat during the transitions, the reaction will not be chemiluminescent.
b) In order to produce sufficient energy to form the electronically excited state, the reaction has to be exothermic.
c) The deactivation pathway should be favorable. During the photon emission process other competitive non-radiative processes such as intra- or inter-molecular energy transfer, molecular dissociation, isomerization or physical quenching should be in low proportion compared to radiative CL pathway.

Two main types of chemiluminescent reaction have been defined in literature [1], as direct (1.1) and indirect (1.2). In the direct CL, the reaction generates the primary excited state molecule (I) which is responsible for the emission of the light.

$$A + B \longrightarrow [\,I\,]^{*} \longrightarrow \text{PRODUCTS} + \text{LIGHT} \qquad (1.1)$$

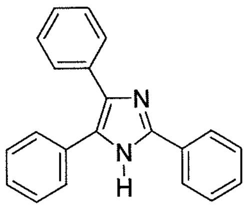
In the indirect type, the excited product of the reaction is not the actual light emitter, but rather transfers its energy to an acceptor (F) which then emits light.

$$A + B \longrightarrow [\,I\,]^{*} + F \longrightarrow [\,F\,]^{*} \longrightarrow F + \text{LIGHT} \qquad (1.2)$$

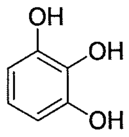
1.1.1 Historical Evolution of CL Materials

Early written references citing luminescence phenomena appeared in the Chinese literature around 1500–1000 b.c. [2]. In fact, all of these first observations were related mainly to living organisms that emit light such as the fireflies, luminous bacteria and protozoa, the sea pansy, the marine fireworm, unicellular organisms such as the dinoflagellates, etc. The widespread luminescence of such living organisms as fireflies and bacteria is based on the oxidation of luciferin in the presence of an enzyme, luciferase. This is a sort of CL called "bioluminescence" which occurs in living organisms.

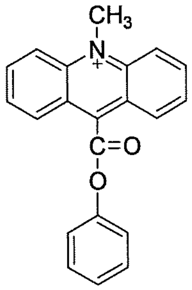
Since the pioneering work of Radziszewski, many chemiluminescent organic compounds have been synthesized. He reported that lophine (2,4,5-triphenylimidazole), the first synthetic chemiluminescent compound, emitted green light when it reacted with oxygen in the presence of strong base [3]. Some organic compounds possesing CL properties are shown in Fig. 1.2.



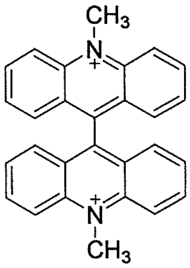
2,4,5-Triphenylimidazole
(Lophine)



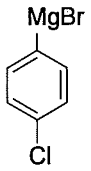
Benzene-1,2,3-triol



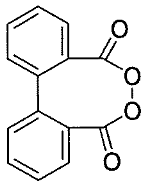
Acridinium Ester



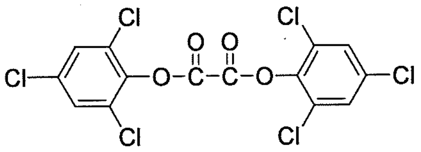
Dimethylbisacridinium
Nitrate (Lucigenin)



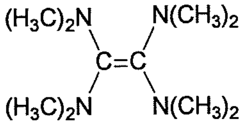
p-Chlorophenyl
Magnesiumbromide



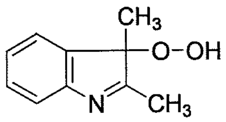
Diphenoyl Peroxide



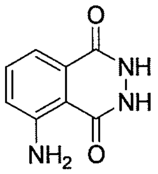
Bis(2,4,6-trichlorophenyl) Oxalate



Tetrakis(dimethykamino) Ethylene



3-Hydroperoxy-2,3-
dimethyl-3H-indole



5-Amino-2,3-dihydrophthalazine-1,4-dione
(Luminol)

Figure 1.2 Some chemiluminescent compounds.

1.1.2 Luminol (Phthalhydrazide)

1.1.2.1 CL of Luminol

Especially, CL as a vibrant class of luminescence has been the subject of extensive research in analytical chemistry due to its high sensitivity, high luminescence efficiency, low cost and simple instrumentation. Chemiluminescent compounds continue to be used as active materials in (electro)chemiluminescent detection with flow injection and liquid chromatography [4-9]. The most widely used active material for CL detection is luminol (5-amino-2,3-dihydro-1,4-phthalazine-dione or 3-aminophthalhydrazide), which is synthesized by German scientist Schimitz in 1908 for the first time [10,11]. After two decades, Albrecht firstly reported its involvement in CL reactions in 1928 [12].

CL reaction of luminol follows the direct path. In alkaline protic solvents (pH=8–11) such as water or alcohols and in the presence of a strong-mild oxidant (in most cases H_2O_2) and a suitable catalyst such as a metal ion, the excited 3-aminophthalate dianion returns to the ground state by releasing its energy in the form of light [13]. Reaction mechanism of luminol is shown in Fig. 1.3.

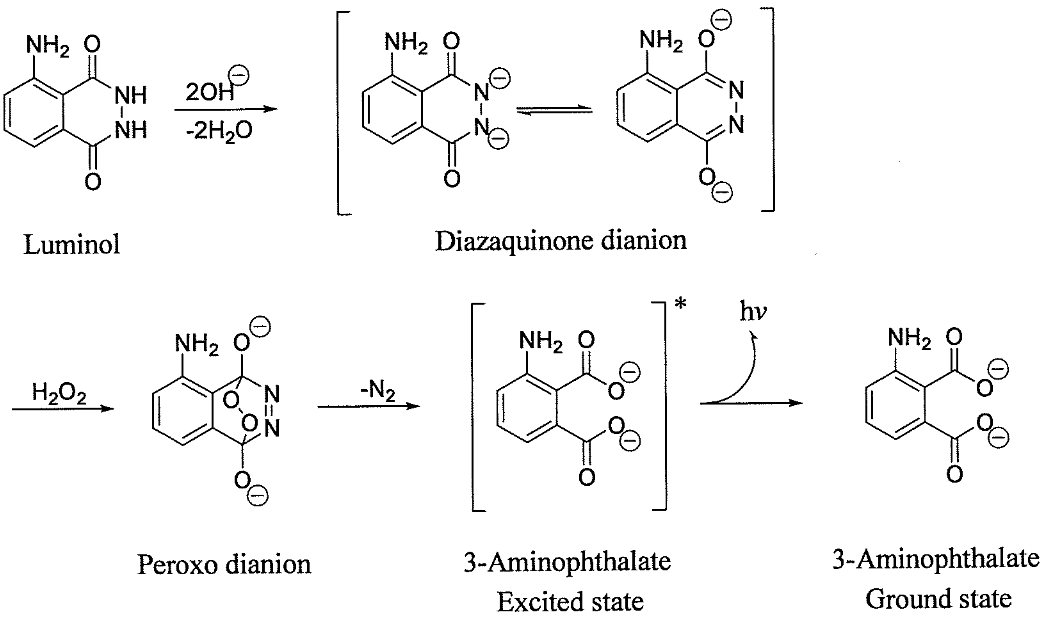
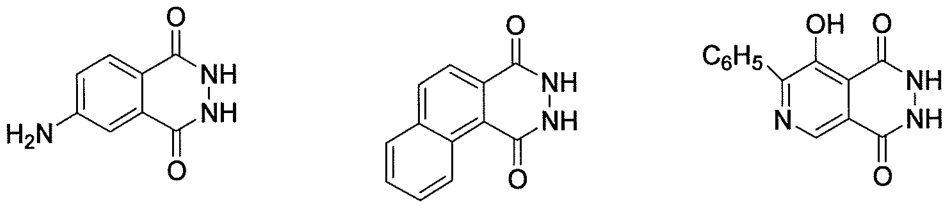


Figure 1.3 CL reaction mechanism of luminol to produce light [14].

Although the CL reaction mechanism was proposed above, the exact role of the catalyst and reaction intermediates are not known exactly. Actually, suggesting a mechanism is difficult because CL reaction of luminol can be catalysed by wide range of transition metal catalysts and metal-complexes, and the optimum conditions differ depending on the catalyst used [15]. Also, the generation of light from the oxidation of luminol depends on several factors like pH, temperature, types of oxidant and metal catalyst and so on [16].

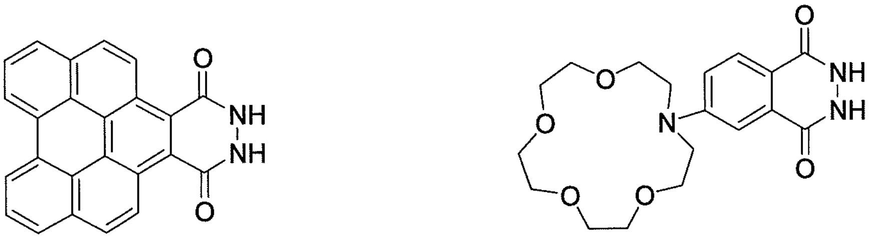
The green-blue light emission of the luminol is observed between 375 nm and 500 nm under different conditions and its quantum efficiency is about 1 % [13,17]. In order to increase the quantum efficiency, some structural modifications on luminol were done successfully (Fig. 1.4) [18-20].



Isoluminol

2,3-Dihydrobenzo[*f*]phthalazine-1,4-dione

2,3-Dihydro-8-hydroxy-7-phenylpyrido[4,3-*d*]pyridazine-1,4-dione



2,3-Dihydroperyleno[1,12-*fgh*]phthalazine-1,4-dione

6-(1,4,7,10-Tetraoxa-13-aza-cyclopentadec-13-yl)-2,3-dihydro-phthalazine-1,4-dione

Figure 1.4 Some luminol derivatives.

1.1.2.2 Electrogenerated CL of Luminol

Electrogenerated CL is a kind of luminescence produced by electrode reactions, it is also called “electrochemiluminescence” (ECL) or “electroluminescence” [21]. The first detailed ECL was reported by Hercules in 1964 [22]. Today, ECL is extensively investigated to clarify its mechanism and origin [23-28].

Actually ECL is a sort of CL and the main difference between them is the initiation of light emission process. In CL, light emission is produced by mixing the emitter with necessary reagents in a reaction vessel while in ECL, light emission is produced by the energetic electron transfer reaction and it is controlled by the electrode potential. ECL has found wide applications in clinical and biomedical diagnostics [23, 29-31]. For example, various chemical and biochemical analytes can be detected with high sensitivity using ECL technique [32-36]. Luminol and tris(2,2'-bipyridyl)ruthenium(II) are the most commonly used ECL luminophores or labels especially in the fields of bioanalytical applications such as enzyme biosensors [26, 27,37-40]. The most important advantage of ECL is that there is a minimum background in signal detection, which allows achievements of very low detection limits. Secondly, it does not suffer from electrochemical interferences in samples when compared to electrochemical detection using amperometric devices, [41], and finally it does not suffer from optical interferences induced by light sources when compared to fluorescence and absorptiometric methods [42].

In ECL, luminol is firstly oxidized and then forms the diazaquinone as shown in Fig. 1.5. Light emission is observed in the presence of H_2O_2, which makes luminol amenable for use in H_2O_2 sensors [43].

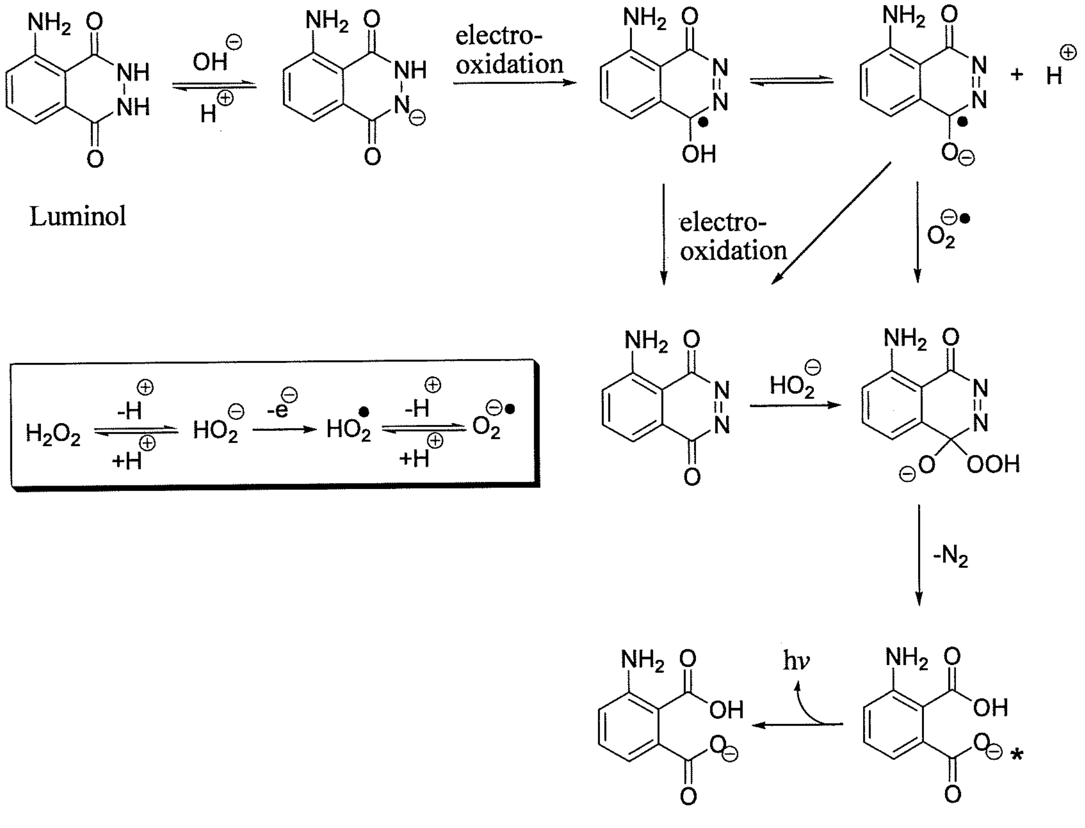


Figure 1.5 A proposed mechanism for ECL reaction of luminol [4,44].

1.1.2.3 Application Areas of Luminol

Nowadays, luminol becomes the subject of many scientific centers. It is mainly used as CL sensor for H_2O_2 [45], in the determination of biologically active compounds like glucose [46], adenine [47], folic acid (water soluble B vitamin) [48], lactic acid [49], dopamine [50], glycolic acid [51], also in routine clinical laboratories for the analysis of immunoassay and DNA probe assays, in the form of a chemiluminescent label or as a chemiluminescent detection reaction for an enzyme label [52], in food analysis [53,54], in pesticide analysis [55], in air pollution analysis [56,57]. Also, a promising derivatives of luminol combined with an azo-crown unit in Fig. 1.4 was used to detect Li^+, Na^+, K^+ cations [58].

In analytical chemistry, CL combined with flow injection analysis has been proved to be a powerful method for the determination of biologically active compounds. For example, a reaction pathway for glucose is illustrated in (1.3) and (1.4) [46]. The glucose in the sample is enzymatically oxidized. The generated H_2O_2 is merged with an alkaline luminol solution and the CL intensity is monitored and related to the glucose concentration in the sample. Similar methods are used for the other biologically active compounds.

$$\text{Glucose} + O_2 \xrightarrow{\substack{\text{Glucose}\\ \text{Oxidase}}} \text{Gluconic Acid} + H_2O_2 \tag{1.3}$$

$$\text{Luminol} + H_2O_2 \longrightarrow \text{3-Aminophthalic acid} + h\nu \tag{1.4}$$

Similarly, several metallic ions such as iron, cobalt, chromium, nickel, copper, and manganese and their complexes catalyse the luminol CL reaction , producing visible CL when exposed to the luminol solution [59-63].

On the other hand, ECL of luminol is also widely employed to develop very sensitive biosensors allowing the detection of analytes at low concentrations and over a wide range [64].

As shown in Fig. 1.6, gold nanoparticles were self-assembled onto silica sol-gel network, and then glucose oxidase was adsorbed on the surface of gold nanoparticles. Glucose is oxidized by the enzyme, glucose oxidase (GOx), which is immobilized into the network, at the end of the reaction H_2O_2 evolves, and due to the interaction with luminol light emission is monitored.

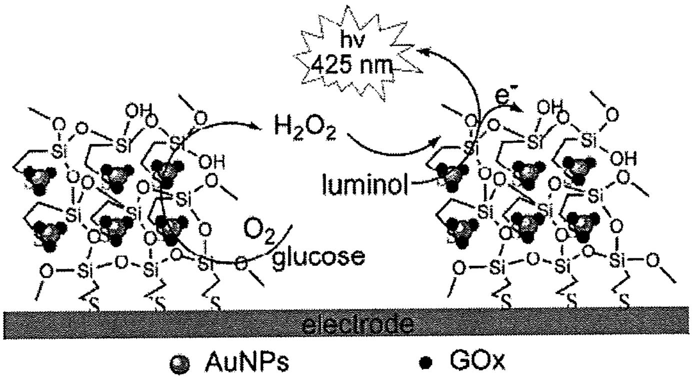


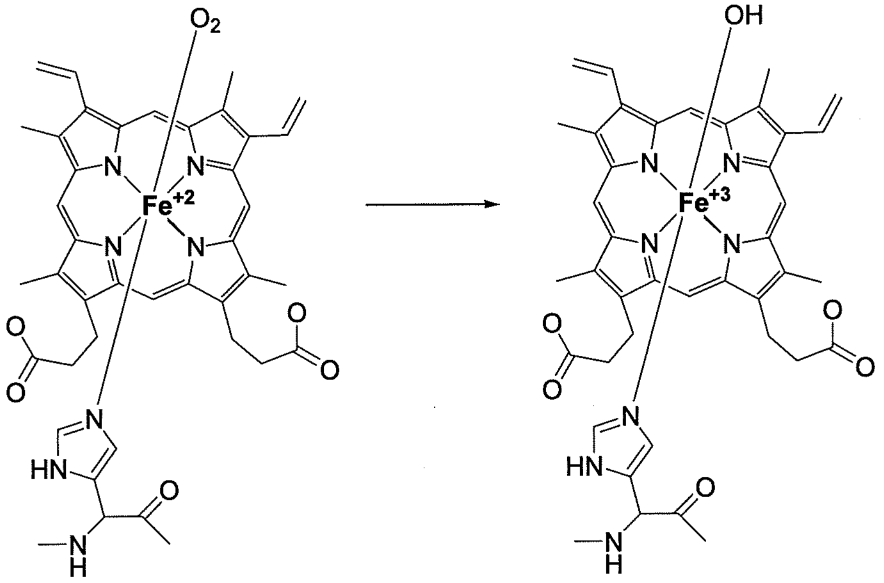
Figure 1.6 Principle of glucose biosensor based on gold nanoparticles (AuNP)-catalyzed luminol ECL.(adapted from [64])

1.1.2.4 Luminol in Forensic Science

Luminol is a cyclic acyl-hydrazide, and shows the typical reactivity of this class of compounds [65]. Properties like photo- and thermal-stability and chemical behaviour in protic polar media make it a good candidate for forensic application. Specht, a forensic scientist, was the first who studied the reaction of luminol with hemin, an iron-containing compound, and investigated its application in blood detection analysis, in 1937 [66]. Then, investigators in the forensic science have effectively used this compound to detect trace amounts of blood left at crime scenes. It has been effectively used for more than 40 years for the detection of bloodstains; for this reason, it has been considered one of the most important and well-known assays in the field of forensic sciences. In order to answer the question "why luminol makes CL when interacted with blood", firstly the content of blood should be examined.

Hemoglobin is the oxygen-carrying molecule found in the erythrocytes of all vertebrates and some invertebrates and is responsible for the red colour of blood.

Within the organism hemoglobin is protected against denaturation by encapsulation in red blood cells and iron ions are kept in the ferrous state, represented as heme in Fig. 1.7. Once outside the organism and deposited on a substrate, blood is subjected to a series of degradation processes, after oxiditation, iron goes to the ferric state (hematin) and the colour turns to brown.



