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**NEW TECHNIQUE FOR CHEMILUMINESCENCE
DETERMINATION OF IRON LEVEL IN THALASSEMIA
PATIENT BY MOBILE PHONE**

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NEW TECHNIQUE FOR CHEMILUMINESCENCE DETERMINATION OF IRON
LEVEL IN THALASSEMIA PATIENT BY MOBILE PHONE

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January 2023

We certify that we have read this thesis and that in our opinion it is fully adequate, in
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ABSTRACT

NEW TECHNIQUE FOR CHEMILUMINESCENCE DETERMINATION OF IRON LEVEL IN THALASSEMIA PATIENT BY MOBILE PHONE

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Master of Science in Chemistry

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January 2023

The current study aims to Creation of a brand-new instrument to assess chemiluminescence intensity. A brand-new photosensor was employed as a light, and the new gadget has the advantages of great sensitivity, low cost, simplicity of design, and ease of usage. The experimental section consists of the design of a new instrument for Chemiluminescence and Bioluminescence by using the direct reaction between luminol and hydrogen peroxide and the determination of optimum conditions of the new device. The light intensity from the oxidation of Luminol was measured by using the Remote XY program installed in a mobile phone which gives data as a results analysis for chemical interaction. The linearity, detection limit ($3\sigma \times \text{noise}$), and correlation coefficient were $R = 0.9997$. It was conducted experiments on 25 samples of Thalassemia patients with the device. The measurement results of the homemade device developed to measure iron in the blood of thalassemia patients were compared with those of the colorimetric Cromatest kit array. It was found that the results were almost similar.

2022, 73 pages

Keywords: Chemiluminescence, Photo sensor, Luminol, Mobile phone, Thalassmia

ÖZET

CEP TELEFONU İLE TALASEMİ HASTASINDA DEMİR SEVİYESİNİN KEMİLÜMİNESANS TAYİNİ İÇİN YENİ TEKNİK

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Bu çalışma, kemilüminesans yoğunluğunu değerlendirmek için yepyeni bir enstrüman oluşturmaya amaçlamaktadır. Işık olarak yepyeni bir fotosensör kullanıldı ve yeni cihaz, yüksek hassasiyet, düşük maliyet, tasarım basitliği ve kullanım kolaylığı gibi avantajlara sahiptir. Deneysel kısım, luminol ve hidrojen peroksit arasındaki doğrudan reaksiyon kullanılarak Kemilüminesans ve Biyolüminesans için yeni bir cihazın tasarımı ve yeni cihazın optimum koşullarının belirlenmesinden oluşmaktadır. Luminol'ün oksidasyonundan kaynaklanan ışık yoğunluğu, kimyasal etkileşim için bir sonuç analizi olarak veri sağlayan bir cep telefonuna kurulu Remote XY programı kullanılarak ölçüldü. Doğrusallık, tespit limiti ($3\sigma \times \text{gürültü}$) ve korelasyon katsayısı $R=0,9997$ 'dir. Cihaz ile 25 Talasemi hastası örneğinde deneyler yapılmıştır. Talasemi hastalarının kanındaki demiri ölçmek için geliştirilen ev yapımı cihazın ölçüm sonuçları kolorimetrik Cromatest kit dizisinin sonuçları ile karşılaştırıldı. Sonuçların hemen hemen benzer olduğu bulundu.

2022, 73 sayfa

Anahtar Kelimeler: Kemilüminesans, Fotoğraf sensörü, Luminol, Cep telefonu, Talasemi.

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LIST OF SYMBOLS

%	Percent
**	Significant
/	Divide
+	Plus
<	Greater than
=	Equal
>	Less than
±	Plus – minus
≤	Greater or equal to
≥	Less or equal to
μg	Microgram
μL	Microliter
dL	Deciliter
g	Gram
kg	Kilogram
L	Liter
m ²	Square meter
mg	Microgram
min	Minute
mIU	Milli-international units
mL	Milliliter
mmol	Millimole
mol	Mole
ng	Nanogram
nm	Nanometer
NS	Non-significant
rpm	Revolutions per minute

LIST OF ABBREVIATIONS

BL	Bioluminescence
CAL	Cathodoluminescence
CL	Chemiluminescence
CRET	Chemiluminescence resonance energy transfer
CRL	Cathode ray tube
EL	Electroluminescence
Hb	Hemoglobin
HLA	Human Leukocyte Antigen
LCD	Liquid crystal display
PL	Photoluminescence
RL	Radioluminescence
SEM	Scanning electron microscope
SL	Sonoluminescence
TL	Thermoluminescence
TrL	Triboluminescence

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1. INTRODUCTION

Luminescence, sometimes known as “cold light,” is the spontaneous emission of light by a substance that is not caused by heat (Virk 2015). Luminescence is the emission of light at both high and low temperatures without the presence of an external source. Luminescence has fascinating tales which span from the earliest times to the year 1900 (from prehistoric to modern societies). The first description of a solid- state luminous substance appears in taken from Chinese literature that was written during the Song period (960–1279 A.D.). According to legend, the Buddhist precious diamond known as “hashi-no-tama” in Japan is self-luminous and can illuminate its surroundings brilliantly. In the Upanishad, most likely written before the sixth-century Svetasvatara finds a reference to fireflies as one among the manifestations of in the third century BC Brahma (Xu and Tanabe 2019).

The materials that display this effect are referred to as “Luminescent materials” or “Phosphors”, which is Greek for “light bearer.” Vincentinus Casciarolo, an Italian alchemist from Bologna, is credited with creating the word "phosphor" in the 17th century. When an electron in an atom is excited from its ground state (the state with the least energy) to its excited state (the state with the most energy), the electron emits light to return to the ground state. Fireflies, along with certain microbes and deep-sea species, are examples of natural luminescence. Researchers from all around the globe have employed this phenomena for quality control, R&D, and a host of other industrial purposes in fields as diverse as archaeology, geology, biomedical engineering, chemistry, and physics (Murthy and Virk 2014).

Luminescence devices, such as lights, televisions, mobile displays, LED lamps, LED televisions, signals, displays, etc., have become indispensable to contemporary urban life. An example of electromagnetic energy is light. Another type of energy must be provided in order to produce light. This can happen through luminescence or incandescence, which are the two most typical methods. Heat energy produces luminous incandescence. If you heat anything up sufficiently, it will start glowing. Incandescence

is the phenomenon in which a metal item in a flame or the electric stove's heater begins to glow "red hot" (Wiedemann 1888).

The current study aims to creation of a brand-new instrument to assess chemiluminescence intensity. A brand-new photosensor was employed as a light, and the new gadget has the advantages of great sensitivity, low cost, simplicity of design, and ease of usage. The purpose of the study is the determination of iron in thalassemia patients by using a new technique with a chemiluminescent reaction.



2. LITERATURE REVIEW

2.1 Luminescence

Luminescence is the emission of longer-wavelength light as a result of the absorption of shorter-wavelength light when radiation impacts a medium. In the case of luminescence, it is the luminescent material itself, and not the incoming radiation, that determines the wavelength of the emitted light. Light of many wavelengths, including the visible, ultraviolet, and infrared, may be emitted. This luminescence, also known as cool emission, is the result of two processes that do not involve the emission of blackbody radiation: light emission occurs when (1) an electrical system is triggered into a higher energy state, and (2) photons (or merely light) are emitted as a result (Xu and Tanabe 2019).

After the radiation has been absorbed, there occurs an emission of light at a specific period (T_c). Using this parameter, we may further divide the luminescence process into fluorescence and phosphorescence, as described in Figure 2.1. According to the length of the emission or luminescence can be divided into two categories (Virk 2015).

2.1.1 Fluorescence

When atoms in a substance are excited, they release electromagnetic radiation, frequently visible light, which is then immediately reemitted within 10⁻⁸ seconds. It is the first arousal. This is usually caused by incoming radiation or particles absorbing energy, such as X-rays or electrons. Because reemission occurs so quickly, the fluorescence ends as soon as the stimulating source is taken away (Xu and Tanabe 2019).

2.1.2 Phosphorescence

Afterglow occurs when radiation is removed from a substance, yet the material

continues to emit light. Unlike fluorescence, which only needs supplemental light, the excitation generates radiation and can last anywhere between 10^{-3} seconds depending on the conditions, days or years (Siraj *et al.* 2016).