HEME
(in the organism)

HEMATIN
(out of the organism)

Figure 1.7 Structure of hemoglobin in the organism (HEME) and outside of the organism (HEMATIN)

When a luminol formulation is applied on a bloodstain, ferric heme groups are able to catalyze both the decomposition reaction of peroxide and the oxidation of luminol and other substrates by peroxide [67-70]. At the end of reaction a blue glow in the dark can be seen easily (Fig. 1.8).

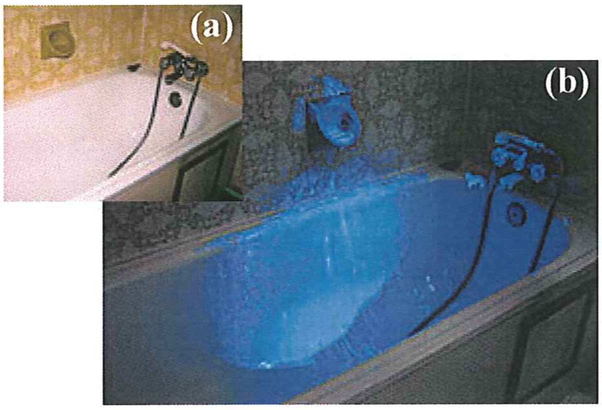


Figure 1.8 (a) Apparence of the crime scene in the day light, no bloodstains can be seen by naked eye and **(b)** apparence after the luminol solution is applied, the intense blue color luminescence indicates the presence of bloodstains which are invisible to the naked eye in the daylight [71].

Luminol also has some drawbacks in forensic science. "Luminol has always been considered by the international forensic community a fascinating but, at the same time, an "obscure" chemical compound" [14]. There are some problematic chemicals which leads to false-positive results even if no blood is present. These kind of chemicals arouse intensification or a generation of a CL emission. Therefore, if these substances are present at the crime scene, the luminol test must not be considered sufficiently specific to permit an unmistakable identification of blood [66,72-75]. Also, it has few detrimental effects on the subsequent DNA recovery and typing [14].

1.2 Conducting Polymers

1.2.1 Brief History of Conducting Polymers

The field of conducting polymers stimulated beginning from the 1970's with the discovery of polyacetylene, which can be made highly conductive by doping [76,77] For the discovery and development of electrically conductive polymers, The Chemistry Nobel Prize in year 2000 was awarded to A.G. MacDiarmid, A. Heeger and H. Shirakawa.

Due to the limitations of polyacetylene, alternative "synthetic metals" were developed to improve solubility and processing properties. Several electron rich conducting polymers including polypyrroles [78-80], polythiophene [81,82], poly(3,4-ethylenedioxythiophene) [83-85], and polyfuran [86], as well as other aromatic polymers such as polyaniline [87], poly(*p*-phenylene vinylene) [88], and poly(*p*-phenylene) [89] were developed and extensively investigated as alternative conducting polymers (Fig. 1.9).

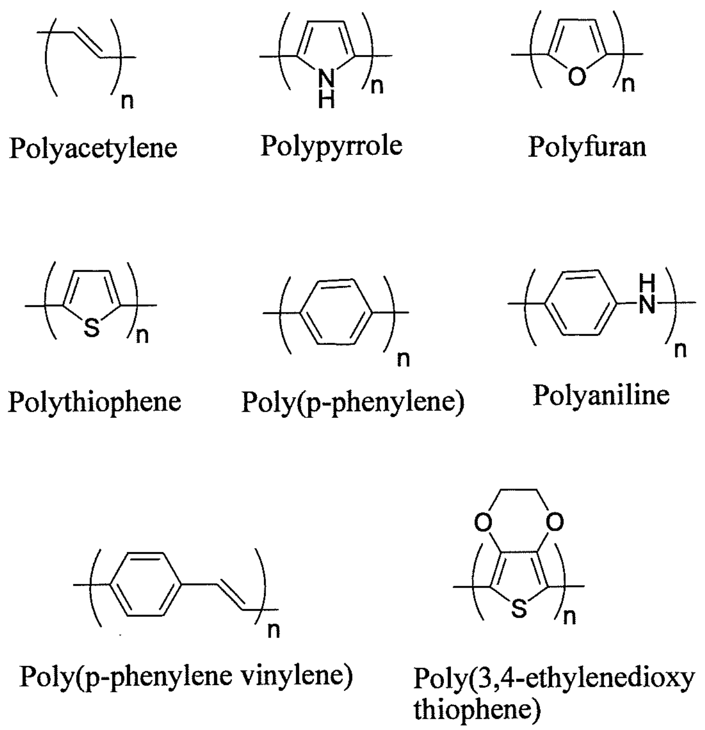


Figure 1.9 Some common conducting polymers.

Among these so-called "first generation" conducting polymers, polythiophenes are of current interest due to relative ease of modification of the starting monomer and the rich chemistry, which thus provides the control of polymer properties (i.e. electronic, optical, conductivity, etc.) in relation to the desired application [90].

1.2.2 Electrochemical Polymerization

Electrochemical synthesis, one of the most widely used methods to synthesize conducting polymers, involves the anodic oxidation of a monomer dissolved in a suitable electrolytic medium by applying an external potential, usually the monomer oxidation potential, to form reactive radical cations. "Nearly all electrochemical polymerizations of conducting polymers appear to follow a *generic reaction pathway*" as shown in Fig. 1.10 [91].

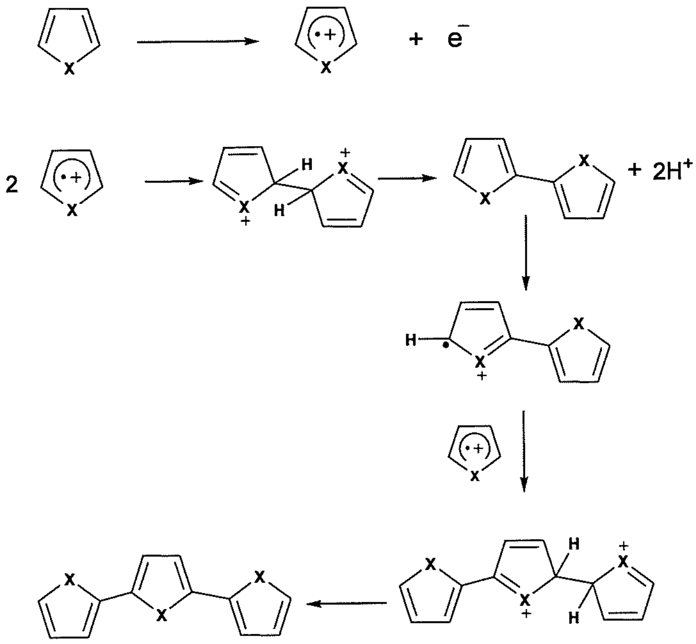


Figure 1.10 Generic reaction pathway for electrochemical polymerization of conducting polymers (X: S, NH, O).

There are some critical factors which affect the elecrochemical polymerization such as; applied potential, supporting electrolyte, solvent medium, temperature and type of electrode [76].

With the help of this tecnique, oxidation potential at which the polymerization takes place and the thickness of the film can be controlled accurately. Therefore, electrochemical polymerization technique offers exiciting opportunities for scientist to produce different conducting polymers prepared from the same monomer by only changing the electrochemical parameters.

1.2.3 Applications of Conducting Polymers

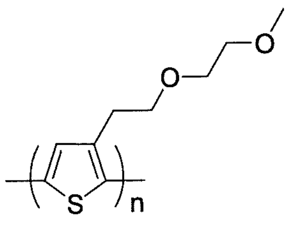
Conducting polymers have attracted considerable attention because of their potential applications in the development of new materials as, for example, (bio)sensors, artificial muscles, optical displays, electrochromic devices, light emitting diodes, memory devices, photovoltaic devices [92-95], electrochemical switches [96,97] and energy storage systems [98-100]. Bearing in mind that, these advanced systems require the salient properties (electronic, optical, conductivity, etc.) of polymers, which can be possible with the modification of starting monomers.

For example, crown ether and/or polyether based polythiophenes have been extensively studied for the recognition of metal cation. Poly[3-(3,6-dioxaheptyl)thiophene] (**1**) is the first example of polyether based polythiophenes, which sensed Li^+ in organic medium via cyclic voltammetry [101-103].

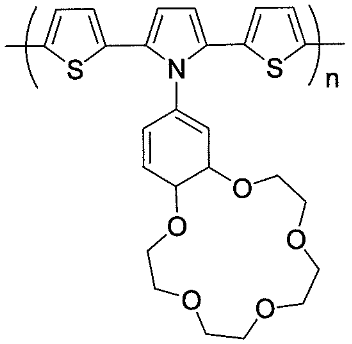
After this study, considerable effort has been devoted to that end where polythiophenes are substituted with crown ethers and/or polyether chains to design modified electrodes usable as electrochemical and bioelectrochemical sensors [104-108].

Recently, Algı *et al.* was synthesized a new processable conducting polymer (**2**) by electrochemical polymerization of 15-crown-5-ether based dithienylpyrrole [109]. The polymer film shows a clear, reversible and selective voltammetric response toward the alkali series (Li^+, Na^+ and K^+) in both neat water and organic medium (ethanol). These prominent features make it an excellent sensor material for any practical use.

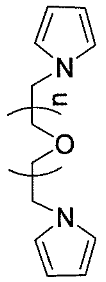
Also, A.M. Önal *et al.* reported a series of N-substituted polybipyrroles bridged by a various length of polyether chain (**3**) for the pre-concentration of rare earth ions [110].



1



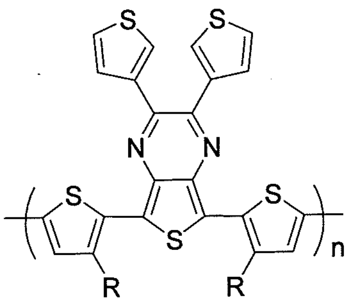
2



3 (n= 1, 2, 3)

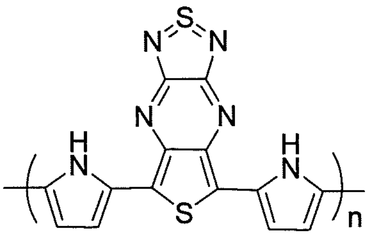
Despite the fact that the earliest electrochromic devices are mostly based on inorganic oxides (tungsten trioxide, iridium dioxide) [111-117], the use of organic conducting polymers as a next generation, has opened new avenues as well as myriad of applications [90] such as electrochromic devices [118,119], smart windows [120, 121], electrochromic mirrors [122,123], optical displays [124], light-emitting diodes [88,125], and camouflage materials [126]. For that reason, they have become very popular. Among these organic compounds, conjugated polymers are of current interest due to their several advantages; e.g. lower cost, higher contrast ratio, multi-colors with the same material, higher stability and longer cycle life with low response time. Recently, for example, Reynolds and *colleagues* have disclosed a programme for the application of conducting polymers in electrochromic devices. They have prepared a series of trimeric hybrid materials by changing the central units (vinyl, thienyl, phenyl, furan, etc) which were substituted with two external 3,4-ethylenedioxythiophene appendages [127,128]. This strategy did not only allowed a fine-tuning of the band gap (E_g) of the polymer films but also provided access to a large variety of red and blue polymeric electrochromic devices. Nevertheless, the third leg (green) of color space (red-green-blue, RGB) has remained elusive.

The problem was that the green polymeric electrochromics should have at least two absorption bands (blue and red) in their neutral state. Furthermore, they should also deplete simultaneously during oxidation [129] and it has been difficult to control both absorption bands by the application of a voltage pulse. Fortunately, a real advance on polymeric electrochromics came in the year 2004 by the work of Wudl and Sönmez *et al.* which brought all the problem to a close by turning the spotlight on the design of hybrid materials (**4**) in which electron rich (donor) and electron poor (acceptor) units were combined [129,130]. Soon after this pioneering work, which definitely paved the way of utilization of these materials in RGB displays, clever and closely related cases have emerged as literal extensions in a clutch of papers from Toppare's laboratory which continuous its expertise in polymeric electrochromics [131-134]. Like RGB colors, various hues of colors can be generated by mixing cyan (C), magenta (M) and yellow (Y) colors. The third leg (cyan) of color space in CMY colors waits for discovery.



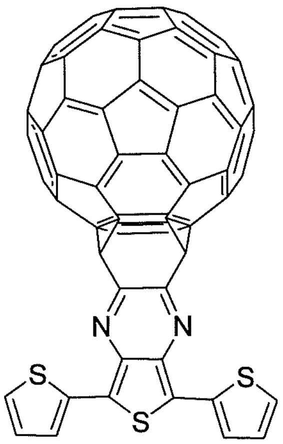
4 (R= H, C_8H_{17})

Also, zero or very low band gap of conducting polymers have attracted great attention due to more electron mobility and transparency. Therefore, highly conductive polymers can be usable for use in practical systems and devices like conductive and antistatics coatings, polymer light emitting diodes, electroluminescent devices and capacitors. Tanaka *et al.* reported a promising polymer (**5**) with a band gap of ~ 0.0 eV [135]. Unfortunately, the results did not convince many scientists, since there is no any detailed characterization about this promising features except for cyclic voltammetry. Therefore, a stable and processable zero band gap conducting polymer is waited for discovery.

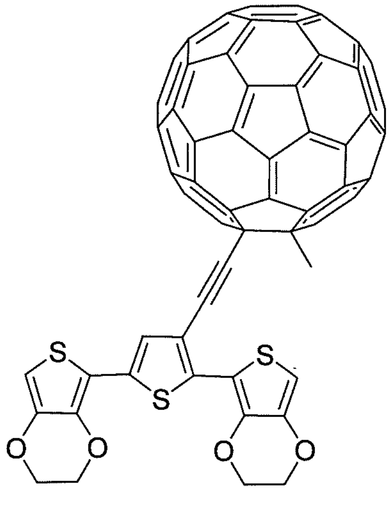


5

Polythiophenes are also extensively used active materials in organic solar cells. Recently, promising terthiophene derivatives (**6** and **7**) based on C_{60} were synthesized and these materials with low band gaps of 0.7 eV for **6** and efficient photochemical response for **7** became more useful for the realization of solar cells [136,137].

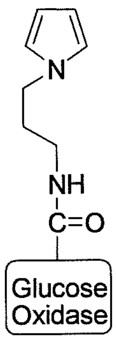


6

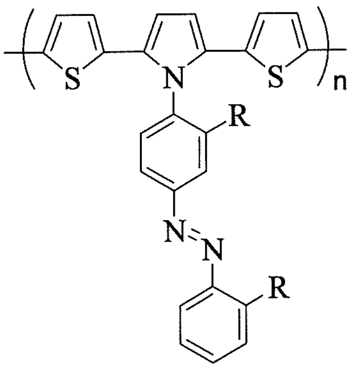


7

In addition, some modifications must be done on the structure of the conducting polymers to be useful in the biomedical applications. For example, glucose oxidase enzyme combined into a polymerizable unit (**8**) can be used as glucose sensor when polymerized electrochemically [138]. On the other hand, the combination of conjugated polymers and azobenzene units can also possess very interesting electrochemical and spectroelectrochemical behaviors since azobenzene and its derivatives have reversible photo-isomerizable units (-N=N-, a reversible trans→cis→trans photoisomerization under UV irradiation) and photochromic properties as well as a second order nonlinear optical properties [139-141]. Recently, Cihaner *et al.* initiated a programe to synthesize and electropolymerize new processable dithienylpyrroles bearing azobenzene derivatives (**9**) [142] to be amenable use in advanced technological applications such as memory and optoelectronic devices [143], optical data storage [144-147], photoswitchable devices [148,149], nonlinear optical devices [150] and sensors [151,152].



8



9 (R= CH_3, OCH_3)

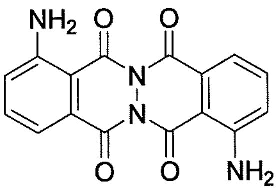
1.2.4 Polyluminol

Luminol is a derivative of aniline and its electro-oxidation generated a polymer film (polyluminol) on the electrode surface [153-156]. It would be possible to benefit from the boons of the electropolymerized luminol in the field of chemical sensors, biosensors, electroanalysis and electrocatalysis [153,154],

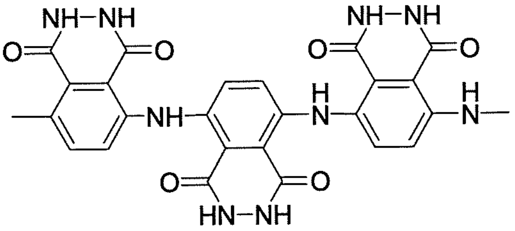
However, reports on the electrochemical polymerization of luminol are not very consistent. According to the results of a study done on electrochemical polymerization of luminol in alkaline solution, it was concluded that hydrazide did not adsorb on Pt electrode [157].

On the other hand, another study showed that luminol could adsorb on Pt in phosphate buffer solution [9]. With the help of these studies, it was thought that the optimum medium for the polymerization of luminol was alkaline medium. However, It was shown that luminol could polymerize on the surface of electrode in acidic solution [154]. As a result, the optimum type of electrode and polymerization medium have been the topic of many researchers, and the obtained results are contradictory.

On the other hand, many questions remain about luminol participation in reaction mechanisms. Whether the obtained product on the electrode was in the form of dimer (**10**) or a polymer (**11**) is still a question.



10



11

Since the results obtained on polymerizing luminol by electrochemical means were not enough to answer this question, a further study was done on polymerizing luminol by chemical means in order to propose a mechanism for its oxidative polymerization [156]. From the results of electrochemical measurements, the luminol oxidized on the phthalimide-group site under the low electrode potentials KIO_3 (E_{ox}= 1.085 V vs NHE) and on the amino group site at high potentials $(NH_4)_2S_2O_8$ (E_{ox}= 2.05 V vs NHE).

Due to the solubility problems, current passing during polymerization was very low. Therefore, luminol forms a very thin polymeric layer on the electrode surface. Since, a free-standing polyluminol film could not be obtained in all of these studies, its physico-chemical properties were not been able to studied.

On the other hand, electropolymerized luminol acts as an active luminophore of the electrochemiluminescent reaction, as the monomer does. However, investigations are very limited due to the polymerization problems [43,158]. According to a study done by Sassolas et al., the polyluminol film formed in a buffered solution with pH=6 acts as an ECL luminophore [43]. H_2O_2 detection is possible in a wide linear range, extending from 7.9×10^{-8} M to 1.3×10^{-3} M. The advantage of this study is not to use any reagent; that is, it is no longer necessary to add luminol in solution. However, it is important to prevent monomer adsorption in polyluminol which can lead to the wrong results. In spite of difficulties in polymerization, these results have great potential as a first step towards the preparation of various more elaborate oxidase-based ECL reagentless biosensors.

1.3 Aim of this work

Taking into account the advantages and disadvantages of the luminol, we have initiated a program aimed at the design and synthesis of a novel chemiluminescent material which can be easily electropolymerized without the destruction of the chemiluminescent unit during polymerization. It was envisaged that suitable chemiluminescent group appended in trimeric monomer systems would make it and its corresponding polymer amenable for use in blood detection in the forensic application, light emitting diodes, electrochromic devices and analytical sensors.

Interestingly, to the best of our knowledge, such a conducting polymer consisting of a terthienyl trimeric monomer with a chemiluminescent unit has not been investigated so far. Herein we wish to unveil our results concerning the synthesis, electropolymerization and characterization of two novel chemiluminescent materials called 2,3-dihydrothieno(3,4-d)pyridazine-1,4-dione (T-Lum) and 5,7-di-thiophen-2-yl-2,3-dihydro-thieno[3,4-d]pyridazine-1,4-dione (TTT-Lum) as well as their applications in “cold light” test at the crime scenes. Furthermore, optical, electrochromic, morphological and electrochemical luminesence properties of the corresponding polymer film (PTTT-Lum) were elaborated.