Short-term phosphorescence ($T_c \sim 10^{-4}$ s) and long-term phosphorescence ($T_c > 10^{-4}$ s), sometimes called thermoluminescence, are two distinct types of the same phenomena (TL). Each technique outlined the knowledge mentioned above is pertinent to science and is useful (Šípoš and Šima 2020).

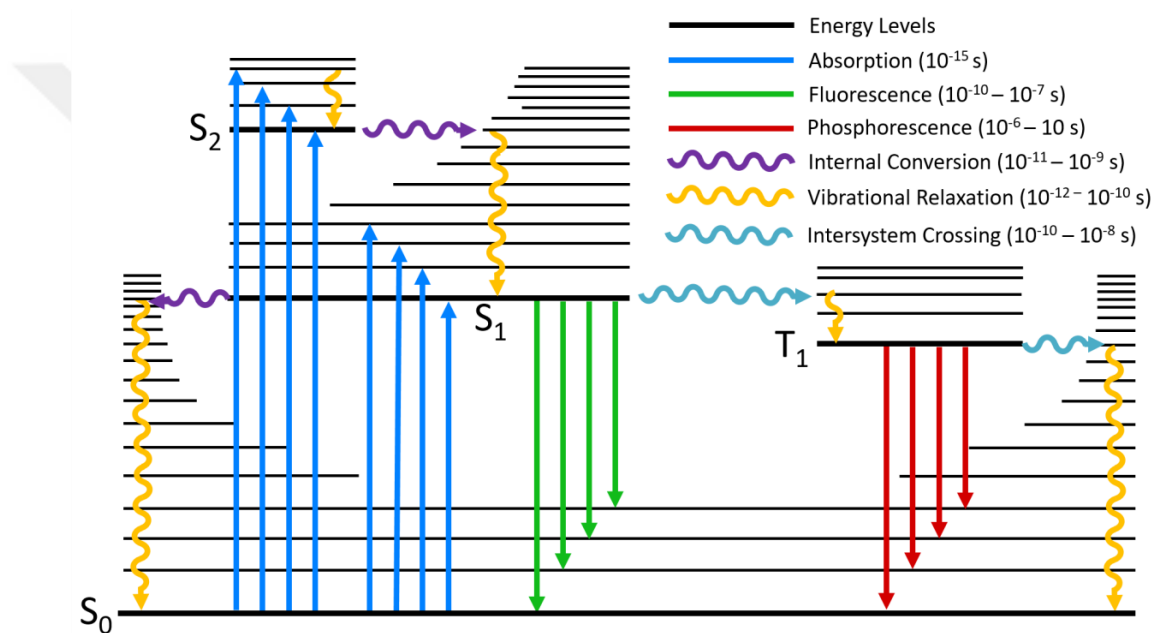


Figure 2.1 The Jablonski diagram (Valeur and Berberan-Santos 2012)

2.1.3 Types of luminescence

Numerous forms of luminescence exist, and they are classified by the sort of energy or catalyst that triggers them (Selvin *et al.* 1994).

Chemiluminescence (CL): Light is produced by the energy released during a chemical reaction (also known as CL). A brilliant blue glow is produced when luminol ($C_8H_7N_3O_2$) reacts with a suitable oxidant (Cha *et al.* 1999).

Bioluminescence (BL): Amazing instances of bioluminescence, commonly called "living light," may be discovered across the ocean's depths. Several marine creatures perform chemical processes that result in visible light emission, allowing them to thrive in the ocean's deep darkness (Cha *et al.* 1999, Latz 2017).

Triboluminescence (TrL): This kind of luminescence, found by chance, uses mechanical energy to produce light. Light emissions may be triggered by separating the material by tearing, ripping, scraping, crushing, or rubbing, much as they are in sugar or silicon crystals. Mechanoluminescence is another name for this phenomenon (Cha *et al.* 1999).

Cathodoluminescence (CaL): In this case, excitation of an electron beam is responsible for the emitted light. A scanning electron microscope (SEM) and cathode ray tubes (CRTs) used in TVs and computers both employ electron guns for this purpose (Bunker 2004).

Electroluminescence (EL): When an electric field is applied to a certain substance, light is produced. LEDs (light-emitting diodes) are by far the most common kind of electroluminescent technology seen in everyday life (Selvin *et al.* 1994).

Photoluminescence (PL): Upon being excited by electromagnetic radiation/photons, a system will release photons, resulting in light emission. The phrase encompasses both fluorescence and phosphorescence, making it less precise. From bleaching agents in laundry detergent to plasma panels in stadiums, the uses for photoluminescence are many. One kind of luminescence emits light for many minutes or even hours before dying out. Long-lasting or persistent luminescence is widely used in traffic control and directional signage (Perry *et al.* 2012).

Thermoluminescence (TL): Another name for it is "thermally stimulated luminescence" (TSL). It's the glow that gets turned on by heat after being exposed to radiation like α , β , γ , or X rays. Not to be confused with heat radiation, thermal excitation only causes energy to be released from a substance after it has been excited by some other means

(Kalita and Chithambo 2019)

Radioluminescence (RL): The process through which a material gives out light in response to being bombarded by ionizing radiation (such as beta particles, X-rays, or gamma rays) (Perry *et al.* 2012).

Sonoluminescence (SL): What we have here is a process wherein light is produced in response to the activation of ultrasonic waves (Canaparo *et al.* 2020).

2.2 Chemiluminescence

Chemiluminescence (CL) is one of the most important methods for examining and measuring various macromolecules. As a consequence of a chemical reaction, the sample emits light (luminescence), which is the foundation for CL methods. The sample, column eluate, and luminescent reagent are continually combined in the mixer. A photomultiplier tube is used to quantify the luminescence once the chemical reaction has reached its peak intensity (Song *et al.* 2019).

Chemical reactions, often involving an oxidation-reduction process, are used to create CL. Producing such emission often involves transforming the heat from an exothermic chemical process into light energy. Some chemical reactions involve the transfer of energy to electrons in chemical bonds. In the process of returning to their ground states, these electrons emit light. Given the time required for some of these processes to complete, the light might be visible for an exceptionally long period. This is referred to as CL. This contrasts with more powerful chemical reactions, where the chemicals produce so high heat emissions that they burn or otherwise glow very hot. This is, to put it simply, incandescence. CL is seen by many organisms, and oxidation is frequently a chemical reaction. As an illustration, the biological molecule Luciferin emits light. This organic molecule, which has the basic symbol LH_2 , consists of two hydrogen atoms bound together (Song *et al.* 2019).

CL is used regularly in biology and analytical chemistry. It is already widely known as a potent analytical instrument because of its inherent capacity for high levels of sensitivity with little background noise, selectivity, and wide linear detection, utilizing inexpensive tools. CL agents vary in biological specificity and usefulness, but common examples include luminol, hydrogen, fluorescein, dioxetanes, and oxalate derivatives and acridinium dyes. Luminol CL (LCL) is used in the fields of medicine, biology, and forensics. Because of its inexpensive cost, high sensitivity, and selectivity, LCL has shown to be a very valuable detection technology (Khan *et al.* 2014).

CL is also quite sensitive in comparison to other analytical techniques. CL calls for far simpler equipment than fluorescence or spectrophotometric approaches, both of which need a light source and monochromator. The equipment needed for CL is fairly simple and affordable. This might make CL a more portable detection technique (Von Lode 2005). "CL" refers to the light produced by any chemical process, whether it takes place in a solid, liquid, or gaseous state. The foundations of CL have been extensively explored in recent books and articles. Direct and indirect chemiluminescent reactions are the most common forms documented in the literature (Figure 2.2). Direct CL can be represented by Equation (1.1) (Siraj *et al.* 2016).



When a molecule is stimulated to a metastable intermediate, it may return to its ground state through a variety of pathways. Figure 2.2 is only one example of a graphical representation of these pathways; other examples include the more simplistic Jablonski diagram, which was used for the first time in the 1930s, and the "energy well" diagram. Any of fluorescence, CL, or phosphorescence may result from a singlet state, but only phosphorescence can result from a triplet state. Chemiluminescent processes produce light that varies in intensity, duration, and wavelength, the latter of which spans the near-ultraviolet to near-infrared spectrum (Barni *et al.* 2007). Typically, the electromagnetic spectrum's visible or near-infrared parts are where the light is emitted.

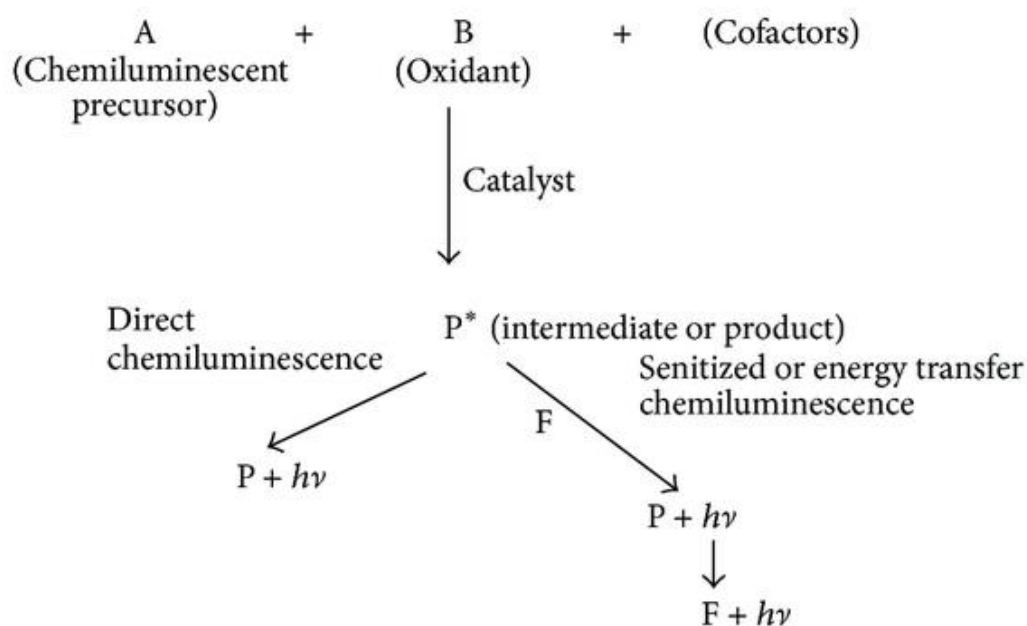


Figure 2.2 Basic mechanisms of chemiluminescent reactions

Despite the fact that impurities may generate nonspecific background signals during detection, which reduces its sensitivity, CL methods have the benefits of being very sensitive, having a large dynamic range, and being applicable over a wide spectral range. The efficacy of most writings may be shown by the strength of chemiluminescent signals (Xue *et al.* 2019).

Heat is the most frequent type of energy generated during the vibrational stimulation of ground states; hence it is produced very selectively. For a chemical reaction to be acceptable for CL detection, it must meet three requirements.

For an electrically stimulated condition to be reached, sufficient energy must be discharged.

The path it travels ought to promote the emergence of such an aroused state. Either the excited state is luminous or has the ability to transfer its energy to a molecule that is.

2.3 The Emission Intensity of CL

The CL reaction starts after the essential reactants have been mixed, and the emission intensity quickly increases. The reactants are consumed as the CL reaction progresses. When reagent concentrations are lowered, the reaction intensity and, by extension, the CL intensity, are lowered as well. As demonstrated in Figure 2.3, which displays the fluctuation in CL emission intensity with time after mixing aliquots of reactants, this demonstrates that the CL emission intensity is essentially transitory. The length of time an emission lasts for varies from a fast flash lasting less than a second to a continuous glow lasting hours, depending on the kind of reaction and, in certain cases, the concentrations of the reactants involved (Campana *et al.* 2001).

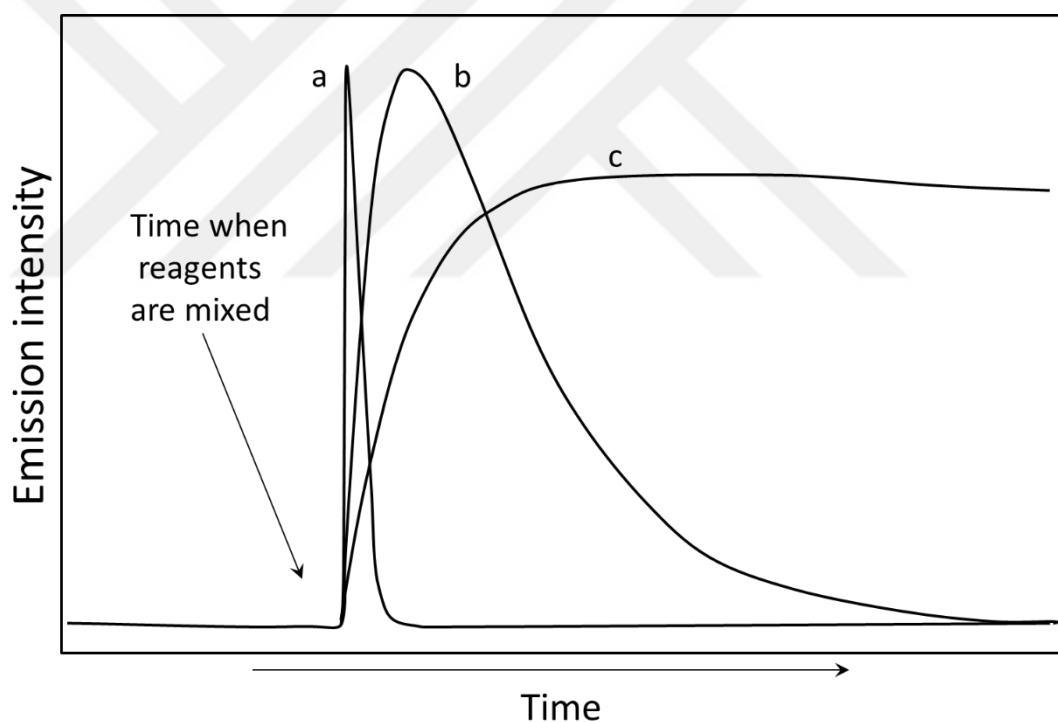


Figure 2.3 Temporal dependence of CL emission for reactions with short (a), medium (b), and long (c) emission durations

2.4 Reagent-Based CL Determinations

To begin with, the analyte plays a role in the CL process, often as a reagent, catalyst, quencher, or enhancer, in the first mode of operation. Only a small subset of these systems, such as luminol, peroxyoxalate, tris-(2, 2'-o-bipyridyl) ruthenium (II), acridinium esters, and cerium (IV), are used for the vast majority of analytical applications. When it comes to CL reagents used in liquid form, luminol is a popular choice (Lahuerta Zamora *et al.* 2001). Hydrogen peroxide is the most common oxidant, however permanganate, hypochlorite, or iodine may also be employed. Catalysts for this reaction are often transition-metal ions such as hexacyanoferrate (III) (II), which contains Fe (II), Mn (II), Co (II), Ni (II), and Cu (Ciriminna *et al.* 2016). The optimum pH for a chemical reaction is between 8 and 11. Using luminol or similar substances, this may be utilized to accurately identify the oxidant, catalyst, or species derivative (Barnett and Frances 2005).

2.5 Luminol Reaction

Figure 2.4 depicts some pivotal moments in the history of Luminol's development and use (Barni *et al.* 2007).

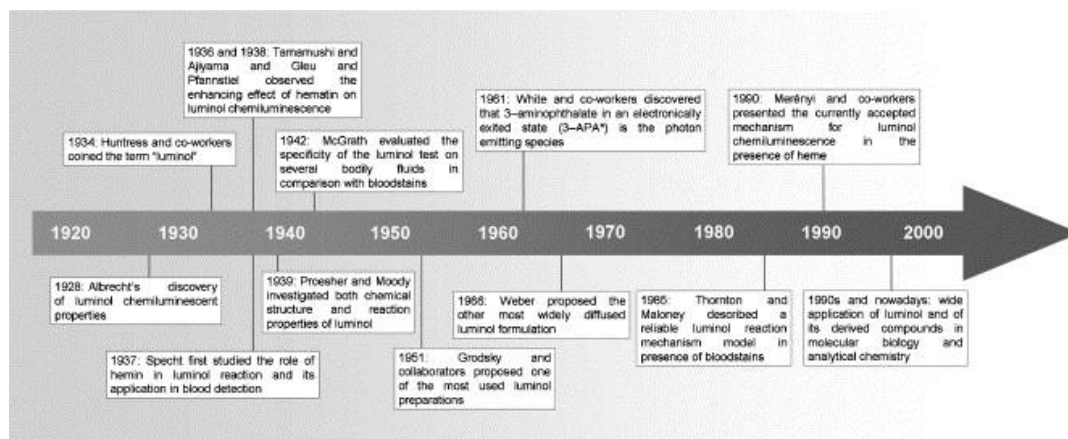


Figure 2.4 An account of the steps used to isolate luminol

Luminol was initially synthesized in 1908 by German chemist Schmitz, while there is significant controversy about who really reported its synthesis first (Barni *et al.* 2007, Wang *et al.* 2013). Albrecht may have been the first to report its role in CL reactions,