CHAPTER 2

EXPERIMENTAL

2.1 Materials

All chemicals were purchased from Aldrich Chemical. For electrochemical studies, $LiClO_4$ dissolved in freshly distilled (over CaH_2 under N_2 atmosphere) acetonitrile (ACN) containing 5% of borontrifluoro diethylether (BF_3-Et_2O) by volume and neat BF_3-Et_2O solutions were used as electrolyte solutions. 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 Ag/AgCl in 3 M NaCl(aq) solution as a reference. Polymer films were prepared either by repetitive cycling or constant potential electrolysis.

For CL measurements, all the working solutions to be tested were freshly prepared. Test solutions for Fe(III) ion analysis were prepared from $K_3Fe(CN)_6.6H_2O$ complex. Dilutions were done by using doubly distilled water. Synthesized monomers were kept under Ar(g). Especially, 2,3-dihydrothieno(3,4-d)pyridazine-1,4-dione (T-Lum) and 5,7-di-thiophen-2-yl-2,3-dihydro-thieno[3,4-d]pyridazine-1,4-dione (TTT-Lum) and their solutions were protected from light and stored in refrigerator.

2.2 Instrumentation

2.2.1 Cyclic Voltammetry

Cyclic voltammetry falls into a class of potentiodynamic experimental methods. It is widely used in laboratories due to the increasing development of computer controlled experimental equipments with automated data collection and due to the development of mathematical description of potentiodynamic curves. Three-electrode setup (working, counter and reference electrodes) is used in this method. In order to ensure sufficient conductivity, electrolyte is added to the test solution. The range of applied potential is determined by the combination of the solvent, electrolyte and specific working electrode material. Electrodes are static and sit in unstirred solutions during recording the cyclic voltammogram.

Cyclic voltammetry is used to find out the electrochemical behavior of electroactive species. In this method, while the potential of the working with respect to reference electrode is swept, the resulting current flow between working and counter electrodes is measured. Thus, the obtained voltammogram displays current vs. potential providing us the information about the electroactivity and redox potential of the substrate. One can also deduce the mechanism of the electrochemical reaction, whether it is reversible or not and whether reaction products are further reduced or oxidized from the cyclic voltammogram.

Following electropolymerization, the electrode coated with polymer is removed from the monomer solution and carefully rinsed with solvent to remove unreacted monomer and electrolyte. The rinsed electrode is then placed in another three-electrode cell filled with monomer-free electrolyte solution to study the redox behaviour of the polymer film.

Cyclic voltammetry and electrolysis experiments were carried out by a Gamry PCI4/300 potentiostat-galvanostat.

2.2.2 Spectroelectrochemistry

Spectroelectrochemical measurements of the conducting polymers provide information about their bandgaps and intraband states created upon doping as well as electrochromic properties at various applied potentials. Spectroelectrochemical properties were investigated on an ITO electrode as well as a platinum wire as counter electrode and a Ag wire as a pseudo-reference electrode (calibrated externally using 5 mM solution of ferrocene/ferrocenium couple) utilizing Hewlett–Packard 8453A diode array spectrometer. In order to equilibrate the redox behavior of the polymer film and to obtain reproducible results, the coated polymer film were switched between its neutral and oxidized states several times before electroanalytical and optical studies.

2.2.3 Characterization of Monomers and Polymers

Fourier Transform Infrared (FTIR) spectra of all the monomers and polymers were recorded on a Bruker Vertex 70 Spectrophotometer using KBr pellet or an Attenuated Total Reflectance (ATR) unit. Nuclear Magnetic Resonance (NMR) spectra of the monomers were done by a Bruker Instrument-NMR Spectrometer (DPX-400) in d-DMSO and $CDCl_3$. Elemental identification of monomers were investigated by a LECO, CHNS-932 instrument. Surface morphologies were inspected by QUANTA 400F Field Emission Scanning Electron Microscope (SEM). Melting point of the target monomer were recorded by a GALLENKAMP 220/240 V, 50/60 Hz, 50 W instrument.

2.2.4 ECL Measurements

ECL is the process where species generated at electrodes undergo electron transfer reactions to form excited states that emit light. Application of a voltage to an electrode in the presence of an organic or inorganic luminophore results in light emission.

Light emission from monomer solution and polymer film (in monomer-free solution) were measured by a homemade system combining cyclic voltammetry 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) as shown in Fig. 2.1. The working electrode is an ITO fitting a UV cuvette. 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.

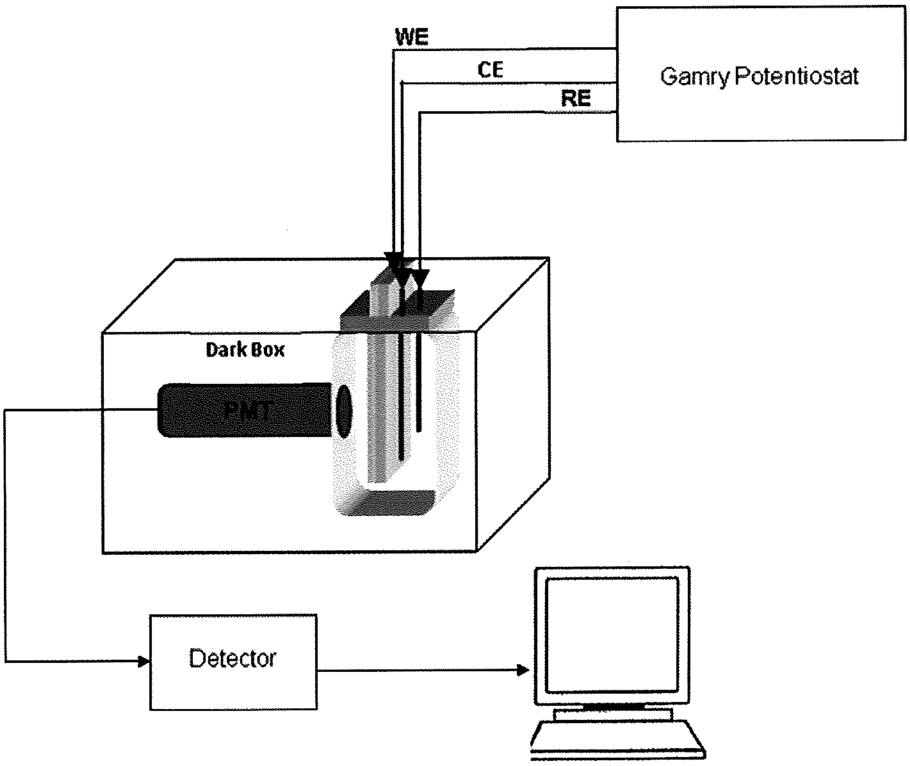


Figure 2.1 Schematic representation of homemade ECL system; WE, working electrode; CE, counter electrode; RE, reference electrode.

2.2.5 CL Measurements

Stopped-flow cell assemblied by PMT is used for measuring the intensity of CL in very fast reactions (Fig. 2.2). In order to measure the sensitivity of T-Lum and TTT-Lum against H_2O_2, metal cations and blood, stopped-flow luminometer was used. The sample (containing metal cation, H_2O_2 or blood) and reagent (containing T-Lum or TTT-Lum dissolved in NaOH aqueous solution) were efficiently mixed in a reaction chamber and rapidly forced to an observation cell, in which the flow is violently stopped, allowing monitoring of the full intensity-time curve.

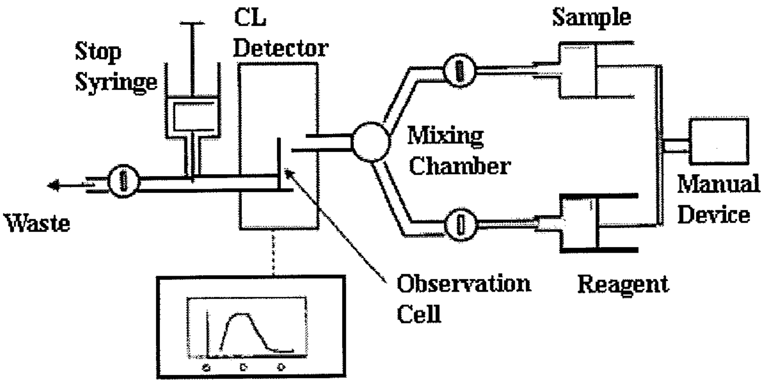


Figure 2.2 Schematic diagram for stopped-flow CL measurements [4].

Measurements were carried out with a Pro-K.2000 Rapid Kinetic System with PMT. Photographs and videos of CL reactions performed in dark were taken by a Power Shot A720IS Canon digital camera.

2.3 Synthesis of Monomers

Reaction pathways for the synthesis of T-Lum and TTT-Lum are given in Fig. 2.3. Also, a detailed experimental procedure is given for all products and most of the products were obtained in high yields.

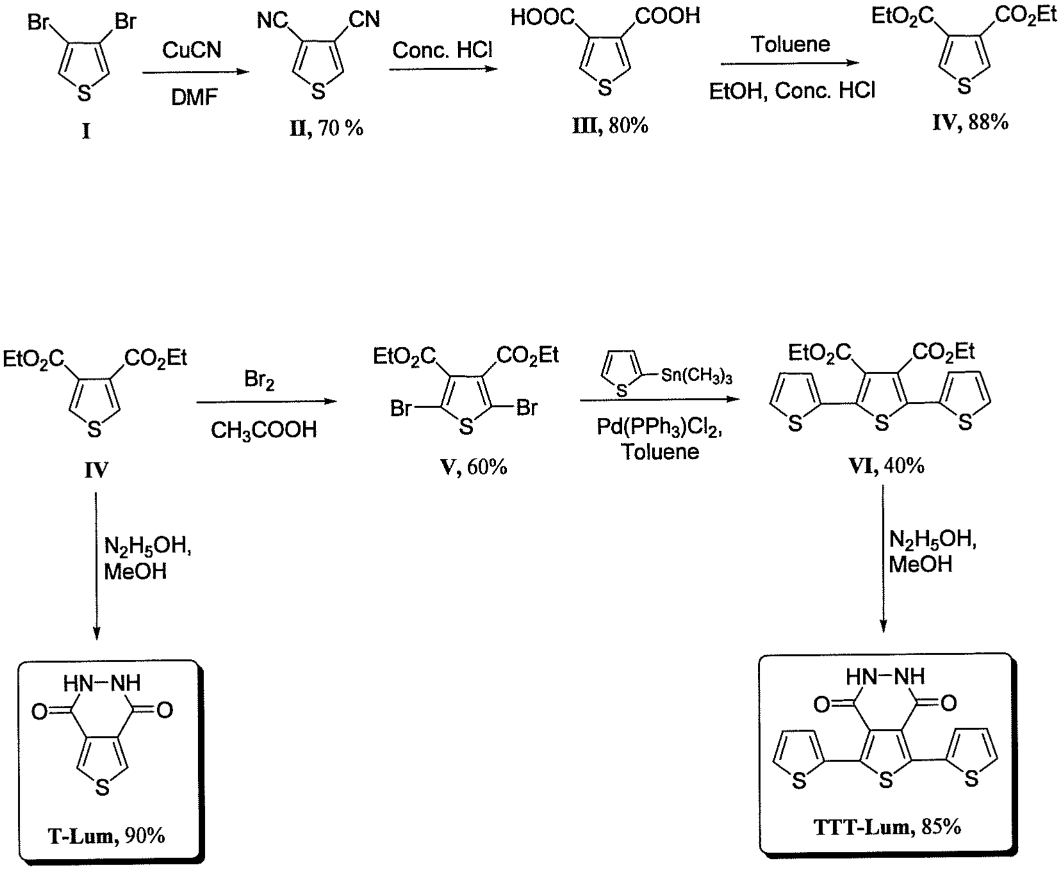
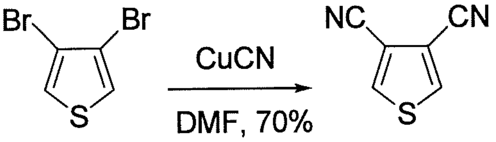


Figure 2.3 Synthetic routes for the synthesis of T-Lum and TTT-Lum.

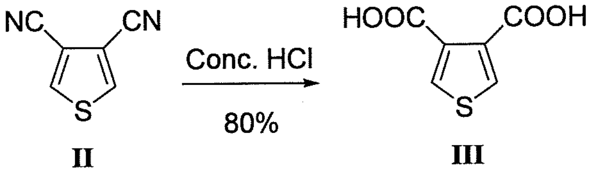
2.3.1 Synthesis of 3,4-dicyanothiophene (II)



I **II**

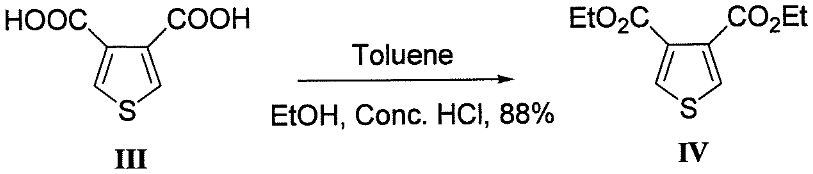
A solution of compound **I** (3 g, 12 mmol) and cuprous cyanide (CuCN, 3.12 g, 34.8 mmol) in dry N,N'-dimethylformamide (3 ml) was refluxed at 150 °C for 4 h under Ar(g). After the dark mixture was allowed to cool, it was poured into a solution of hydrated $FeCl_3$ (12 g) in HCl (21 ml, 1.7 M). The mixture was maintained at around 60-70 °C for 30 min. After the solution was allowed to cool, the raw product was taken into CH_2Cl_2 (15 ml) phase. Extraction was done four times for the aqueous phase (4x15 ml). Then, the organic phase washed with two 1.2 ml portions of HCl (6 M), water, saturated $NaHCO_3$ solution, and again with water. Then, the final solution was dried ($MgSO_4$) and concentrated under vacuum to give a slightly yellow product. Then, further purification was done by recrystallizing from acetonitrile to give a white crystalline product **II** (1.16 g, 8.7 mmol) in a yield of 70 % [159]. ^1H 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, 1299-1262, 1163, 1108.

2.3.2 Synthesis of thiophene-3,4-dicarboxylic acid (III)



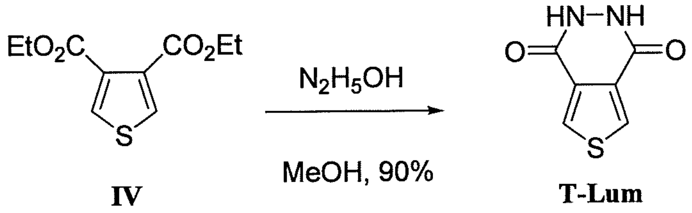
Compound **II** (0.7 g, 5.2 mmol) was refluxed in concentrated HCl (13 ml) for 6 h. After the reaction was completed, the mixture was cooled and the precipitated product was collected and recrystallized from water-ethanol (2:1) to give a white solid product **III** (0.72 g, 4.2 mmol) in a yield of 80% [160]. ^1H 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, 1690-1620, 1461, 1292, 1237, 1181-1145, 949.

2.3.3 Synthesis of diethyl thiophene-3,4-dicarboxylate (IV)



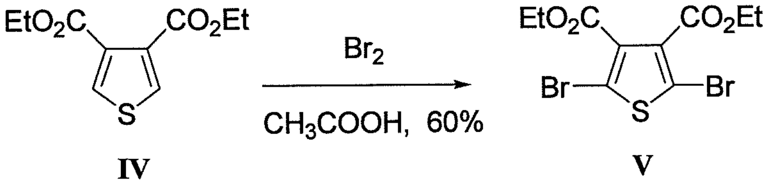
Compound **III** (0.2 g, 1.16 mmol) was refluxed (5 h) in ethanol (8.2 ml), dry toluene (4.1 ml) and concentrated HCl (0.04 ml). A Dean-stark apparatus was attached to the system containing ethanol-toluene mixture (1:1). It was withdrawn and renewed every hour. After the esterification reaction was completed, 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 (4x2 ml). Organic phase was dried ($MgSO_4$) and solvent was removed under reduced pressure to give a light yellow oily liquid **IV** (0.23 g, 1 mmol) in a yield of 88%. [161]. ^1H NMR (400 MHz, $CDCl_3$): δ/ppm: 1.34 (t, J= 7.1 Hz, 6H), 4.33 (q, J=6.9 Hz, 4H), 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

2.3.4 Synthesis of 2,3-dihydrothieno(3,4-d)pyridazine-1,4-dione (T-Lum)



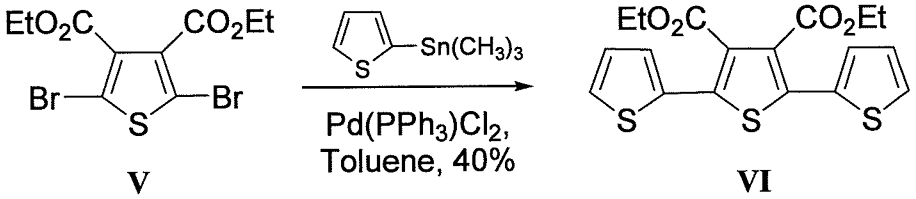
Compund **IV** (0.19 g, 0.83 mmol) was allowed to react with hydrazine hydrate (0.16 g, 2.56 mmol) in methanol (2 ml). The reaction mixture was refluxed in the hot water bath bath at 65-70 ^0C for 4 h. After the reaction was completed, the reaction mixture was cooled to room temperature and concentrated under vacuum. The crude solid compound was dissolved in minimum amount of hot water containing 1% NH_4OH by volume and precipitated by adding concentrated HCl to the solution. Finally, the obtained solid compound was washed with cold water, alcohol and then dried at room temperature to give 0.126 g of T-Lum as a white crystalline product in a yield of 90%. [162]. ^1H NMR (400 MHz, DMSO): δ/ppm: 8.33 (s, 2H). ^{13}C NMR (100 MHz, DMSO): δ/ppm: 128.4, 131.6, 154.2. IR (KBr, cm^{-1}): 3075, 2990, 2924, 2700 (broad), 1693, 1645, 1557, 1461, 1318-1296

2.3.5 Synthesis of diethyl 2,5-dibromothiophene-3,4-dicarboxylate (V)



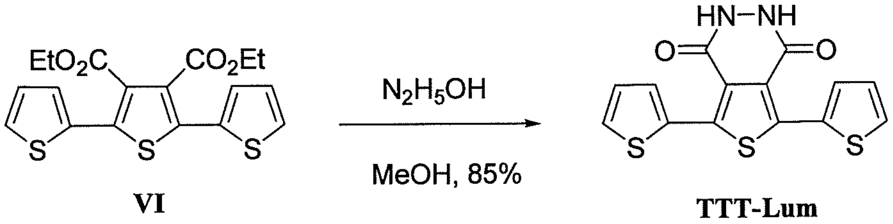
0.064 ml (1.25 mmol) bromine was added drop by drop to a mixture of **IV** (58 mg, 0.25 mmol) in acetic acid (1 ml). Then, the mixture was refluxed at 70 °C untill all the starting materials were consumed (TLC). After the reaction was complete, the flask was cooled and saturated $NaHCO_3$ solution was added to remove acetic acid and then the solution was extracted with dichloromethane. Organic phase was dried ($MgSO_4$) and solvent was removed under reduced pressure to give a red liquid **V** (59 mg, 0.15 mmol) in a yield of 60% [163]. ^1H NMR (400MHz, $CDCl_3$): 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.