but this debate has since died down. Blood detection using hemin, an iron-containing protein generated from heme, was first investigated by forensic scientist Specht of the University Institute for Legal Medicine and Scientific Criminalistics in Jena, Germany. Liquid phase CL analysis was cutting edge technology. Research into the chemical composition and reaction properties of luminol led to accurate predictions of its keto-enolic tautomerization in alkaline solutions and its full protonated form in acidic solutions by Moody and Prosher (Ciscato *et al.* 2012). They got to the conclusion that even three years of old dry and deteriorated blood resulted in an increase in CL emission intensity and duration compared to fresh blood. They also discovered that bloodstains could be frequently cleaned with CL by misting Luminol solution over them, particularly if the bloodstains had dried. The luminol test was found to be highly specific for blood and rather insensitive to the other biological fluids studied by McGrath (1942).

Since these first investigations, many additional efforts have been made to clarify the reaction mechanism involving luminol, with a sizable effort by Mer'enyi and co-workers in the 1980s culminating in a summary publication in 1990. For analytical purposes, luminol is still the most popular chemiluminescent material. Despite extensive investigation, the precise mechanisms at work in many CL systems, both biological and non-biological, are still largely unknown. Undoubtedly the most well-known solution chemiluminescence reaction is the luminol reaction, which may be used to quantify a number of analytes (Iranifam 2013). However, permanganate and hypochlorite may also be used to oxidize luminol (3-aminophthalhydrazide) in aqueous alkaline conditions, producing 3-aminophthalate, nitrogen, and a bright blue-violet light (Nelstrop *et al.* 2001).

A thorough comprehension of the reaction mechanism may lead to the development of novel analytical CL systems, which may improve the CL response and the detection limits. Figure 2.5 shows the mechanism reaction of Luminol (Barni *et al.* 2007).

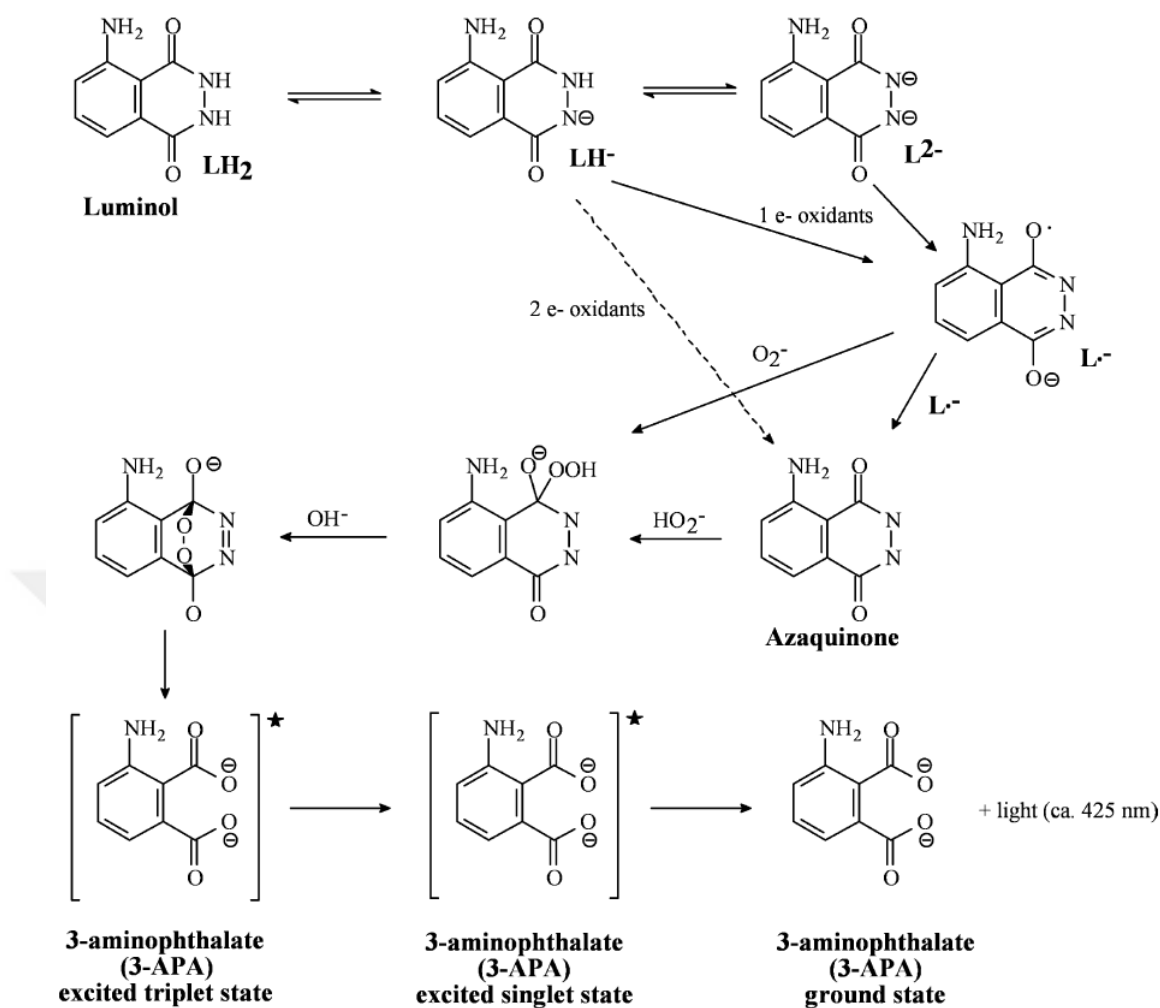


Figure 2.5 Reaction mechanism for luminol CL (Barni *et al.* 2007)

2.6 Application Chemistry

Chemical and biological sensing apps for smartphones are relatively new, but they've already made a wide range of investigations possible (Christodouleas *et al.* 2015). In order for smartphone technology to help chemists with chromatography, the image that is obtained must first be able to be measured. The key device of a smartphone is the camera. One approach is to use standard photographs that have been recorded with smartphone spectrophotometers that measure the absorption of colored liquids using the Beer-Lambert Law. Recent advancements in smartphone technology have spurred a great deal of study in the disciplines of chemical and biological sensing (Iqbal and Eriksson 2013).

Smartphones are high-tech cell phones with operating systems capable of handling complicated computing tasks, such as email, online browsing, and downloading and running mobile applications. As of October 2022, 6.64 billion smartphones are used worldwide. Google's Android operating system currently powers 71 percent of smartphones, followed by Apple's iOS (28%) and Microsoft's Windows (1%). Operating systems are used to categorize smartphones in general. There has been an increasing tendency in both industry and academia to create sensors that integrate with cell phones in order to take advantage of their portability, connection, usability, and pervasiveness. This is a result of applications' rising popularity and the free availability of application development tools (Bankmycell 2022).

2.6.1 Software-based smartphone sensors

One way to make a sensor based on a smartphone a reality is to find suitable software programs that leverage sensors that are already available in cell phones to take measurements. Numerous apps (available for download or purchase from stores like Google Play for Android or the App Store for Apple devices) may transform handheld sensors into convenient electronic devices. An application that monitors a user's heart rate and enables color image analysis using colorimetry or photometry has been developed by looking at the color channel. Spectrometers or test strip readers are just two examples of the devices used in the important chemical procedure known as chromatography. To keep track of processes, chemists typically utilize color, but they also need equipment to get more accurate results (Pelegri *et al.* 2010).

For use in telemedicine applications, (Martinez *et al.* 2008) have developed a program that lets users snap a picture of an assay and add notes on it. Then, doctors may use remote imaging technology to analyze the picture. A microsensor developed by (Delaney *et al.* 2011) measures chemical radiation using a paper chip and a smartphone camera. (López-Ruiz *et al.* 2012) created a portable sensor to measure oxygen levels in the air by using an illuminated membrane put over a smartphone camera.

The smartphone application algorithm for iOS (iOS) and Android was developed by

(Yetisen *et al.* 2014) in the Department of Chemical Engineering and Biotechnology, University of Cambridge, UK (Android). The software turns a smartphone into a reader that can accurately and consistently measure pH, protein, and glucose using commercial urine colorimetric tests. The results showed linear responses in the mg/dcL (5.0 - 9.0 and 0 - 100) and mg/dcL (0 - 300), respectively, ranges. Numerous colorimetric sensors based on smartphones have been used to evaluate the effectiveness of water treatment. (Copperwhite *et al.* 2011) created a UV dosimeter that takes color photographs of a colorimetric sensor substrate with a smartphone. Similar to this, by snapping color images of pH detector test strips, it has been developed a smartphone-based pH meter, (Sumriddetchkajorn *et al.* 2013) have created a program that visualizes a water sample blended with a chromatographic reagent to measure the amount of chlorine present.

2.6.2 Smartphone sensor attachments

Using specialized hardware add-ons that are physically attached to the phone is an alternative method of obtaining smartphone sensors. Building the building with the measuring equipment already installed and connected to a smartphone for remote control is a common practice. An oscilloscope, spectrum analyzer, and power meter, for instance, were recently released by Oscium Analyzers and can be used with an iPhone or iPad by connecting to the serial connection (Tonneau *et al.* 2015).

Sanofi's blood glucose monitors are another illustration of a sensor that may be connected to a smartphone via the serial port. pH meters were developed by both Sanofi and Sensorex. Nearly all mobile devices have a 3.5 mm audio input/output connection, which is also made to support a wide range of smartphone sensor attachments, including oximeters (Hudson *et al.* 2012).

The creation of smartphone sensor accessories has improved the scalability of components now used in smartphones (Daponte *et al.* 2013). For instance, by including additional external optical components, smartphone cameras may be considerably enhanced. A smartphone camera may be adapted into a handheld microscope, as shown by (Breslauer *et al.* 2009). The UCLA research group (Zhu *et al.* 2011) have created a

lens and optics that can be clamped together to make a high-performance microscope and flow cytometer (Coskun *et al.* 2013). The analytical tools that may be used with a mobile phone that are now on the market are shown in Figure 2.6.



Figure 2.6 Examples of commercially available mobile phone-based portable analytical equipment

A - the pH sensor with Bluetooth technology and the electrochemical portable glucose meter that utilizes a blood glucose test stripe and connects straight into the headphone jack to show results on the phone's screen.

2.7 Arduino

A variety of makerspace projects, both simple and complex, can employ an open-source programmable circuit board known as an Arduino. A microprocessor on this board may be intended to handle and recognize physical objects. The Arduino is capable of exchanging data with many other types of outputs, such as lights, motors, and screens, thanks to its ability to process data from sensors and inputs. Makers and makerspaces have flocked to Arduino due to the platform's versatility and low cost, making it ideal for developing interactive hardware projects. In 2005, Italian inventor Massimo Banzi created Arduino to provide people who aren't engineers a straightforward, low-cost way to build their own custom electronics.

The open-source board has been distributed under a Creative Commons license, allowing anybody to use it to create their own board. There are hundreds of Arduino - compatible clones and modifications available online, but only the official boards may be called Arduino (Zlatanov 2015). In figure 2.4, it was given the image of an original Arduino board.

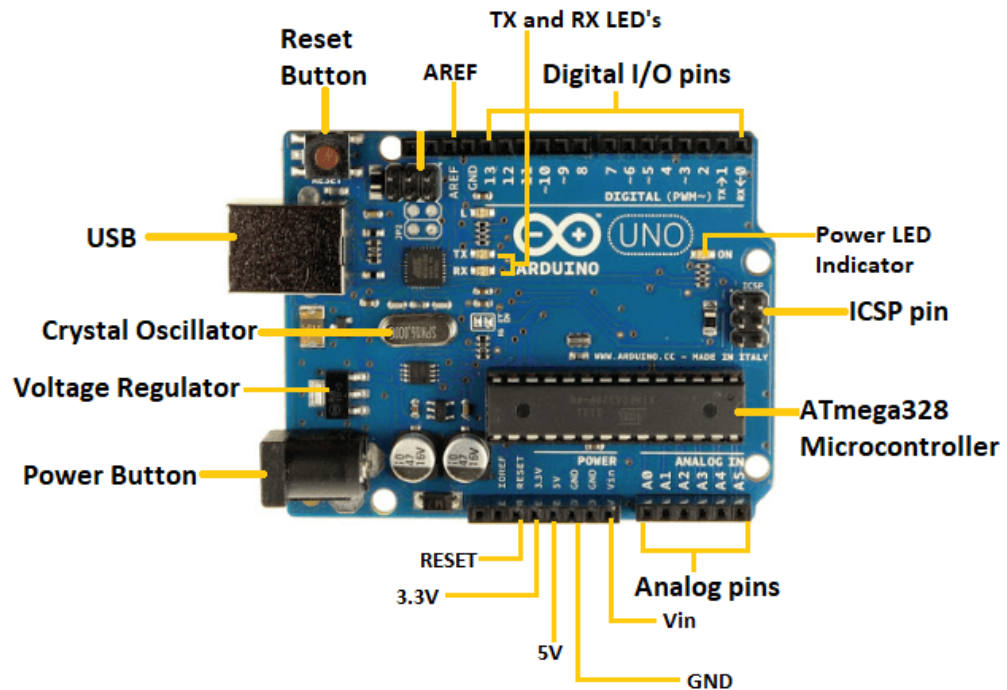


Figure 2.7 The components of Arduino board

Arduino is a platform that has developed a strong following among electronics enthusiasts (Mellis *et al.* 2007). Unlike the majority of earlier programmable circuit boards (UNO 2012), the Arduino's software uses a condensed form of C++, and there is no additional hardware required to upload new code to the board. Instead, you may do it with a USB cable (Stroustrup 1995). It also gives you a simpler environment that bypasses the microcontroller's features into a more user-friendly package, making programming easier to learn (Gibb 2010).

An Arduino Board (UNO 2012) can be classified into two parts: (1) Hardware: The Arduino board's (UNO 2012) hardware is made up of numerous parts that work

together to make it function. (2) The Arduino IDE (Boxall 2021): Software A set of instructions known as software tells the hardware what to do and how to accomplish it.

Components

1. Arduino Uno.
2. LED.
3. Speaker.
4. Infrared Proximity Sensor.
5. Breadboard.

The Uno is a widely used Arduino board. In spite of the fact that it wasn't the first board ever constructed, it has much more supporting materials and sees far more action than any other board. Due to the Arduino Uno's widespread adoption, there is an abundance of resources available online to assist you get going or get out of a bind. The Uno is quite popular because of its amazing features and ease of use (Kamruzzaman *et al.* 2011).

In order for Arduino to be able to feel its environment, a sensor must be added to it. There are many distinct kinds of sensors, and each one has a specific purpose. The list below includes some of the sensors that are most frequently used in projects (Khusainov *et al.* 2013).

1. Distance ranging sensor
2. PIR motion sensor
3. Light sensor
4. Degree of the flex sensor
5. Pressure sensor
6. Proximity sensor
7. Proximity sensor
8. Sound-detecting sensor
9. RGB and gesture sensor
10. Humidity and temperature sensor

2.7.1 Arduino in chemistry

Analytical chemists may now link their computers and lab equipment with ease and efficiency thanks to modern microcontroller boards. The abundance of training materials, appropriate sensors, and electromechanical devices makes it fun and empowering to learn how to utilize microcontrollers. Advantages of Arduino microcontroller boards for laboratory automation are discussed in the course materials. Moreover, it provides examples of lessons and activities that may be used to instruct students in their application throughout the study of analytical chemistry (Mabbott 2014). Simple spectrometer data acquisition and heat cycler management are two examples. Because of their adaptability, these instruments will soon become standard equipment for analytical chemists (Aricu *et al.* 2020).