2.3.6 Synthesis of diethyl 2,5-di(thiophen-2-yl)thiophene-3,4-dicarboxylate (VI)



Compound **V** (60 mg, 0.155 mmol) was treated with $Pd(PPh_3)Cl_2$ catalyst (35 mg, 0.05 mmol) in the presence of 2-(trimethylstannyl)-thiophene (26 mg, 1.19 mmol) and dry toluene (40 ml) as solvent under inert atmosphere and the solution was heated under reflux untill 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 an oily liquid **VI** (36.5 mg, 0.09 mmol) in a yield of 40% [164]. ^{1}H NMR (400MHz, $CDCl_3$): δ/ppm: 1.28 (t, J= 7.0 Hz, 6H), 4.28 (q, J=7.1 Hz, 4H), 7.06 (dd, J= 3.8 Hz, 2H), 7.32 (d, J= 4.0 Hz, 2H), 7.39 (d, J= 5.4 Hz, 2H). ^{13}C NMR (100 MHz, $CDCl_3$): δ/ppm: 13.9, 61.6, 127.4, 127.8, 128.7, 130.0, 132.6, 138.0, 163.7. IR (KBr, cm^{-1}): 702, 1020, 1208, 1723, 2982, 2845, 3106.

2.3.7 Synthesis of 5,7-di-thiophen-2-yl-2,3-dihydro-thieno[3,4-d]pyridazine-1,4-dione (TTT-Lum)



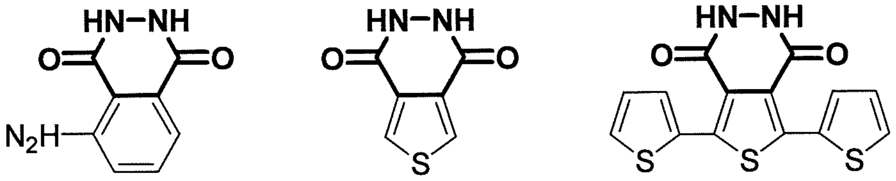
N_2H_5OH (0.19 gr, 3 mmol) was added to compound **VI** (0.392 g, 1.0 mmol) dissolved in methanol (2.4 ml) and the reaction mixture was heated at 65-70 ^{o}C in hot water bath for 4 h. After the reaction was completed, the reaction mixture was cooled to room temperature and concentrated under vacuum. The crude solid compound was dissolved in minimum amount of hot water containing 1% NH_4OH by volume and precipitated by adding concentrated HCl to the solution. Then, the solid compound was washed with cold water, alcohol and dried at room temperature. Finally, a yellow solid compound, TTT-Lum, was obtained in a yield of 85% [162]. M.p.: 289-290 ^{o}C. ^{1}H NMR (400 MHz, DMSO): δ/ppm: 7.115 (t, J = 4.36, Hz, 2H), 7.69 (d, J = 2.48 Hz, 2), 7.75 (t, J = 5.06 Hz, 2H). ^{13}C NMR (100 MHz, DMSO): δ/ppm: 124.4, 127.2, 129.6, 130.2, 131.7, 136.8, 153.0. IR (KBr, cm^{-1}): 700, 827, 1125, 1225, 1280, 1381, 1472, 1641, 3103, 3387. Elemental Anal. Calcd. for $C_{14}H_8N_2O_2S_3$: C, 50.58; H, 2.43; N, 8.43; O, 9.63; S, 28.90. Found: C, 50.69; H, 2.63; N, 8.31; O, 9.37; S, 29.00.

CHAPTER 3

RESULTS & DISCUSSIONS

3.1 CL Properties

Initially, the CL properties of T-Lum and TTT-Lum were investigated. Since both of the monomers have the same chemiluminescent group with luminol (Fig. 3.1), it is expected to observe a CL reaction in alkaline medium in the presence of H_2O_2 and with/without a catalyst.



Luminol T-Lum TTT-Lum

Figure 3.1 Chemical structures of luminol, T-Lum and TTT-Lum.

As expected, we were delighted to note that a CL was observed from the reaction of T-Lum or TTT-Lum dissolved in 0.1 M NaOH aqueous solution with an oxidant like H_2O_2. This reaction mixture emits bright light in the dark and this glow can easily be sensed by the naked eye (see Fig. 3.2)

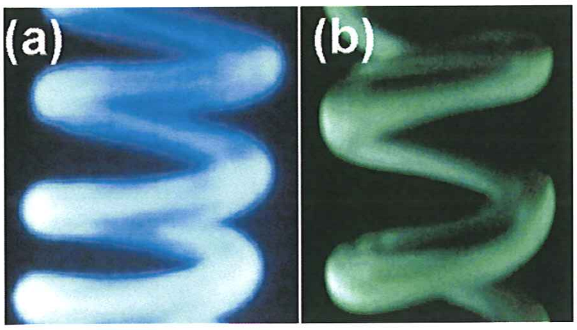


Figure 3.2 The emitted light obtained from the CL reaction of **(a)** luminol and **(b)** TTT-Lum dissolved in alkaline solution with H_2O_2 in the presence of Fe^{+3} as the catalyst.

An emission mechanism proposed for T-Lum and TTT-Lum monomers, which are analogous with the luminol, is given in Fig. 3.3 [14].

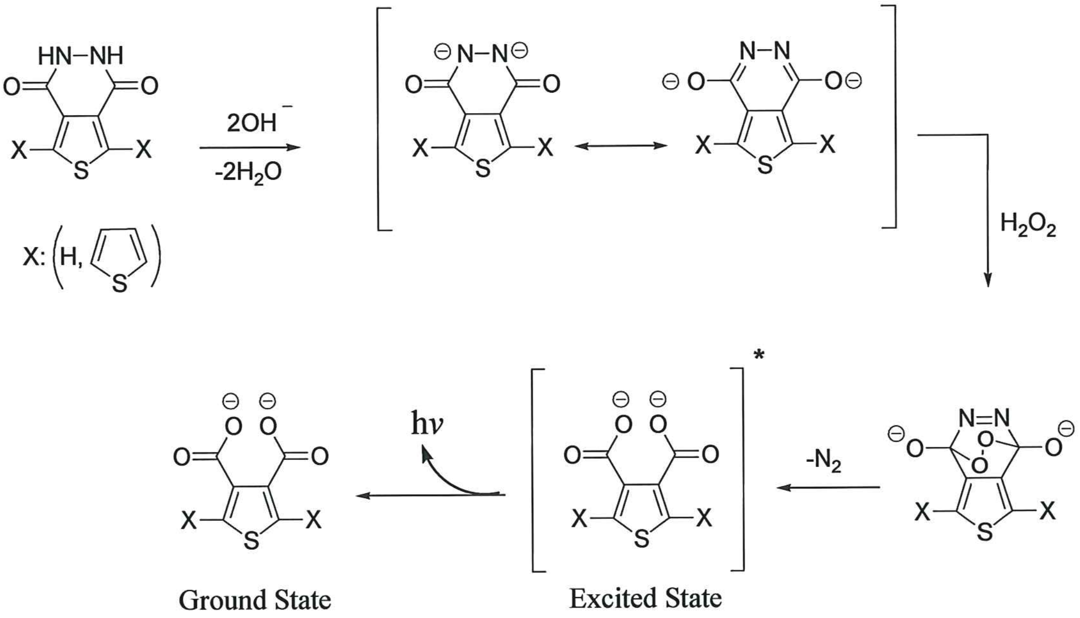


Figure 3.3 Proposed CL reaction mechanism for both T-Lum and TTT-Lum.

From the emission spectrum of T-Lum, TTT-Lum and luminol, it can be easily concluded that T-Lum and TTT-Lum emit at higher wavelengths than luminol. Thus,

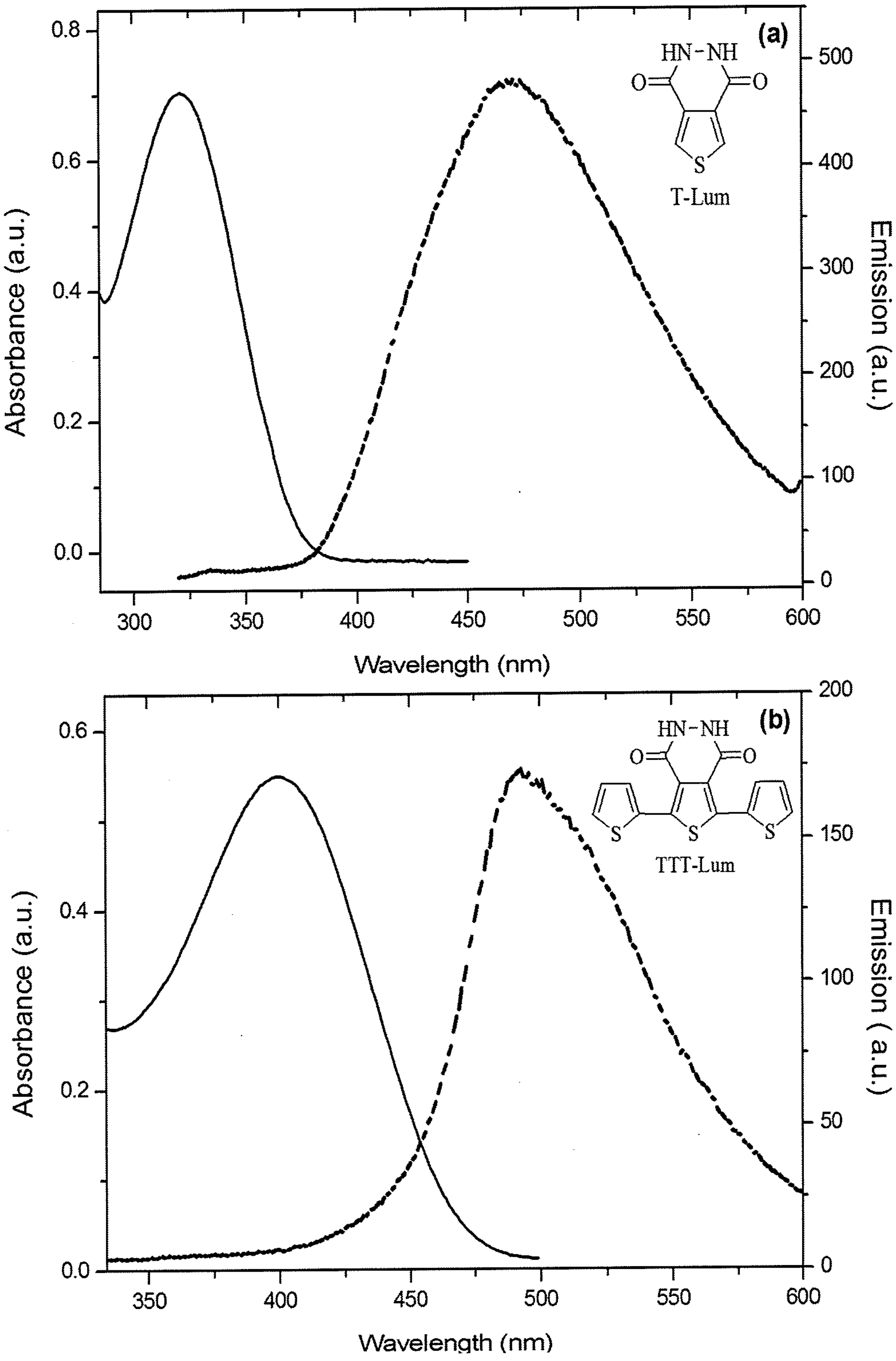


Figure 3.4 The absorption and emission spectra of **a)** T-Lum (excited at 321 nm) and **b)** TTT-Lum (excited at 400 nm), in DMSO.

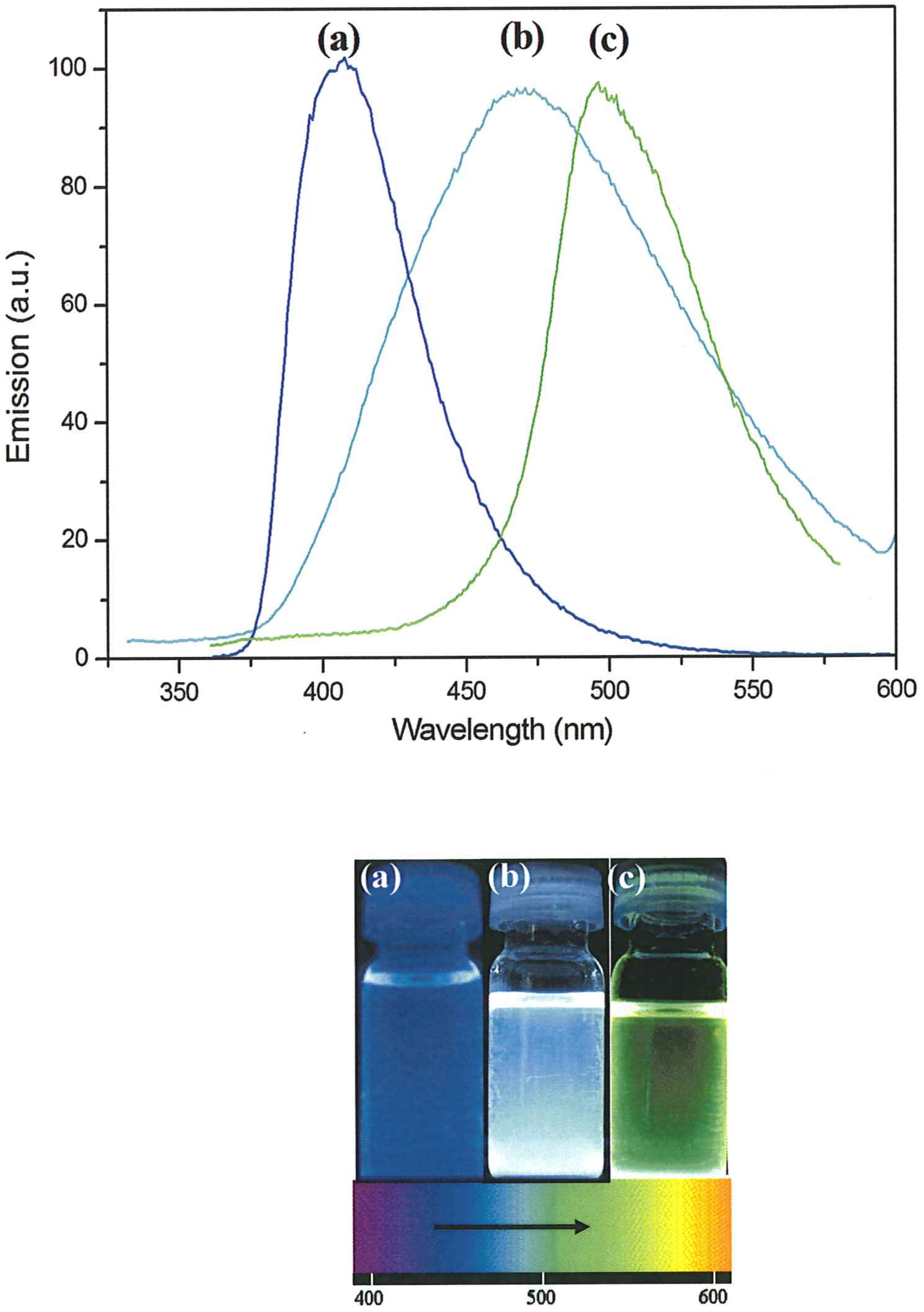


Figure 3.5 Comparison of emission spectra of **a)** Luminol, **b)** T-Lum and **c)** TTT-Lum in DMSO.

the possibility of emitted light to destroy DNA is less when compared to luminol. Absorption and emission spectra of T-Lum ($\lambda_{\mathrm{max,abs}}$= 321 nm, $\lambda_{\mathrm{max,emis}}$=471 nm) and TTT-Lum ($\lambda_{\mathrm{max,abs}}$= 400 nm, $\lambda_{\mathrm{max,emis}}$=495 nm) were shown in Fig. 3.4 and comparison with luminol is shown in Fig. 3.5.

3.1.1 Sensitivity of TTT-Lum Towards H_2O_2

The CL reaction is started when the chemiluminescent active material comes into contact with H_2O_2 in alkaline media, but the intensity of light emitted is so low and the reaction is so slow that it is not possible to sense it with naked eye (Fig. 3.6). The bright light can only be seen with the help of a catalyst (metal cations, complexes containing Fe^{+3}, blood, etc) which speeds up the reaction.

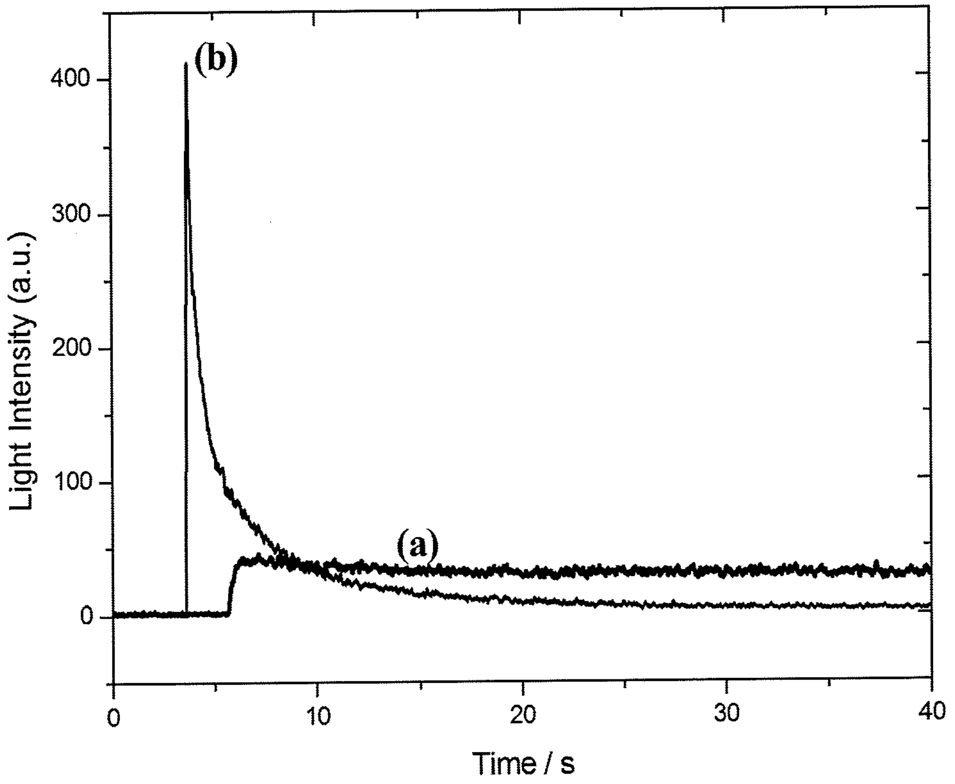


Figure 3.6 (a) Slow light emission in the presence of H_2O_2 only, **(b)** Instantaneous light emission with the addition of Fe^{+3}.

Thus, since the monomers have chemiluminescent properties like luminol, they can also be used for the determination of H_2O_2. By using stopped-flow CL instrument (Fig. 2.2) a series of measurements were done for different concentrations of H_2O_2 to find the lowest detectable value (Fig. 3.7). It is found that $7.5x10^{-6}$ M H_2O_2 can be detected by using 10^{-5} M TTT-Lum.