There is a laboratory unit for introduction to electronics available for the undergraduate course in chemical instrumentation. Students assemble a functional photometer out of affordable electronic components. The students wire the photometer up to a microcontroller and write Arduino code to process the signal, determine the absorbance, and output the results on a liquid crystal display (LCD). In this lab, students measure hexavalent chromium levels in standard solutions using instruments they designed and constructed themselves; they then calculate the instrument's sensitivity, detection limit, and dynamic range. They also used their equipment to calculate the relative amounts of Keeping an eye on the temperature throughout a reaction or physical process is a must in many experimental thermochemical laboratories. There are already other options available to meet this need, but they are too costly for most high school and introductory college students. As a result, a lot of papers talk about an affordable and useful data collection tool built with open-source Arduino software. A number of these pieces detailed how to assemble an Arduino-based calorimeter, a device often used in chemistry labs to measure heat. In order to determine the exothermic dissolving heat (H) for NaOH in water and hexavalent chromium in an unnamed water sample, the team employed an LM35 transistor as a temperature sensor paired to an Arduino UNO microcontroller for temperature detection during testing (Vallejo *et al.* 2020).

In our daily work, we regularly need demonstrative measuring equipment with displays that are large enough to be seen from all sides of the classroom (thermometers, pH meters, etc.). There are works that show off some of the Arduino platform's educational applications. This microcontroller board can be used to quickly and cheaply create measurement devices for student-run chemistry experiments and presentations. ChemDuino is an initiative to enhance chemistry education via the use of Arduino hardware and software (including Wiring and OneWire). It is a combination of the words "Chemistry" and "Arduino," (Papadimitropoulos *et al.* 2021).

The hardware needed to construct various instruments has been previously reported: a pressure gauge, a pH meter, and thermometers with ranges from -80 to 120 degrees Celsius and from 200 to 1400 degrees Celsius (Kubínová and Šlégr 2015).

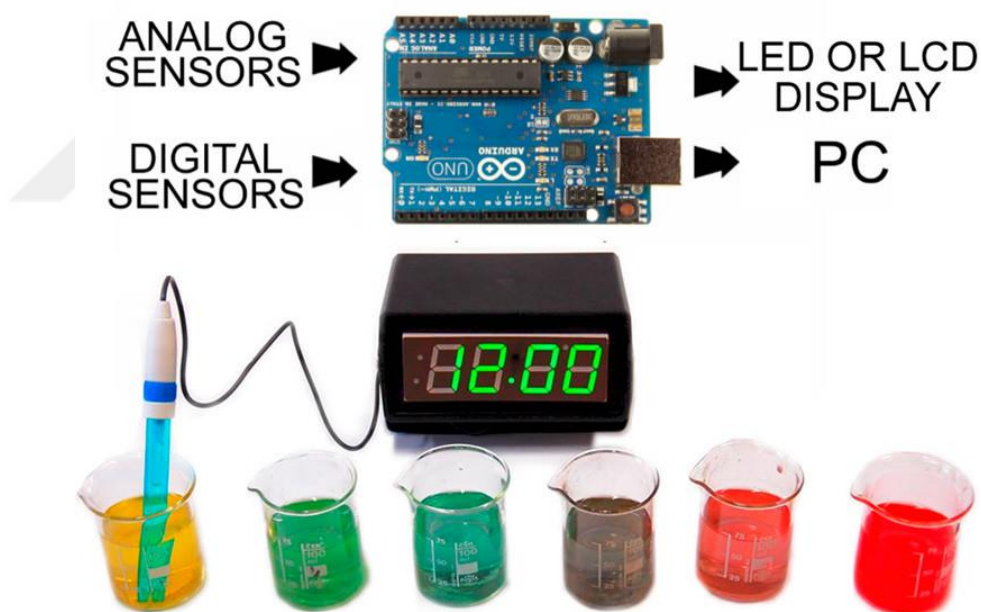


Figure 2.8 Arduino in chemistry as pH meter with LED display of pH values (Kubínová and Šlégr 2015)

2.8 Bioluminescence

Enzyme electrodes were created in 1962 by scientist Leland C. Clark, ushering in a new era of biosensors. Since then, experiment communities from a variety of fields, such as

physics, chemistry, and biotechnology, have created trustworthy and advanced biosensing systems for application in agriculture, medicine, and defense as well as the identification and avoidance of bioterrorism (D'souza 2001).

A biosensor is an instrument that has a transducer and a bioreceptor as its main parts. Bioreceptors may be any kind of biological material, such as cells, tissues, bacteria, organelles, cell receptors, enzymes, antibodies, nucleic acids, etc., and they are responsible for selecting the analyte of interest. To convert the detecting event into a measurable signal, a physicochemical detector will use a transducer. The performance of a biosensor is dependent on the biochemical specificity of the biologically active material. Vital factors include the material's specificity, storage, operability, and environmental stability. Applications for biosensors can be found in the biomedical field, business, and even the military. Due to the huge commercial potential, blood glucose sensing has been the main application so far (Girigoswami and Akhtar 2019).

Biomolecules such as enzymes, antibodies, receptors, organelles, and microorganisms have been used in biological sensing, alongside animal and plant cells and tissues. Microbes have been integrated with amperometric, potentiometric, calorimetric, conductimetric, colorimetric, luminescent, and fluorescent transducers to construct biosensor devices. There are definite benefits to using chemiluminescence (CL) as a detecting mechanism for creating biosensors including a quick test time and great sensitivity. For a variety of substrates, the CL detection limit is in the region of picomoles or nanomoles (Zauner *et al.* 2011).

Typically, enzymes found in biosensors are employed in an immobilized form on various solid substrates. Although the precise species of CL that produce light has not yet been determined, superoxide and H_2O_2 have been shown to interact preferentially with luminol. A class of heme-containing enzymes known as peroxidases catalyzes processes utilizing H_2O_2 as the oxidant. Mammalian tissues and some plants have these enzymes. These behaviors imply that peroxidases may serve as cellular activators; these enzymes may be produced by other cells in the body (Verma and Kumar 2019).

Living microbes emitting light is known as “bioluminescence.” The lux gene that controls luminescence in bacteria has been utilized as a reporter in a wide variety of research, both in an inducible and constitutive capacity. An inducible promoter controls expression of the lux reporter gene in response to changes in the concentration of an interest chemical. Therefore, the component concentration may be quantified by measuring the bioluminescence's intensity. Constant, constitutive expression of the reporter gene is ensured by its connection to promoters that are functional as long as the organism is alive and undergoing metabolic activity (Lei *et al.* 2006).

It has been demonstrated that both kinds of reporters are helpful for the creation of biosensors. In the study, a novel bioluminescence method for the enzymatic detection of glycerol following lipoprotein lipase hydrolysis was developed to assess triglycerides (Close *et al.* 2010).

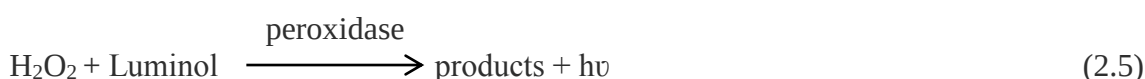
2.9 Chemiluminescence Enzyme-Based Biosensors

Chemiluminescence (CL) is a method for enzyme-based optical biosensing in which a chemical reaction results in the emission of light (luminescence). Based on the properties of peroxidase-mediated oxidation of Luminol by H₂O₂, Seitz and his colleagues developed an H₂O₂ biosensor for use in alkaline solution. The following Equation (2.2) and Equation (2.3) were typical CL-related reactions that were seen (Choi 2004).



A biosensor's CL reaction was generated with the combination of Luminol and peroxidase. As a peroxidase, HRP may catalyze the reaction of hydrogen peroxide and the chemiluminescent reagent to generate CL. G-quadruplex-hemin DNAzyme with hemin in certain locations might also operate as a peroxidase in enzyme-based

chemiluminescence biosensors. Additionally, oxidases were added as direct H_2O_2 transducers or exogenous H_2O_2 producers for additional chemiluminescence detection. The following Equation (2.4) and Equation (2.5) illustrate how linked enzyme reaction systems work (Sun *et al.* 2019).



However, ways for obtaining bioanalysis by quenching chemiluminescence were also presented. Nonradioactive ET is used in chemiluminescence resonance energy transfer (CRET) to transmit energy between two nearby molecules (often within 10 nm of each other). The process of CRET involves the oxidation of the fluorescent acceptor, such as graphene or organic dyes, which subsequently stimulates the chemiluminescent donor (Deshpande 2001).