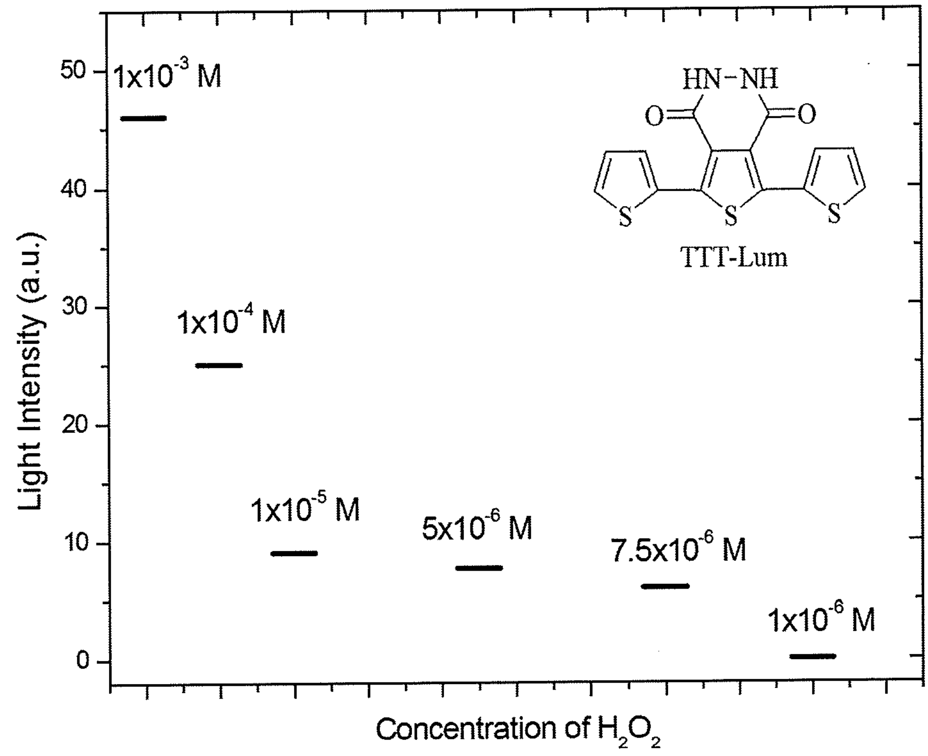
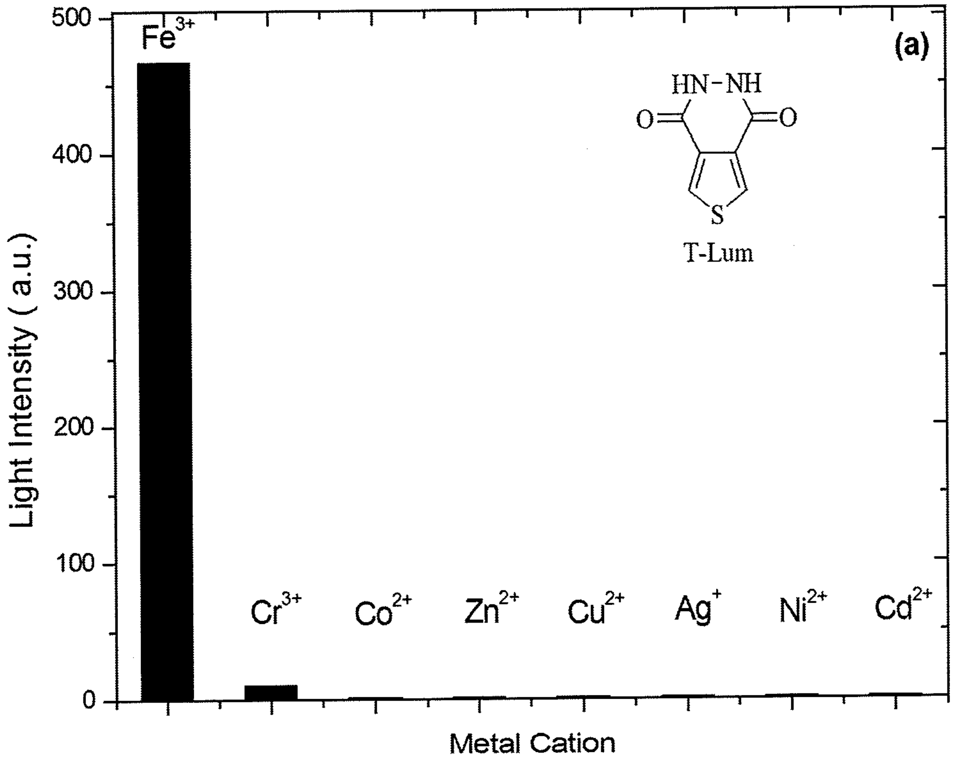


Figure 3.7 The intensity of the light obtained from the CL reaction between $1.0x10^{-5}$ M TTT-Lum dissolved in 0.1 M NaOH solution with different concentrations of H_2O_2.

3.1.2 Sensitivity of T-Lum and TTT-Lum Towards Metal Cations

It is well-known that the chemiluminescent process between luminol and H_2O_2 can be speeded up by using a large family of metalic cations as catalyst [4]. In order to probe the effect of metal ions to the CL of T-Lum and TTT-Lum, we tested a variety of metal ions (Ag^+, Zn^{+2}, Ni^{+2}, Cu^{2+}, Cd^{+2}, Co^{2+}, Cr^{+3}, and Fe^{+3}) which are known to be highly effective catalyst in the case of luminol.



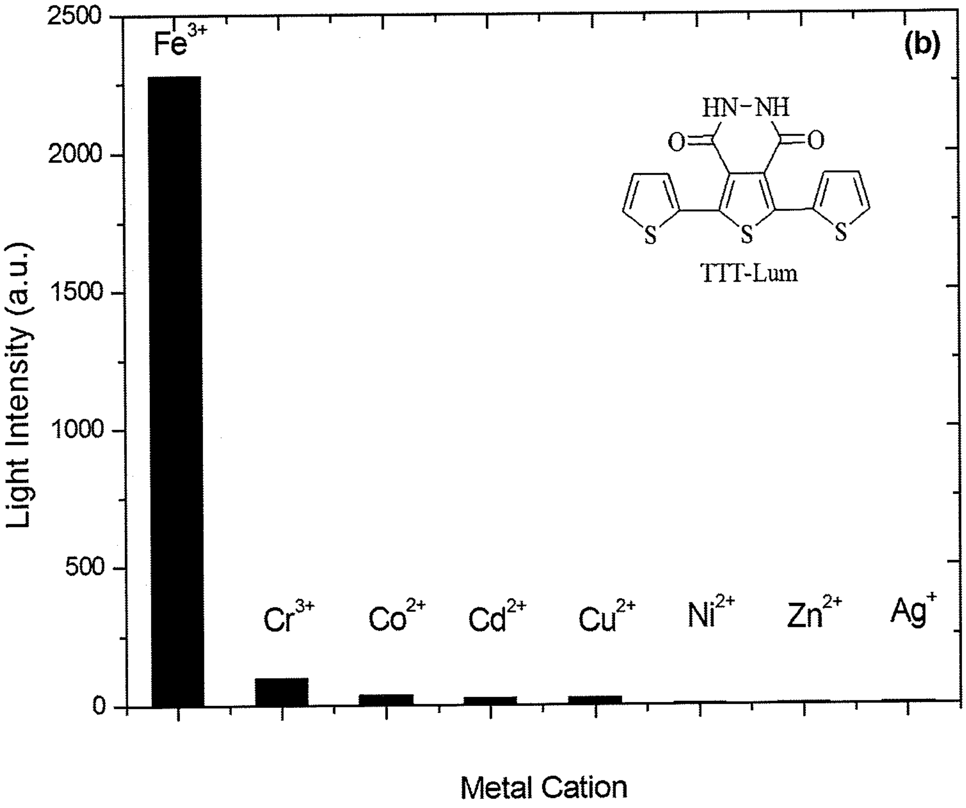


Figure 3.8 The intensity of the light obtained from the CL reaction between **(a)** $1.0\text{x}10^{-5}$ M T-Lum and **(b)** $1.0\text{x}10^{-5}$ M TTT-Lum, dissolved in 0.1 M NaOH solution with $1.0\text{x}10^{-4}$ M H_2O_2 in the presence of different metal cations (10^{-2} M).

Although, luminol was sensitive to all of the cations shown above; it is found that both T-Lum and TTT-Lum are not sensitive to Ni^{+2}, Zn^{+2} and Ag^{+}, while they are very sensitive towards Fe^{3+} ion when compared to other ions (Fig. 3.8). After prooving that T-Lum and TTT-Lum are more sensitive towards Fe^{3+} cation when compared to luminol, detection limit for Fe^{+3} cation was investigated.

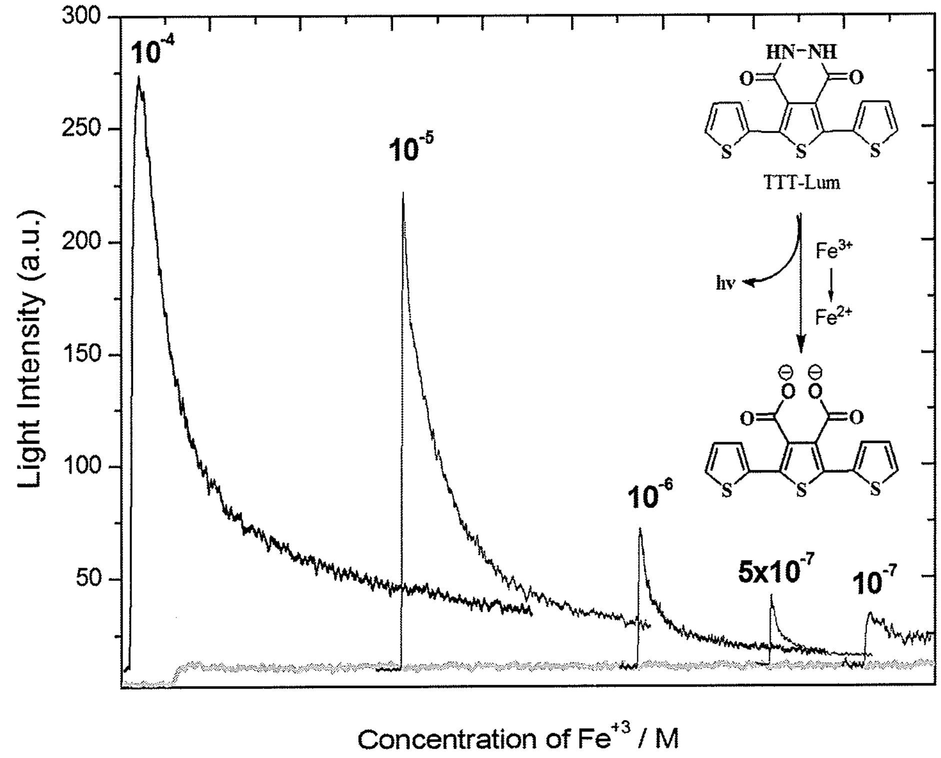


Figure 3.9 The intensity of the light obtained from the CL reaction between $1.0x10^{-5}$ M TTT-Lum dissolved in 0.1 M NaOH solution with $1.0x10^{-4}$ M H_2O_2 in the presence of various concentrations of Fe^{+3} cation. — represents the background signal.

It was found that Fe^{+3} can be detected down to $1x10^{-7}$ M with 10^{-5} M TTT-Lum. (Fig. 3.9) It is noteworthy that TTT-Lum is a promising chemiluminescent material which is highly sensitive towards Fe^{+3} ion which makes it amenable for use in the applications of forensic science when compared to luminol.

3.1.3 Application of TTT-Lum in Forensic Science

Based on the foregoing results, it would be interesting to know if TTT-Lum can be used in the detection of bloodstains since the hemoglobin in the blood of the organism includes Fe^{+2} ion which can be oxidized to Fe^{+3} out of the organism. Keeping this in mind, a freshly prepared TTT-Lum, NaOH and H_2O_2 solution was sprayed on bloodstains in the dark which otherwise couldn't be sensed by naked eye under day light. As expected, the emission of green color was observed which unambigiously confirmed the presence of bloodstains in Fig. 3.10. Furthermore, it is found that TTT-Lum is very sensitive to blood samples even at very low concentrations (See Fig. 3.11).

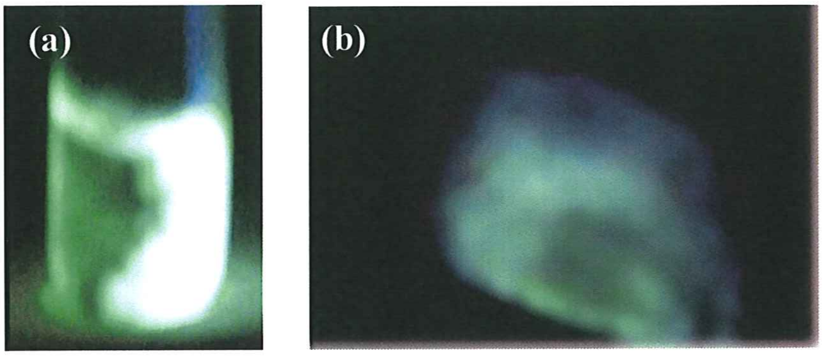


Figure 3.10 (a) The green glow obtained when TTT-Lum solution is applied to the solution containing several times diluted blood solution **(b)** The light obtained when TTT-Lum solution sprayed on the bloodstains in the dark.

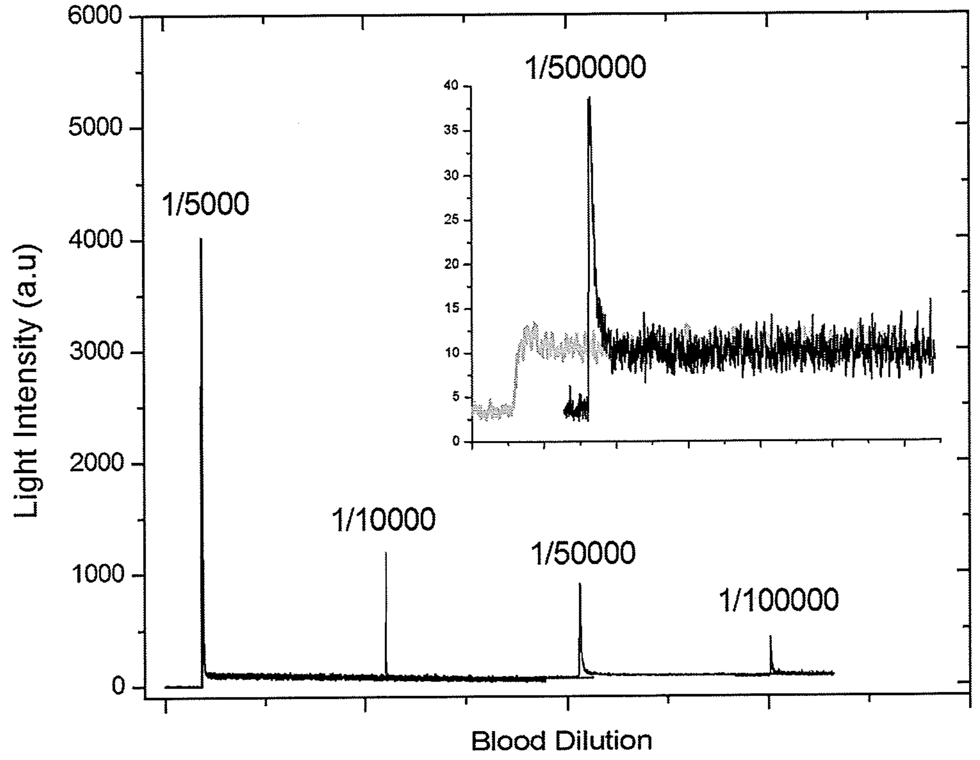


Figure 3.11 The intensity of the light obtained from the chemiluminescent reaction between $1.0\mathrm{x}10^{-5}$ M TTT-Lum dissolved in 0.1 M NaOH solution with $1.0\mathrm{x}10^{-4}$ M H_2O_2 in the presence of different dilution ratio of blood with water. — represents the background signal .

Another advantage compared to luminol is that TTT-Lum is not very sensitive toward Cu^{+2} cation (Fig.3.12). As mentioned before, there are some problematic chemicals which lead to false-positive results even if no blood is present. These kinds of chemicals cause intensification or generation of a CL emission. Therefore, if these substances are present at the crime scene, the luminol test must not be considered sufficiently specific to permit an unmistakable identification of blood. One of these chemicals is Cu^{+2} cation which has a high possibility to be present anywhere. Since luminol is very sensitive to this cation beside Fe^{+3} (blood), it can lead to false results during the identification of bloodstains at the crime scenes. However, TTT-Lum is not as much sensitive as luminol towards Cu^{+2} cation which makes it a good candidate for forensic application.

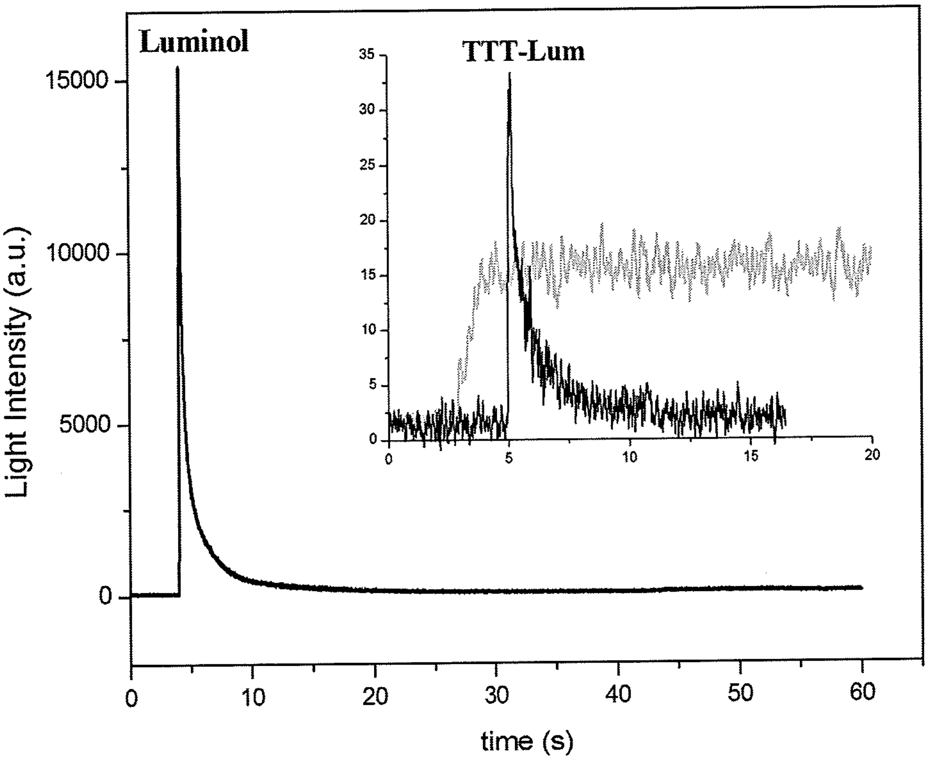


Figure 3.12 The intensity of the light obtained from the chemiluminescent reaction between 1.0x10^{-5} M TTT-Lum (inset) and luminol, dissolved separately in 0.1 M NaOH solution with 1.0x10^{-4} M H_2O_2 in the presence of Cu^{+2} cation (10^{-2} M). — represents the background signal .

3.2 Electropolymerization of TTT-Lum and Characterization of Its Polymer (PTTT-Lum)

Our aim was to synthesize a molecule consisting of an easily polymerizable thiophene in one side and a chemiluminescent group on the other. For this purpose, we firstly synthesized T-Lum and elaborated its redox behavior via cyclic voltammetry.

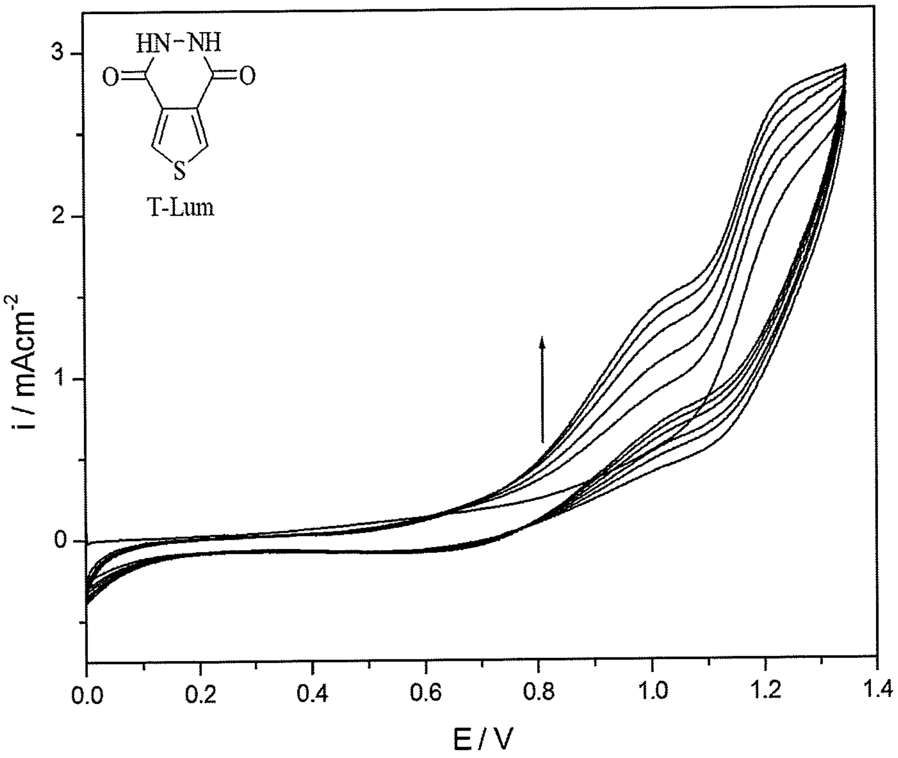


Figure 3.13 Cyclic voltammogram of 10^{-3} M T-Lum in 0.1 M $LiClO_4$/ACN solution vs. Ag/AgCl at 100 mV/s.