Immobilized enzyme CL flow-through sensors are widely utilized nowadays. Immobilizing fungal peroxidase in silica and graphite pastes (Janasek and Spohn 2000) aided in the synthesis of CL. Glucose oxidase and lactate oxidase are immobilized in a poly-(carbamoylsulfonate) hydrogel that is adsorbed on the graphite paste modified by FRP, allowing for rapid and highly sensitive detection of oxidase substrates. Using an enzyme-functionalized column or a flow cell directly coated with HRP, similar flow-through CL biosensors allow CL production from reactions between injected samples and reagents in the flowing carrier solution before it reaches the detector. As reported in (Marquette and Blum 2000), the first CL biosensor was developed by directly tagging anti-2,4-D polyclonal antibodies with HRP to enable p-iodophenol-enhanced chemiluminescent detection.

As can be seen in Figure 2.9, a homogeneous chemiluminescent immunoassay approach was described by (Akhavan-Tafti *et al.* 2013) by using particular binding compositions indicated by an acridan-based chemiluminescent material and HRP, respectively. When

a trigger solution is added, the chemiluminescent chemical and the HRP are brought into close contact, resulting in the creation of a chemiluminescent signal without the need of intermediate separation steps. (Lee *et al.* 2012) developed a graphene-based CRET biosensor using a CRET platform, and it was used to assess C-reactive protein.

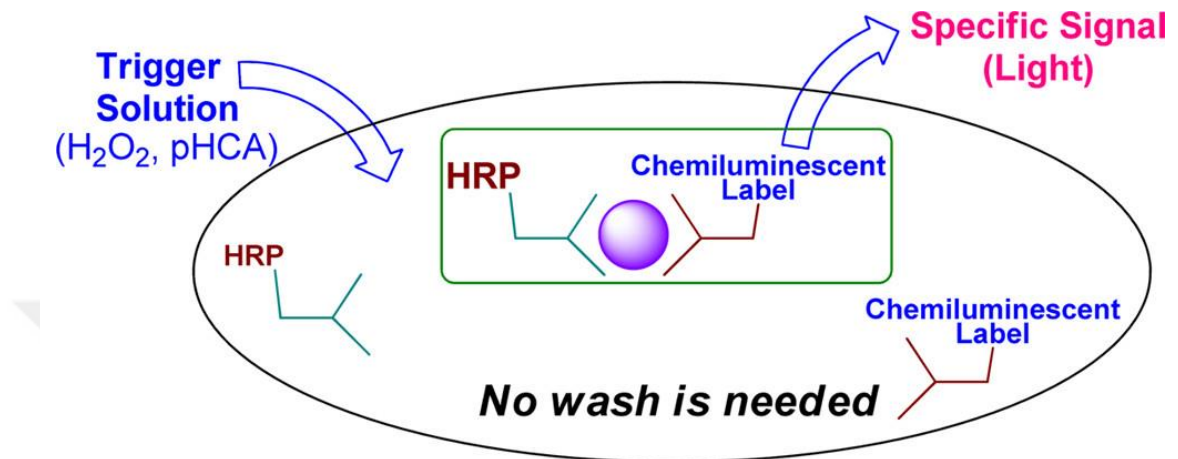


Figure 2.9 Diagram depicting the homogenous chemiluminescent immunoassay procedure (Akhavan-Tafti *et al.* 2013)

A homogeneous DNA probe and DNAzyme structure have been created inside the same DNA scaffold (Zhang *et al.* 2021, Bi *et al.* 2015) created a cascaded-CRET method for Luminol-H₂O₂-HRP that utilized a similar idea to DNAzyme-catalyzed Luminol-H₂O₂. When a hairpin probe structure recognizes a target, the architecture of the probe is in an “open” shape, turning on the CRET signal.

Thalassemias are a heterogeneous group of inherited disorders caused by a deficiency in either the alpha or beta chains of hemoglobin synthesis (Hb). Hemoglobin is the protein found in red blood cells responsible for transporting oxygen across the body. This material consists of two proteins, called alpha and beta. The formation of red blood cells is compromised if the body does not produce enough of either of these two proteins which results in anemia that manifests in infancy and persists throughout adulthood. Since thalassemia is an inherited condition, at least one parent must carry the disease’s gene. It results from either a genetic mutation or the loss of specific important gene segments (Carsote *et al.* 2022).

Caused by a loss of the gene encoding the alpha-globin chain, alpha thalassemia results in abnormally low or nonexistent synthesis of alpha-globin chains. There are four variations of the alpha globin gene, and the severity of the illness ranges from moderate to severe depending on how many deletions there are. The most severe variant, four allele deletion, results in no alpha globin production and the formation of tetramers from extra gamma chains that were present throughout fetal development. It causes hydrops fetalis and is incompatible with life. The smallest and most clinically silent variant is one allele deletion.

The beta-globin gene experiences point mutations that cause beta-thalassemia. The beta-gene mutation is divided into three subtypes based on its zygosity. The underproduction of beta chains is the result of a heterozygous mutation, and this kind of thalassemia is known as beta-thalassemia minor. It seldom causes any noticeable symptoms and is rather modest. Complete lack of beta chains (beta-zero thalassemia) is the outcome of a homozygous mutation in the beta-globin gene, which is the cause of beta thalassemia major. Its clinical manifestations include, but are not limited to, jaundice, growth retardation, hepatosplenomegaly, endocrine disorders, and severe anemia requiring frequent blood transfusions. Between these two extremes is a spectrum of sickness known as beta-thalassemia intermedium, characterized by minimal to moderate clinical manifestations. These two variants are referred classified as intermediate beta-thalassemia and have mild to moderate clinical symptoms (Ahmadpanah *et al.* 2019).

Here are two examples of genes that have been mutated, both of which may cause moderate to severe disease symptoms: This condition is called thalassemia major, or more often Cooley anemia. Most infants born with two mutated beta-hemoglobin genes are healthy at birth; however, moderate to severe symptoms don't appear until six months later, when adult hemoglobin (Hb) replaces fetal hemoglobin (Hb-gamma). This disorder is called thalassemia major or Cooley anemia. Infants with two mutant beta hemoglobin genes are often healthy at birth, but begin to have symptoms around the age of six months when adult hemoglobin (Hb) replaces fetal hemoglobin (Hb-gamma). Because the alpha-beta chain imbalance is not as severe, persons who inherit both forms of thalassemia have a milder clinical course than those who inherit just beta thalassemia.

Sickle cell trait coexistence: Beta-thalassemia is a major hemoglobinopathy that causes sickle cell disease when present with sickle cell trait. Contrary to the sickle cell trait, where HbA is the primary Hb, the major Hb in the co-existence condition is HbS, which can make up more than 60% of Hb depending on the kind of sickness (beta-zero or beta-plus0) (Carsote *et al.* 2022).

Another frequent Hb variation in Southeast Asian people is (HbE). Given that individuals with thalassemia in this region are frequently found to have HbE, there is an association between it and a beta-thalassemia phenotype.

2.9.1 Etiology

Since thalassemia is autosomal recessive, it can only be passed on to offspring if both parents have the disease or are carriers. It's brought on by alterations to the DNA code, such deletions or mutations. Increasingly common in clinical settings are two new terms describing the Hb genes that cause insufficient transfusion-required and non-transfusion-required thalassemias. All basic classifications fall into one of these two categories depending on whether or not they require regular blood transfusions. Over 200 mutations have been found to be responsible for thalassemias. Thalassemia is caused by deletions in the alpha-globin gene or by point mutations in the splicing site and promoter region of the beta-globin gene on chromosome 11 (Ahmadpanah *et al.* 2019).

2.9.2 Epidemiology

Worldwide, the Mediterranean region, the Middle East, the Indian subcontinent, and Southeast Asia have the highest rates of thalassemia (Li 2017). The southern Chinese provinces of Guangdong, Guangxi, Fujian, Yunnan, Guizhou, and Sichuan have the highest prevalence of this inherited blood disorder (Xu *et al.* 2004, Li *et al.* 2014, Yin *et al.* 2014). Thalassemias are very common in the Chinese province of Fujian, which situated near the country's southeast coast (Xu *et al.* 2013). However, prenatal diagnosis

and genetic counseling are significantly hampered by the absence of a systematic investigation of the incidence and molecular characteristics of thalassemia and hemoglobinopathy in this region. Therefore, the present in-depth investigation was designed to detect thalassemia gene mutations and describe the genotypes of unusual thalassemias and hemoglobinopathies in Fujian Province, Southeast China during a 10-year period (2008-2017). Pregnant women in the region might benefit from genetic counseling and prenatal hemoglobinopathy and thalassemia diagnosis if such a study were to be conducted, since it could help reduce the number of babies born with severe thalassemia (Lai *et al.* 2017).