T-Lum as luminol has solubility problems, it is partially soluble in ACN, nitromethane and dichloromethane but completely soluble in DMSO and N,N-dimethylformamide and H_2O which are commonly used solvents in reduction reactions. During the anodic scan one oxidation peak was observed around 1.2 V in Fig. 3.13. A reduction peak around 1.1 V and a new oxidation peak around 1.0 V were observed during the reverse scan. This new oxidation peak gained intensity after repetitive scans; however, no film formation was observed on the electrode. The reason may be the breakage of HN—NH bond before the oxidation of thiophene ring, thus preventing the polymer formation on the electrode.

Therefore, in order to overcome polymerization and solubility problems, a new molecule was designed. A unique combination, consisting of a more easily polymerizable terthienyl system in one side and a chemiluminescent group on the other can be amplified to create an environmentally robust, photo and electroactive polymeric material where the transduction of chemical information into both electrical and optical signals will be possible. Attachment of additional thiophene rings would not only increases the conjugation but also increase the solubility and decrease the oxidation potential.

For this this purpose, we synthesized TTT-Lum and elaborated its redox behavior via cyclic voltammetry. During the first anodic scan, three irreversible oxidation peaks were observed at 1.10 V, 1.35 V and 1.57 V, respectively (Fig. 3.14).

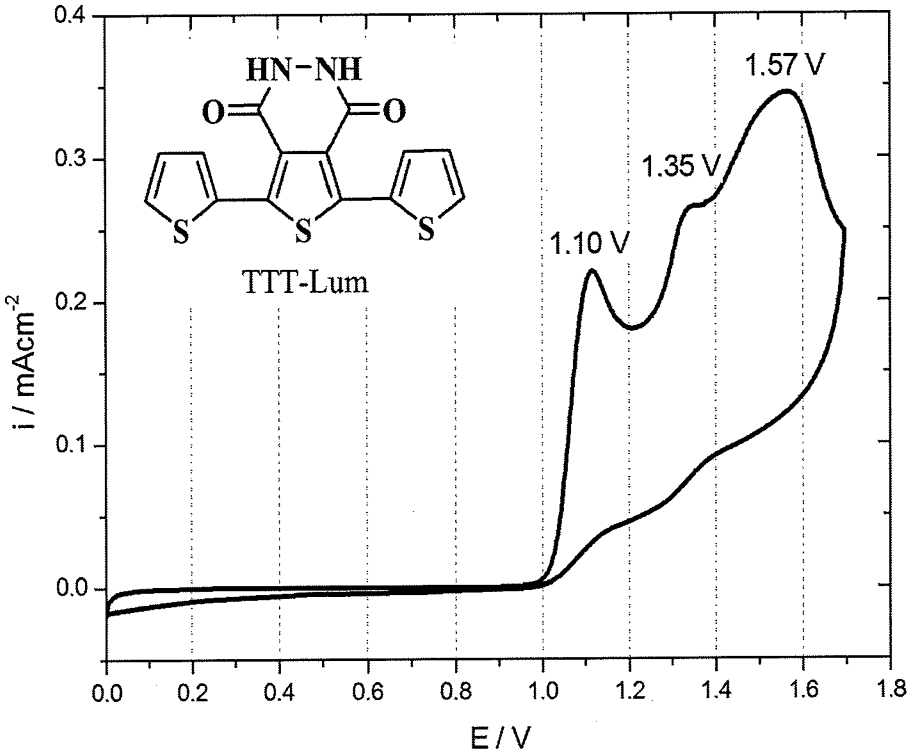


Figure 3.14 Cyclic voltammogram of $5.0\text{x}10^{-4}$ M TTT-Lum on an ITO electrode in 0.1 M $LiClO_4$/ACN containing 5% BF_3-Et_2O by volume at a scan rate of 25 mV/s.

The first irreversible oxidation peak at 1.10 V was attributed to the oxidation of trimeric thiophene rings, 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, electroluminescent emission generated by the oxidation of chemiluminescent group was monitored by a photo multiplier tube (PMT). As depicted in Fig. 3.15, TTT-Lum generated a light under an applied potential around 1.2 V. In order to get a more exact value, constant potential was applied to the same working solution (Fig. 3.15). The light emission was started exactly greater than 1.18 V, after the first anodic oxidation potential. Upon extending this potential value, the intensity of the generated light increases.

These results suggest that chemiluminescent group on the central ring of terthienyl system will remain unchanged if the anodic scan is ceased at 1.15 V. Thus, repetitive anodic scans resulted in the formation of electroactive polymer film bearing a reversible redox couple on the electrode surface in Fig. 3.16.

Electropolymerization of TTT-Lum was also possible 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 and the electropolymerization can be achieved at lower potential by repeating potential scanning (Fig. 3.17a). The electrodeposition of the polymer film on ITO took place easily and rapidly in BF_3-Et_2O. The polymer film showed nearly a well-defined reversible redox couple (0.70 V) in monomer-free electrolyte solution. In addition, the peak currents of the polymer film were intensified linearly as a function of scan rates, indicating a well-adhered polymer film and a non-diffusional redox process (Fig. 3.17b and 3.17c).

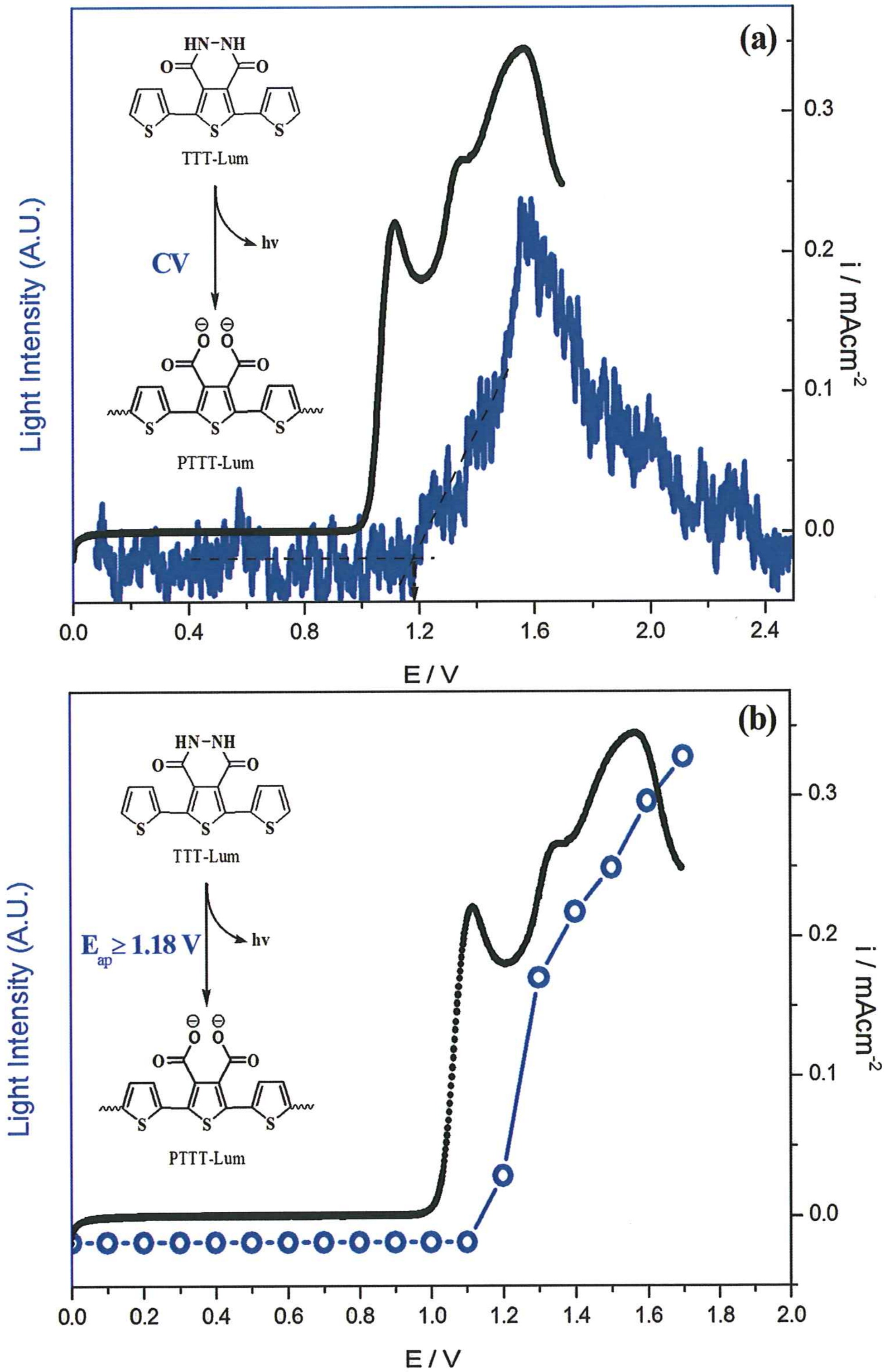


Figure 3.15 Cyclic voltammogram of 5.0×10^{-4} M TTT-Lum on an ITO electrode in 0.1 M $LiClO_4$/ACN containing BF_3-Et_2O by volume at a scan rate of 25 mV/s (—) as well as the generated light emission during potential scanning **(a, —)** and under a pulse of constant potential **(b, —O—).**

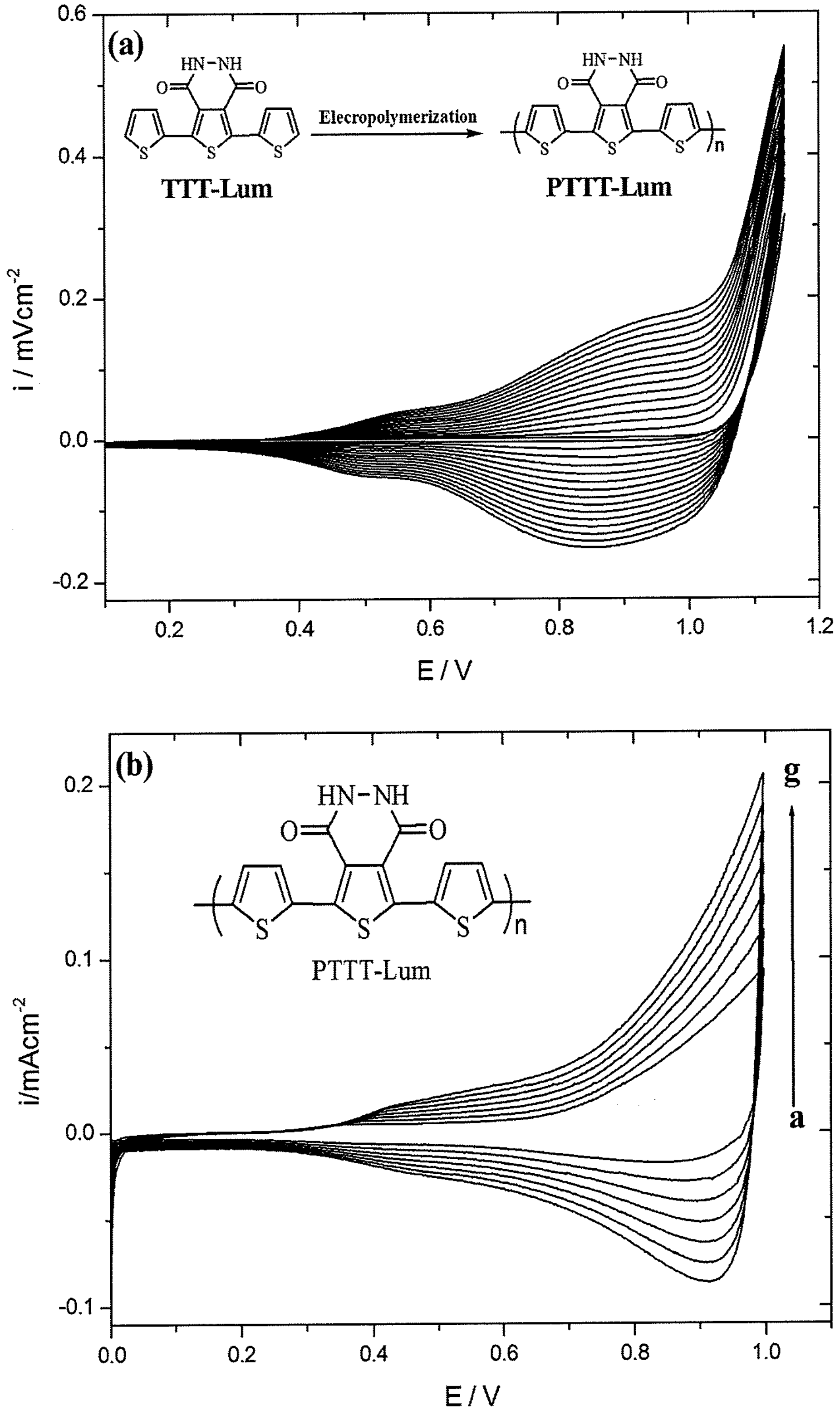


Figure 3.16 a) Repeated scan electropolymerization of 1.0x10^{-3} M TTT-Lum on an ITO electrode in 0.1 M $LiClO_4$/ACN containing BF_3-Et_2O by volume at a scan rate of 100 mV/s. **b)** Scan rate dependence of PTTT-Lum film in 0.1 M $LiClO_4$/ACN at different scan rates: (a) 20; (b) 40; (c) 60; (d) 80 and (e) 100; (f) 120; (g) 140 mV/s.

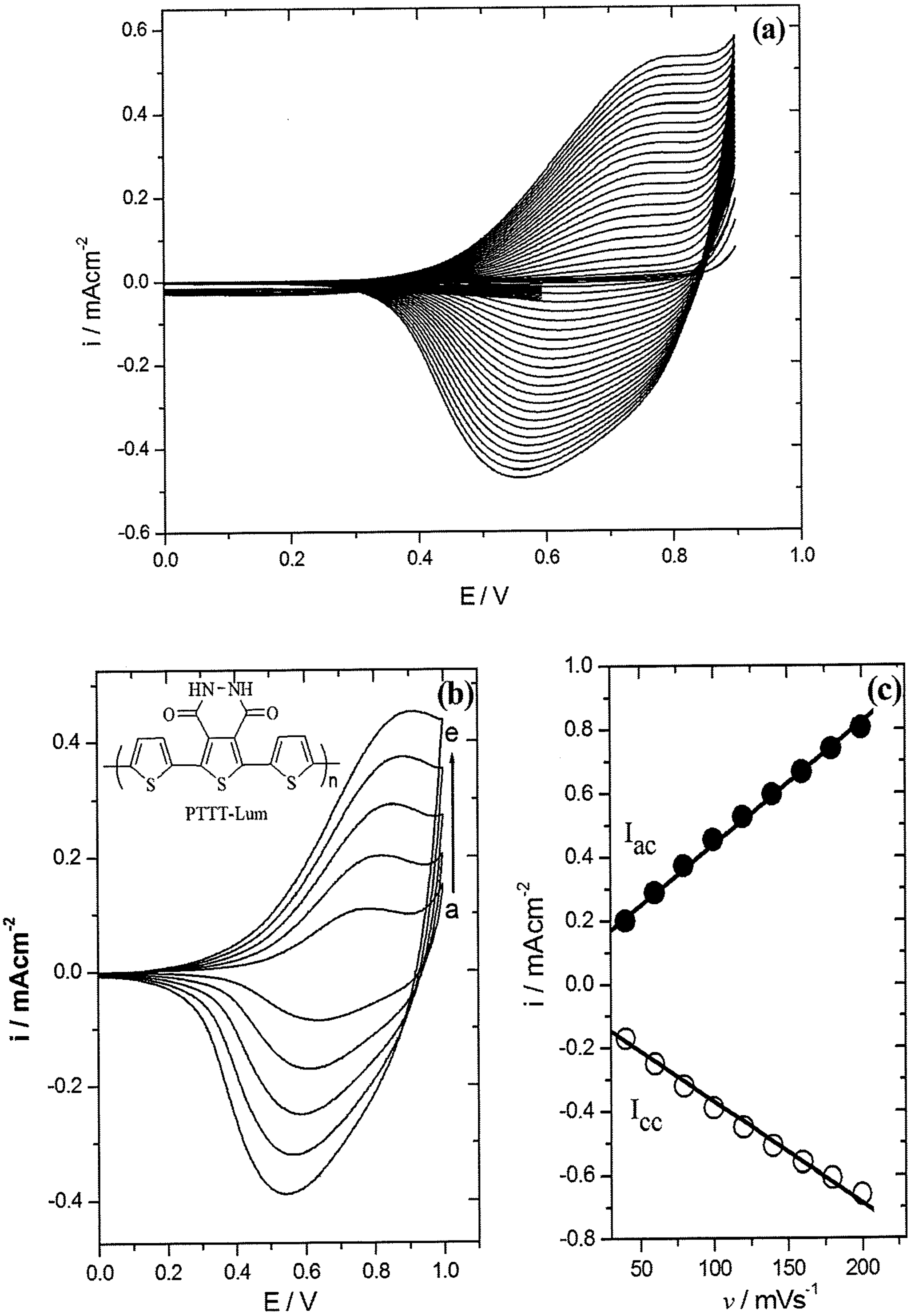


Figure 3.17 **(a)** Repeated scan electropolymerization of $1.0\text{x}10^{-3}$ M TTT-Lum on an ITO electrode in BF_3-Et_2O solution at a scan rate of 100 mV/s and **(b)** Scan rate dependence of PTTT-Lum film in BF_3- Et_2O at different scan rates: (a) 20; (b) 40; (c) 60; (d) 80 and (e) 100 mV/s. **c)** Relationship of anodic and cathodic current peaks as a function of scan rate for neutral and oxidized PTTT-Lum in BF_3- Et_2O.

3.2.1 Characterization of PTTT-Lum

Fig. 3.18 shows the FTIR spectra of TTT-Lum and its corresponding polymer, PTTT-Lum. FTIR spectrum of PTTT-Lum revealed that electropolymerization mainly proceeded via α-coupling of the external thiophene rings and chemiluminescent units remained unchanged during polymerization. The peaks at 700 cm^{-1} (α-H of thiophene rings) and 824 cm^{-1} (β/β'-H of thiophene rings) are the characteristics of TTT system [165]. The absence of the peaks at 700 cm^{-1} and 824 cm^{-1} in the polymer spectrum confirmed the α-coupling between the external thiophene rings. However, the other peaks (i.e., 1636 cm^{-1} (C=O groups), 3404 cm^{-1} (N-H groups)) in the spectrum of PTTT-Lum were remained unchanged upon polymerization [156]. Moreover, the presence of ClO_4^- dopant was confirmed by the peaks at 1085 cm^{-1} and 627 cm^{-1} in the polymer spectrum.

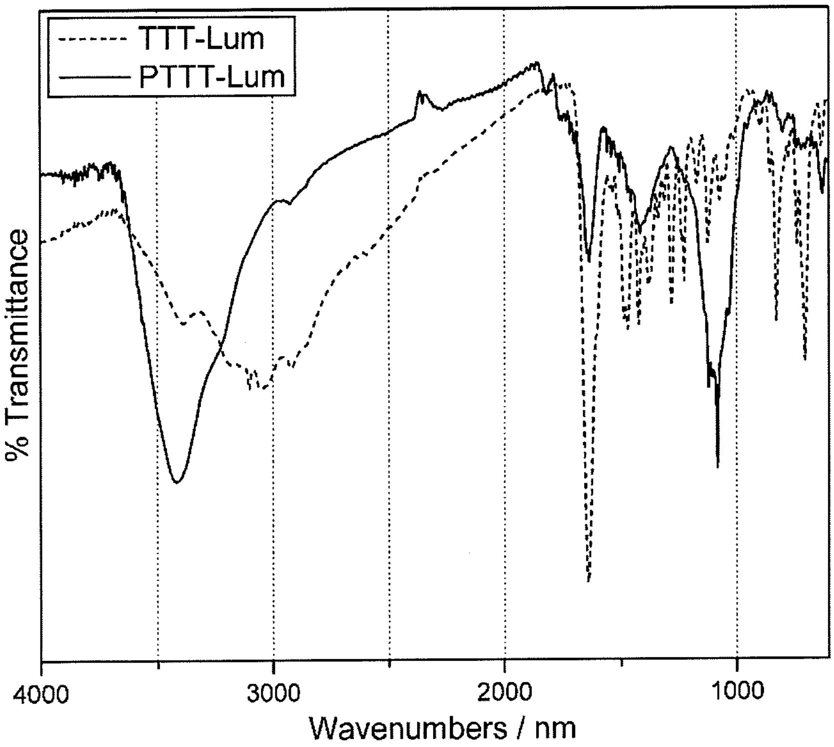


Figure 3.18 FTIR spectra of TTT-Lum and its corresponding polymer, PTTT-Lum.

Both ACN/BF_3-Et_2O mixture and neat BF_3-Et_2O solution were used in order to get a PTTT-Lum film with desired thickness (see Fig. 3.19a). Apart from the cyclic voltammetry data which suggested that the chemiluminescent units were broken after a certain potential, SEM analysis was used to reveal the fate of the chemiluminescent unit. For this aim, different polymer films were synthesized at two different potentials: 1.10 V (for the oxidation of terthienyl system) and 1.3 V (for oxidation of both terthienyl system and/or chemiluminescent unit), and then their SEM photographs were compared. It was confirmed that polymer films have completely different morphologies. The first one (Fig. 3.19b) has a *cauliflower* type morphology which is one of the main characteristics of the conducting polymers where the polymer growth process takes place after the nucleation step. However, the *cauliflower* morphology of the polymer film synthesized at higher potential (1.3 V) was destroyed due to the oxidation of chemiluminescent units and overoxidation of polymer film (Fig. 3.19c).

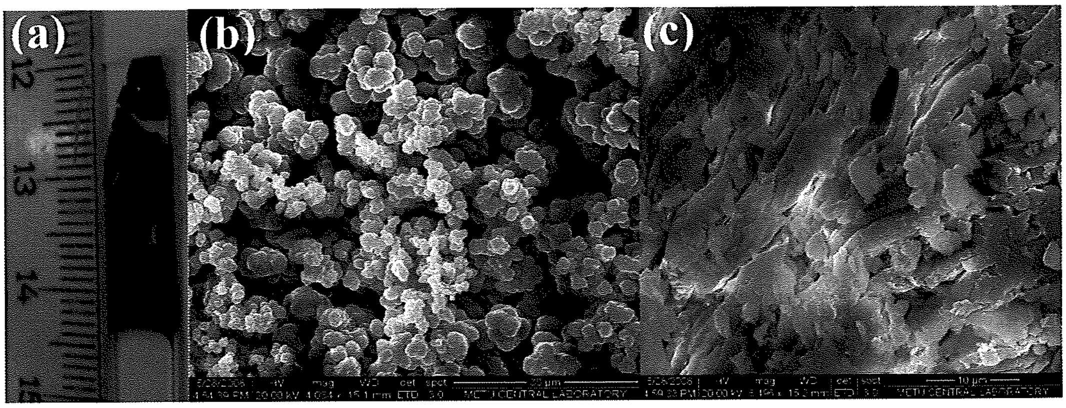


Figure 3.19 **(a)** Electrochemically obtained PTTT-Lum on ITO at 1.1 V. SEM photographs of PTTT-Lum synthesized via constant potential electrolysis at **(b)** 1.1 V and **(c)** 1.3 V.

3.2.2 Electro-optical Properties of PTTT-Lum

The electro-optical properties of the PTTT-Lum were elucidated using the changes in electronic absorption spectra upon doping process (see Fig. 3.20). The polymer film was initially neutralized in monomer-free solution at 0.0 V. Then, a well-defined absorption band of π-π* transition at 536 nm (2.31 eV) was observed in its neutral form and the band gap (E_g) was found to be 1.74 eV by the commencement on the low energy end of π-π* transition.

Upon oxidation, the polymer film exhibited an electrochromic behavior; a color change from light green to purple during p-doping. At low doping levels, the intensity of the π-π* transition band of PTTT-Lum started to decrease which is accompanied with a new band formation at 903 nm due to polaron formation. Upon further oxidation, the band at 536 nm was completely diminished and the polaron band showed a blue shift (732 nm) and then reached the maximum intensity. During oxidation, a broad band at around 1100 nm also intensified due to the bipolaron formation.

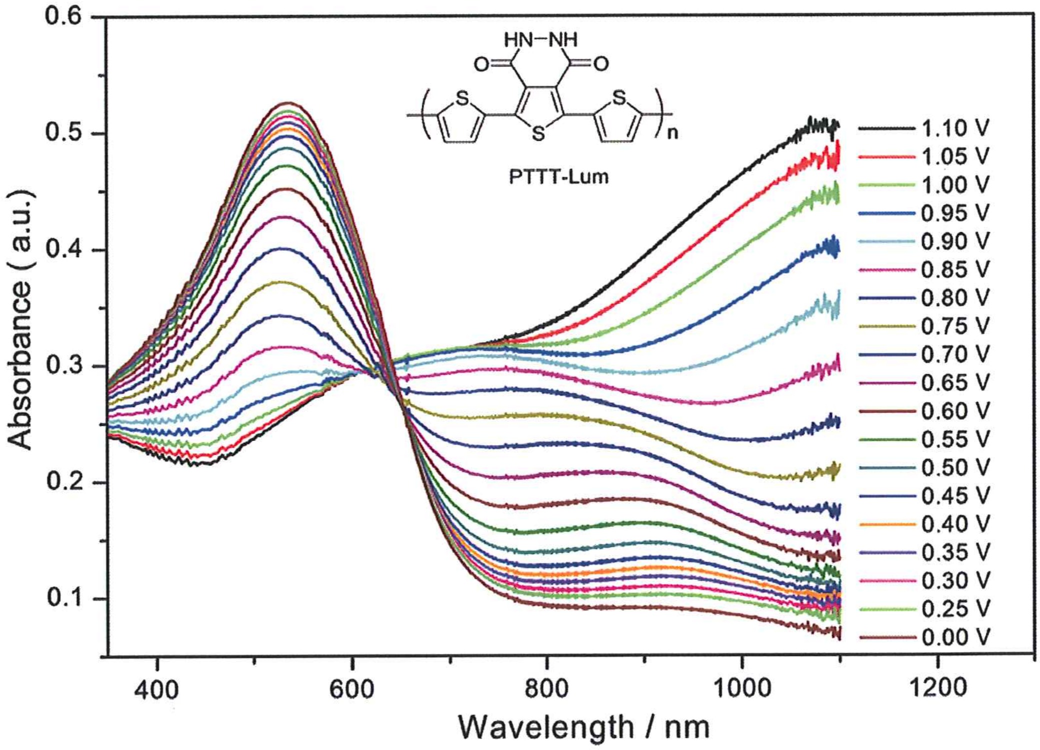


Figure 3.20. Electronic absorption spectra of PTTT-Lum on ITO in BF_3-Et_2O solution at various applied potentials between 0.0 V and 1.10 V.

3.3 ECL Activity of TTT-Lum and PTTT-Lum Under Applied Potential

As shown in section 3.2, the monomer medium was found to be ECL active. However, this property was dependent on the type of solvent used. It was ECL active in ACN and 0.1 M NaOH(aq) medium (Fig 3.21) but inactive in BF_3-Et_2O. The light intensity changes as a function of applied voltage after a threshold level (Fig. 3.15.). Similar results were obtained for the 0.1 M NaOH(aq) medium. The results showed an increasing trend; as the applied voltage increases the light intensity increases.

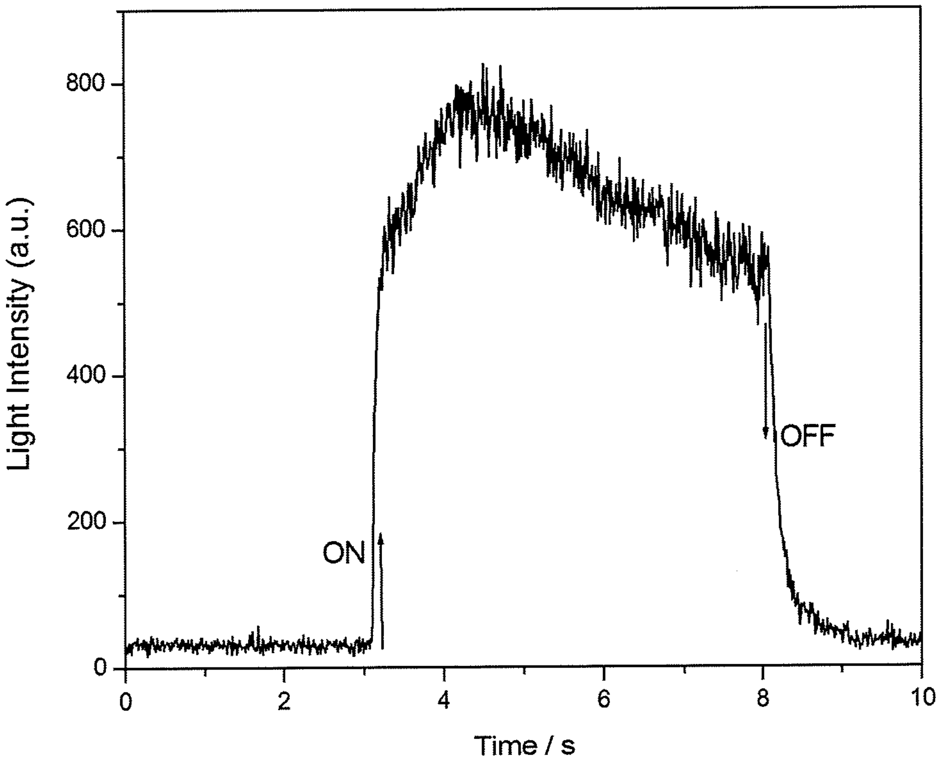


Figure 3.21 Voltage dependence of ECL of TTT-Lum in 0.1 M NaOH medium; 1.2 V was applied for 5 s.

According to the obtained results, PTTT-Lum was found to be ECL active in both ACN and BF_3-Et_2O media. In order to prove the stability of the PTTT-Lum, 1.5 V is applied constantly to the PTTT-Lum film (10 mC/cm^2, synthesized in ACN medium) for about 100 s (Fig. 3.22). The film started to loose its activity after about 85th s, indicating that the stability of the film was very high.

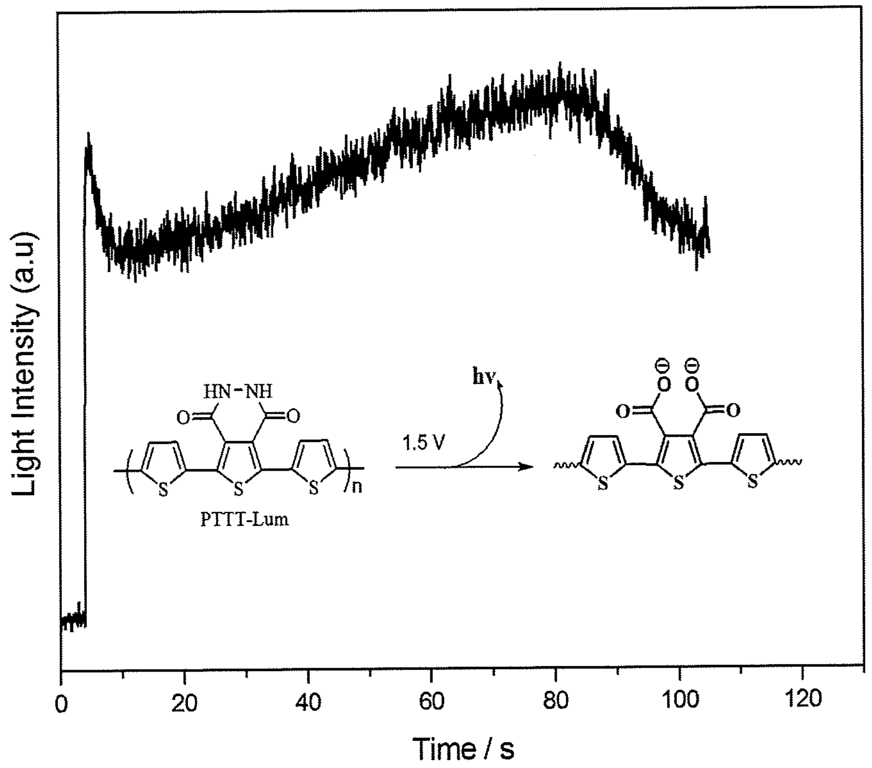


Figure 3.22 ECL of PTTT-Lum in monomer-free 0.1 M $LiClO_4$/ACN medium at 1.5 V for 100 s.

Beside constant potential electrolysis, square wave potential method was also used. The PTTT-Lum film was observed to be not stable in first five cycles, after that the film found to have nearly constant light intensity (Fig. 3.23a). The ECL activity of the film mostly remained unchanged over 1000 cycles (Fig. 3.23b).

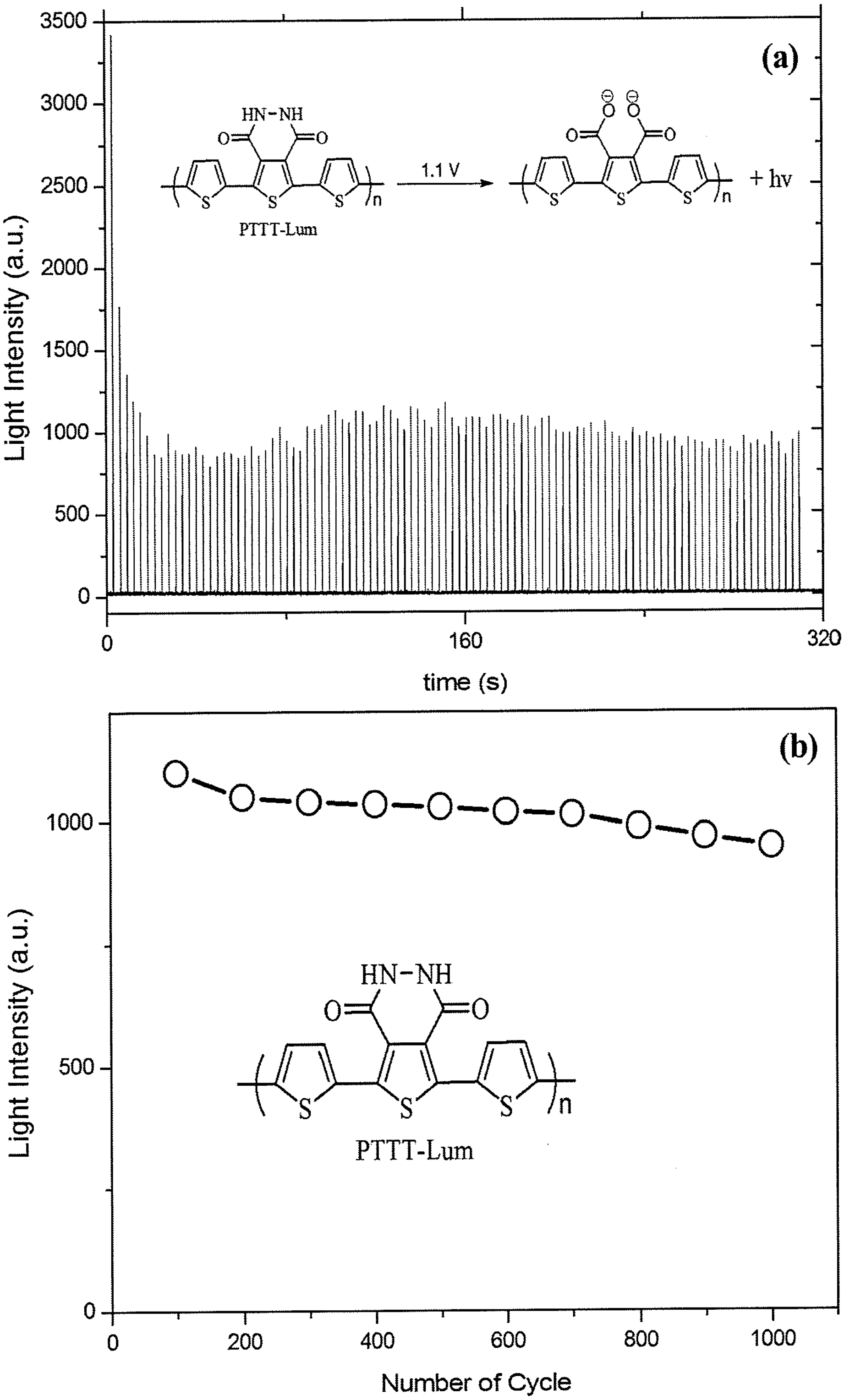


Figure 3.23 **(a)** ECL of PTTT-Lum in monomer-free BF_3-Et_2O medium. Square wave potential experiment, applied voltages were set as 1.1 V (0.1 s) and 0.0 V (3.0 s). **(b)** Representation of ECL activity over 1000 cycles.

CHAPTER 4

CONCLUSIONS

Two novel chemiluminescent monomers (T-Lum and TTT-Lum) were synthesized and one of them, TTT-Lum based on terthienyl system, was successfully electropolymerized (PTTT-Lum). Both monomers have the ability of CL and ECL. Also, they can be efficiently used to detect the bloodstains; therefore, they can find application in forensic science due to their high sensitivity to Fe(III) ion. On the other hand, PTTT-Lum bearing chemiluminescent unit has a very stable and well-defined reversible redox couple (0.9 V). The polymer film has a band gap of 1.74 eV with an absorption band at 536 nm in its neutral state as well as electrochromic behavior during p-doping process. In addition to synthesize an unique monomer, TTT-Lum, which has chemiluminescent units and can be easily polymerized without any destruction to the chemiluminescent units, its corresponding polymer is also an unique polymer which is ECL-active. Therefore, this study circumvents all of the aforementioned problems (solubility and limited thickness of the polymer film) with luminol and polyluminol through a rational design and engineering of a processable material TTT-Lum and its polymer PTTT-Lum. As a result, the works along this line will open the new doors for the design and engineering of new chemiluminescent electroactive materials which will find applications in analytical, forensic and materials sciences.

REFERENCES

1. A.K. Campbell, Chemiluminescence: Principles and Applications in Biology and Medicine, VCH ; Chichester, E. Horwood (1988).
2. E.N Harvey, A History of Luminescence from the Earliest Times until 1900. Philadelphia: American Philosophical Society (1957).
3. B. Radziszewski, Berichte d Chemischen Gesellschaft 10 (1877) 70.
4. A.M. García-Campaña, W.R.G. Baeyens, eds., Chemiluminescence in Analytical Chemistry, Marcel Dekker, Inc. (2001).
5. A.J. Bard, ed., Electrogenerated Chemiluminescence, Marcel Dekker, Inc., (2004).
6. G. Xu, J. Zhang, S. Dong, Microchem. J. 62 (1999) 259.
7. J. Yuan, A.M. Shiller, Anal. Chem. 71 (1999) 1975.
8. M. Yamaguchi, H. Yoshida, H. Nohta, J. Chromatogr. A 950 (2002) 1.
9. S. Sakura, Anal. Chim. Acta. 262 (1992) 49.
10. A. Schmitz, Uber des hydrazyde der trimensinsaure und der hemimellitsaure,Inaug.-Dissert., Heidelberg (1913).
11. J.I. Thornton, R.S. Maloney, Calif. Assoc. Crim. Newslett. (September) (1985) 9.
12. H.O. Albrecht, Z. Physik. Chem. 136 (1928) 321.
13. M. M. Rauhut, A. M. Sernsel, B. G. Roberts, J. Org. Chem. 31 (1966) 2431.
14. F. Barni, S. W. Lewis, A. Berti, G. M. Miskelly, G. Lago, Talanta 72 (2007) 896.
15. A. Larena, M. Valero, E. Bernabeu, Optica Pura Y Aplicada 16 (1983) 91.
16. N.W. Barnett, P.S. Francis, Chemiluminescence: Liquid-Phase, Encyclopedia of Analytical Science, 2nd ed., Elsevier Academic Press, London (2005).
17. J. Lee, H. H. Seliger, Photochem. Photobiol. 4 (1965) 1015.
18. K. D. Gundermann, W. Horstman, G. Bergman, Ann. 684 (1965) 127.
19. C. C. Wei, E. H. White, Tet. Lett. (1971) 3559.

20. H. Masuya, K. Kondo, Y. Aramaki, Y. Ichimori (to Takeda Chemical Industries, Ltd.), Eur. Pat. Appl. 491, 477 (1992).
21. IUPAC,1996,68,2238.
22. D. M. Hercules, Science, 145 (1964) 808.
23. L. R. Faulkner, A.J. Bard, in: A.J. Bard (Ed.), Electroanalytical Chemistry, vol. 10, Marcel Dekker, New York, (1977).
24. L. R. Faulkner, Methods Enzymol. 57 (1978) 494.
25. R.S. Glass, L.R. Faulkner, In Chemical and Biological Generation of Excited States, Adam, W.; Cilento, G., Eds., Academic Press, New York (1982) Ch 6.
26. A. W. Knight, G. M. Greenway, Analyst, 119 (1994) 879.
27. W. Y. Lee, Mikrochim. Acta 127 (1997) 19.
28. A. J. Bard, J. D. Debad, J. K. Leland, G. B. Sigal, J. L. Wilbur, J. N. Wohlstadter, In Encyclopedia of Analytical Chemistry: Applications, Theory, and Instrumentation, R. A. Meyer Ed., John Wiley & Sons: New York, (2000) p. 9842.
29. K.A. Fähnrich, M. Pravda, G.G. Guilbault, Talanta 54 (2001) 531.
30. S. Kulmala, J. Suomi, Anal. Chim. Acta 500 (2003) 21.
31. M.M. Richter, Chem. Rev. 104 (2004) 3003.
32. R. Pyati, M.M. Richter, Annu. Rep. Prog. Chem., Sect. C 103 (2007) 12.
33. C.A. Marquette, L.J. Blum, Anal. Bioanal. Chem. 385 (2006) 546.
34. X-H.N. Xu, Y.B. Zu, in: X-H.N. Xu (Ed.), New Frontiers in Ultrasensitive Bioanalysis, John Wiley & Son, New York (2007) Ch.11.
35. X.Q. Liu, L.H. Shi, H.J. Li, W.X. Niu, G.B. Xu, Electrochem. Commun. 9 (2007) 2666.
36. Y. Li, H.L. Qi, Y.G. Peng, J. Yang, C.X. Zhang, Electrochem. Commun. 9 (2007) 2571.
37. X.B. Yin, E.K. Wang, Anal. Chim. Acta 533 (2005) 113.
38. B.A. Gorman, P.S. Francis, N.W. Barnett, Analyst 131 (2006) 616.
39. C.A. Marquette, L.J. Blum, Anal. Bioanal. Chem. 390 (2008) 155.
40. S.M. Borisov, O.S. Wolfbeis, Chem. Rev. 108 (2008) 423.
41. B. Leca, L.J. Blum, Analyst 125 (2000) 789.
42. G.P. Jirka, T.A. Nieman, Mikrochim. Acta 113 (1994) 339.

43. A. Sassolas, L. J. Blum, B. D. Leca-Bouvier, Anal. Bioanal. Chem. 390 (2008) 865.
44. A.W. Knight, Trends Anal. Chem. 18 (1999) 47.
45. A. Tahirovic´, A. Cˇopra, E. Omanovic´-Miklicˇanin, K. Kalcher, Talanta 72 (2007) 1378.
46. P. Panoutsou, A. Economou,Talanta 67 (2005) 603.
47. E. Liu, B. Xue, J. Pharmaceut. Biomed. Anal. 41 (2006) 649.
48. S. Zhao, H. Yuan, C. Xie, D. Xiao, J. Chromatogr. A 1107 (2006) 290.
49. F. Wu, Y. Huang, C. Huang, Biosens. Bioelectron. 21 (2005) 518.
50. B. Li, Z. Zhang, Y. Jin, Biosens. Bioelectron. 17 (2002) 585.
51. B. Li, Z. Zhang, Y. Jin, Anal. Chem. 73 (2001) 1203.
52. L.J. Kricka, Review, Anal. Chim. Acta. 500 (2003) 279.
53. M.J. Navas, A.M. Jime´nez, Food Chem. 55 (1996) 7.
54. S. Girotti, E. Ferri, S. Ghini, A. Roda, P. Pasini, G. Carrea, R. Bovara, S. Lodi, G. Lasi, J. Navarro, P. Raush, Quı ´m. Anal. 16 (1997) S111.
55. L. Ga´miz-Gracia, A. M. G. ´a-Campan˜ a, J. J. Soto-Chinchilla, J. F. Huertas-Pe´rez, A. Gonza´lez-Casado, Trends Anal. Chem. Vol. 24 (2005) No. 11.
56. H. C. McKee, J. Air Pollut. Control Assoc. 26 (1976) 124.
57. J. E. Sigsby, F.M. Black, T.A. Bellar, D.L. Klosterman, Environ. Sci. Technol. 7 (1973) 51.
58. A. Tsuji, M. Matsumoto, M. Maeda, L. J. Kricka, P. E. Stanley, Proceedings of the 13th International Symposium on Bioluminescence&Chemiluminescence Progress and Perspectives, World Scientific Publishing Co. Pte. Ltd. (2005) pg: 183.
59. K.D. Gundermann, Angew. Chem. (Int. Edn.) 4 (1965) 566.
60. L.A. Burgoyne, U.S. Patent 5,496,562 (1996).
61. W.R. Seitz, D.M. Hercules, Anal. Chem. 44 (1972) 2143.
62. H. Kojima, K. Nonoyama, Aichi Kyoiku Daigaku Hokoku Shizen Kagaku 40 (1991) 21.
63. J.M. Lin, X. Shan, S. Hanaoka, M. Yamada, Anal. Chem. 73 (2001) 5043.
64. X. Liu, W. Niu, H. Li, S. Han, L. Hu, G. Xu, Accepted Manuscript, Electrochem. Commun., PII: S1388-2481(08)00250-6,

65. H.H. Paradies, Berichte Der Bunsen-Gesellschaft-Phys. Chem. Chem.Phys. 96 (1992) 1027.
66. W. Specht, Angew. Chem. 50 (1937) 155.
67. Z. Genfa, P.K. Dasgupta, Anal. Chem. 64 (1992) 517.
68. M.A. Motsenbocker, Y. Ichimori, K. Kondo, Anal. Chem. 65 (1993) 397.
69. H.K. Chung, P.K. Dasgupta, J.N. Marx, Talanta 40 (1993) 981.
70. D.A. Svistunenko, Biochim. Biophys. Acta 1707 (2005) 127.
71. http://www.bluestar-forensic.com
72. M. Grodsky, K. Wright, P.L. Kirk, J. Crimin. Law Criminol. Police Sci. 42 (1951) 95.
73. T.E. Yeshion, in: P.E. Stanley, L.J. Kricka (Eds.), Proceedings of the 6th International Symposium on Bioluminescence and Chemiluminescence, John Wiley & Sons Ltd., Chichester (1991) pp. 379.
74. L. Ewetz, A. Thore, Anal. Biochem. 71 (1975) 564.
75. B.A. Burke, K. Golestaneh, H. Samson, J. Chem. Educ. 76 (1999) 65.
76. C.K. Chiang, C.R. Fincher, Y.W. Park, A.J. Heeger, H. Shirakawa, F.J. Louis, S.C. Gau, A.G. MacDiarmid, Phys. Rev. Lett. 39 (1977) 1098.
77. H. Shirakawa, E. J. Louis, A.G. Macdiarmid, C.K. Chiang, A.J. Heeger, J.Chem. Soc., Chem. Commun. (1977) 578.
78. A. F. Diaz, K. K. Kanazawa, G. P. Gardini, J. Chem. Soc., Chem. Commun. (1979) 635.
79. A. F. Diaz, J. I. Castillo, J. A. Logan, W. Lee, J. Electroanal. Chem. 129 (1981) 115.
80. K. Lee, A. J. Heeger, Synth. Met. 84 (1997) 715.
81. A. F. Diaz, Chem. Scr. 17 (1981) 142.
82. G. Tourillon, F. J. Garnier, J. Electroanal. Chem. 135 (1982) 173.
83. Bayer AG Eur. Patent 339,340, (1988).
84. F. Jonas, L. Schrader, Synth.Met. 41-43 (1991) 831.
85. G. Heywang, F. Jonas, Adv. Mater. 4 (1992) 116.
86. A. Gandini, M. N. Belgancem, Prog. Polym. Sci. 22 (1997) 1203.
87. A. G. MacDiarmid, A. Epstein, J. Farad. Discuss. Chem. Soc. 88 (1989) 317.
88. J. H. Burroughes, D. D. C. Bradley, A. R. Brown, R. N. Marks, K. MacKay,

R. H. Friend, P. L. Burn, A. B. Holmes, Nature 347 (1990) 539.

89. G. Grem, G. Leditzky, B. Ullrich, G. Leising, Adv. Mater. 4 (1992) 36.
90. T.A. Skotheim, J.R. Reynolds (Eds). Handbook of conducting polymers-conjugated polymers. CRC Press Taylor and Francis Group, 3rd ed. (2007).
91. P. Chandrasekhar, Conducting Polymers, Fundamental and Applications, Ashwin-Ushas Corp., Inc., (1999).
92. J.W. Thackeray, H.S. White, M.S. Wrighton, J. Am. Chem. Soc. 89 (1985) 5133.
93. S. Glenis, G. Horowitz, G. Tourillon, F. Garnier, Thin Solid Films 111 (1984) 93.
94. H. Koezuka, A. Tsumura, T. Ando, Synth. Met. 18 (1987) 699.
95. A. Tsumura, H. Koezuka, T. Ando, Synth. Met. 25 (1988) 11.
96. F. Garnier, G. Tourillon, M. Gazard, J.C. Dubois, J. Electroanal. Chem. 148 (1983) 299.
97. K. Kaneto, K. Yoshino, Y. Inuishi, Jpn. J. Appl. Phys. Part 2 22 (1983) L412–L414.
98. S. Panero, P. Prosperi, B. Klaptse, B. Scrosati, Electrochim. Acta 31 (1986) 1597.
99. K. Kaneto, K. Yoshino, Y. Inuishi, Jpn. J. Appl. Phys. Part 2 22 (1983) L567–L568.
100. T. Yamamoto, J. Chem. Soc., Chem. Commun. (1981) 187.
101. M. Lemaire, R. Garreau, J. Roncali, D. Delabouglise, H. Korri-Youssoufi, F. Garnier., New. J. Chem. 13 (1989) 863.
102. J. Roncali, R. Garreau, D. Delabouglise, F. Garnier, M. Lemaire., J. Chem. Soc., Chem. Commun. (1989) 679.
103. L.H. Shi, F. Garnier, J. Roncali., Synth. Met. 41-43 (1991) 547.
104. P. Bäuerle, S. Scheib. Adv Mater 5 (1993) 848.
105. P. Bäuerle, G. Götz, M. Hiller, S. Scheib, T. Fischer, U. Segelbacher, M. Bennati, A. Grupp, M. Mehring, M. Stoldt, C. Seidel, F. Geiger, H. Schweizer, E. Umbach, M. Schmelzer, S. Roth, H.J. Egelhaaf, D. Oelkrug, P. Emele, H. Port., Synth. Met. 61 (1993) 71.
106. S. Scheib, P. Bäuerle., J. Mater. Chem. 9 (1999) 2139.

107. P. Bäuerle, S. Scheib. Acta. Polym. 46 (1995) 124.
108. A. Berlin, G. Zotti, S. Zecchin, G. Schiavon., Synth. Met. 131 (2002) 149.
109. F. Algı, A. Cihaner, Tet. Lett. 49 (2008) 3530.
110. B. Köksel, A. Cihaner, M. Kaya, M. Volkan, A. M. Önal, J. Appl. Polym. Sci. 108 (2008) 2707.
111. D.N. Buckley, L.D. Burke, J. Chem. Soc., Faraday Trans. 1, 71 (1975) 1447.
112. D.N. Buckley, L.D. Burke, J.K. Mukahy, J. Chem. Soc., Faraday Trans. 1, 72 (1976) 1896.
113. L.D. Burke, T.A.M. Thomey, D.P.J. Whelan, Electroanal. Chem. 107 (1980) 201.
114. W.C. Dautremont-Smith, Displays 3 (1982) 3.
115. C.G. Granqvist, Sol. Energy Mater. Sol. Cells 60 (2000) 201.
116. C.G. Granqvist, E. Avendano, A. Azens, Thin Solid Films 442 (2003) 201.
117. E. Avendano, L. Berggren, G.A. Niklasson, C.G. Granqvist, A. Azens, Thin Solid Films, 496 (2006) 30.
118. I. Schwendeman, R. Hickman, G. Sonmez, P. Schottland, K. Zong, D. Welsh, J.R. Reynolds, Chem. Mater., 14 (2002) 3118.
119. H. Meng, D. Tucker, S. Chaffins, Y. Chen, R. Helgeson, B. Dunn, F.Wudl, Adv. Mater. 15 (2003) 146.
120. A. Pennisi, F. Simone, G. Barletta, G. Di Marco, L. Lanza, Electrochim. Acta 44 (1999) 3237.
121. R. Rauh, Electrochim. Acta 44 (1999) 3165.
122. R. G. Mortimer, Chem. Soc. Rev. 26 (1997) 147.
123. D.R. Rosseinsky, R.J. Mortimer, Adv. Mater. 13 (2001) 783.
124. K. Bange, T. Gambke, Adv. Mater. 2 (1990) 10.
125. A. Kraft, A.C. Grimsdale, A.B. Holmes, Angew. Chem. Int. Ed. 37 (1998) 402.
126. P. Chandrasekhar, B.J. Zay, G.C. Birur, S. Rawal, E.A. Pierson, L. Kauder,T. Swanson, Adv. Funct. Mater. 12 (2002) 95.
127. G.A. Sotzing, J.R. Reynolds, P.J. Steel, Chem. Mater 8 (1996) 882.
128. L.B. Groenendaal, J. Friedrich, D. Freitag, H. Pielartzik, J.R. Reynolds, Adv. Mater. 12 (2000) 481.

129. G. Sonmez, C.K.F. Shen, Y. Rubin, F. Wudl., Angew. Chem. Int. Ed. Engl 43 (2004) 1498.
130. G. Sonmez, H.B. Sonmez, C.K.F. Shen, R.W. Jost, Y. Rubin, F.Wudl., Macromolecules 38 (2005) 669.
131. A. Durmus, G.E. Gunbas, P. Camurlu, L. Toppare, Chem. Commun. (2007) 3246.
132. A. Durmus, G.E. Gunbas, L. Toppare, Chem. Mater. 19 (2007) 6247.
133. A. Durmus, G.E. Gunbas, L. Toppare, Adv. Mater. 20 (2008) 691.
134. G.E. Gunbas, A. Durmus, L. Toppare, Adv. Funct. Mater. 18 (2008) 2026.
135. S. Tanaka, Y. Yamashita., Synth. Met. 84 (1997) 229.
136. T. Yamazaki, Y. Murata, K. Komatsu, K. Furukawa, M. Morita, N. Maruyama, T. Yamao, S. Fujita., Org. Lett. 6 (2004) 4865.
137. G. Sonmez, C.K.F. Shen, Y. Rubin, F. Wudl., Adv. Mater. 17 (2005) 897.
138. B.F.Y. Yon-Hin, M. Smolander, T. Crompton, C.R. Lowe, Anal. Chem., 65 (1993) 2067.
139. G. Sudesh Kumar, D.C. Neckers, Chem. Rev. 89 (1989) 1915.
140. J. A. Delaire, K. Nakatani, Chem. Rev. 100 (2000) 1817.
141. J.-I. Anzai, T. Osa, Tetrahedron 50 (1994) 4039.
142. A. Cihaner, F. Algı, Electrochim. Acta, under revision (2008).
143. V. Shibaev, A. Bobrovsky, N. Boiko, J. Photochem. Photobiol. A: Chemistry 155 (2003) 3.
144. R. Hagen, T. Bieringer, Adv. Mater, 13 (2001) 1805.
145. V. Weiss, A.A. Friesern, V.A. Krongauz, Opt. Lett. 18 (1993) 1809.
146. Y.-K. Han, B.-S. Ko, Opt. Mater. 21 (2002) 621.
147. S. Kawata, Y. Kawata, Chem. Rev. 100 (2000) 1777.
148. M. Bauer, W. M. Müller, U. Müller, K. Rissanen, F. Vögtle, Liebigs Ann. (1995) 649.
149. K.G. Yager, C.J. Barrett, J. Photochem. Photobiol. A: Chemistry 182 (2006) 250.
150. M. Dumont, G. Froc, S. Hosotte, Nonlinear. Opt. 9 (1995) 1915.
151. T. Srikhirin, A. Laschitsch, D. Neher, D. Johannsmann, Appl. Phys. Lett. 77 (2000) 963.

152. P. Uznanski, J. Pecherz, J. Appl. Polym. Sci 86 (2002) 1459.
153. G.-F. Zhang, H.-Y. Chen, Anal. Chim. Acta. 419 (2000) 25.
154. S.-M. Chen, K.-C. Lin, J. Electroanal. Chem. 523 (2002) 93.
155. Y.-T. Chang, K.-C. Lin, S.-M. Chen, Electrochim. Acta. 51 (2005) 450.
156. E.P. Koval'chuk, I.V. Grynchyshyn, O.V. Reshetnyak, R.Y. Gladyshevs'kyj, J. Błażejowski, Eur. Polym. J. 41 (2005) 1315.
157. B. Epstein, T. Kuwana, J. Electroanal. Chem. 15 (1967) 389.
158. C.H. Wang, S.M. Chen, C.M. Wang, Analyst 127 (2002) 1507.
159. D. W. H. MacDowell, J. C. Wisowaty, J. Org. Chem. Vol. 37 No. 11 (1972).
160. C. Galvez, F. Garcia, J. Garcia, J. Soldevila, J. Heterocyclic Chem. 23 (1986) 1103.
161. K-Yi. Tserng, L. Bauer, J. Org. Chem. Vol. 40 No. 2 (1975).
162. R. G. Jones, J. Am. Chem. Soc. 78 (1956) 159.
163. M. Pomerantz, H. Yang, Y. Cheng, Macromolecules 28 (1995) 5706.
164. J. Cao, J.W. Kampf, M.D. Curtis, Chem. Mater. 15 (2003) 404.
165. J.P. Ferraris, T.R. Hanlon, Polymer 30 (1989) 1319.