2.9.3 History and physical

The symptoms of thalassemia might vary widely from one case to the next. There may be clues revealed by a comprehensive history and physical examination that the patient isn't aware of (de Montalembert *et al.* 2017).

2.9.3.1 Skin

Anemia can cause the skin to become pale, and intravascular hemolysis can cause hyperbilirubinemia, which can cause jaundice. Patients typically list anemia-related fatigue as their initial presenting symptom. Examination of the extremities may reveal ulcerations. Multiple transfusions can cause chronic iron deposition, which can lead to golden (Wang *et al.* 2017).

2.9.3.2 Musculoskeletal

Before the introduction of chelation therapy, a unique osteoarthropathy manifested in hypertransfused individuals. Hemosiderosis has been linked to anomalies in the synovium and cartilage, which are likely brought on by an excess of iron, an aberrant calcium metabolism, a deficiency in vitamin D, and hyperuricemia. Over the following ten years, a new general pattern of skeletal alterations evolved with the introduction of

iron-chelation therapy in the late 1970s (De Virgillis *et al.* 1988). Iron chelator medications may harm bones and joints, resulting in dysplastic abnormalities in the spine and long bone metaphyses, growth retardation, and arthropathy. Numerous mechanisms, such as aberrant metaphyseal collagen formation, osteoblast inhibition, a direct toxic effect, and the loss of minerals other than iron, could be at play (e.g., copper). Nowadays, post-chelation dysplastic bone characteristics are more frequently observed than those brought on by marrow growth or extramedullary hemopoiesis (Chan *et al.* 2002). The literature, therefore, advises thorough observation of the potentially harmful consequences of chelation therapy, highlighting the dangers of chelation over-treatment and potential individual idiosyncrasies (De Sanctis *et al.* 2018).

2.9.3.3 Cardiac

Chronic transfusions can lead to iron accumulation in cardiac myocytes, which can throw off the heart's rhythm and cause a variety of arrhythmias. Chronic anemia can also cause overt heart failure (Iqbal *et al.* 2018).

2.9.3.4 Abdominal

Bilirubin gall stones may form as a result of chronic hyperbilirubinemia, which also causes the classic colicky pain of cholelithiasis. Chronic iron buildup in these organs, as well as extramedullary hematopoiesis, can cause hepatosplenomegaly. Chronic hemolysis brought on by improperly controlled hematopoiesis leads to splenic infarcts or autophagy (Pilo and Angelucci 2019).

2.9.3.5 Hepatic

Hepatic involvement is common in thalassemia patients, in large part because of their constant need for transfusions. Cirrhosis and chronic liver failure may be caused by iron poisoning or transfusion-associated viral hepatitis (Anurathapan *et al.* 2019).

2.9.3.6 Slow growth rates

A child's ability to grow might be hampered by anemia, and thalassemia can postpone puberty. The focus should be given to the child's age-appropriate growth and development (Anurathapan *et al.* 2019).

2.9.3.7 Endocrinopathies

An excessive amount of iron can cause the body's organ systems to deposit iron, which impairs their ability to operate. Iron accumulation in the thyroid, parathyroid, or pancreas can cause diabetes mellitus, hypothyroidism, or hypoparathyroidism, respectively. Chronic arthropathies are brought on by the deposits in the joints. The substantia nigra is a region of the brain where iron accumulates and which has been linked to Parkinson's disease and other physical health problems in people who have had no previous exposure to the condition. Hemochromatosis is characterized by a wide variety of symptoms (Pilo and Angelucci 2019).

2.9.3.8 Therapeutic options for thalassemia

Routine blood transfusions and iron chelating drugs, pharmacological activation of the β -globin gene, allogeneic transplantation, and single-dose cures utilizing gene therapy that do not need immunosuppression are all conventional therapies for thalassemia (de Dreuzy *et al.* 2016).

Regular transfusion causes the body to become overloaded with iron, which negatively impacts the heart and liver. To reduce the severity of thalassemia, many pharmaceutical medicines that increase HbF are employed. Hydroxyurea is an effective and inexpensive choice that restores normal function to several signaling pathways, increasing HbF production and decreasing the requirement for blood transfusions in patients with sickle cell disease (de Montalembert *et al.* 2017).

Thalassemia allogenic (hemopoietic cell) transplantation is a potential treatment; nevertheless, there are a number of obstacles that impede its use: Blood-forming stem-cell transplantation (HSCT) Lack of Human Leukocyte Antigen (HLA)-identical donors, graft rejection, and iron toxicity are all potential causes of transplant rejection or failure (Wang *et al.* 2017).

The most recent technique of treatment without mortality, graft rejection or clonal dominance problems is lentiglobin gene therapy. However, some patients have experienced delayed platelet engraftment (Iqbal *et al.* 2018).

Each of the aforementioned steps is crucial to the advancement of science and technology and offers its own unique benefits. In this research, we focused on a subset of luminescence known as CL by analyzing a CL-specific reagent and a data-displaying device.

3. MATERIALS AND METHODS

3.1 Materials

The chemicals and reagents, instruments, procedures, and how a homemade apparatus was developed to employ them in determined optimal conditions were all explained in this chapter.

3.1.1 Chemicals and reagents

In this experiment, only high-quality, analytical-grade substances were employed. In this study, deionized water with a conductivity of less than 0.2 S was also employed, and tests were carried out between 25 °C and 30 °C.

An exact 1 liter of a 0.1 M Na_2CO_3 (Merck) buffer solution was made by dissolving 10.50 grams of Na_2CO_3 and 20 grams of 0.5M NaOH.

We made a 0.02 M solution of Luminol (5-amino-2,3-dihydro-1,4 phthalazinedione) (Sigma-Aldrich, St. Louis, MO, USA) by dissolving 0.3544 g of Luminol (97%) in 100 mL of 0.1M Na_2CO_3 solution (the buffer) (pH 10).

0.1 M Potassium ferric cyanide, were taken 0.6580 g of potassium ferric cyanide and put it in a clean beaker that has non-ionic water and it is completely dissolved, it was put it in a container of 20 mL and complete the package and thus the material was prepared. Potassium ferric cyanide is a catalyst.

0.5 M hydrogen peroxide was made by diluting 30 mL of 30% hydrogen peroxide ($d=1.13 \text{ g/mL}$) with 250 mL of deionized water (Sigma, St. Louis, MO, USA).

3.1.2 Instrumentation

Two different measurement techniques were applied in this study. The first is a unique computer-based direct system for CL sensing, and the second is a novel mobile phone detector for direct CL systems. The Ministry of Science and Technology's Chemistry Department laboratory and research laboratory worked together to design and build the two systems.

3.1.3 Patch L system (Di-CL)

A homemade CL sensing system is shown in Figure (3.1), and an overview of the detector and CL connection devices that were employed in this work is shown in Figure (3.2). The analyzing signal was sent to the mobile phone using an Arduino board and Bluetooth technology. The sample was injected into the reaction tube using a syringe, and the photocell detected the visible light emitted from the reaction vessel. It then converted this light into a signal using an Arduino, sent it to a mobile device via Bluetooth, and provided signal data. Mobile devices captured the CL intensity as the peak height (Huawei Y7 prim). In the laboratory, a homemade system was created, and a test tube (10 mm) served as the reaction vessel.

3.2 Methods

In this technique, 0.1M luminol solution was placed in a test tube (1 mL). 1 mL is the sample volume of hydrogen peroxide injected into the injection valve and into the test tube. Depth of about 2 cm the reaction vassal was inserted in the specific holder in our device before injecting the potassium ferric cyanide (the oxidant) and the next step the potassium ferric cyanide was injected after that the light-emitting was measured by Mobile-Phone Di-CL system. The light intensity is the CL resulting from the interaction between Luminol with potassium ferric cyanide. An amplifier (10Ω) was used through a selector, attached to Arduino to control the sensitivity of the device for light intensity. If we used a computer the results were read as digital data with an Excel program. The

recorder height peak on the mobile screen is related to concentration.

3.2.1 Mobile-phone and PC as novel detector for batch CL system (Mobile-phone Di-CL)

The DIY Di-CL system using a mobile phone as a detector is depicted in Figure 3.1. The detector was an i-mobile HUAWEI - Y-7 prime -LDN-L21 smart phone (Support Web Browsing Android HTML Web kit) running Google Android version 8.0.0. An excellent platform and Android, which are simple to work with and create for, make up the hardware of mobile devices.

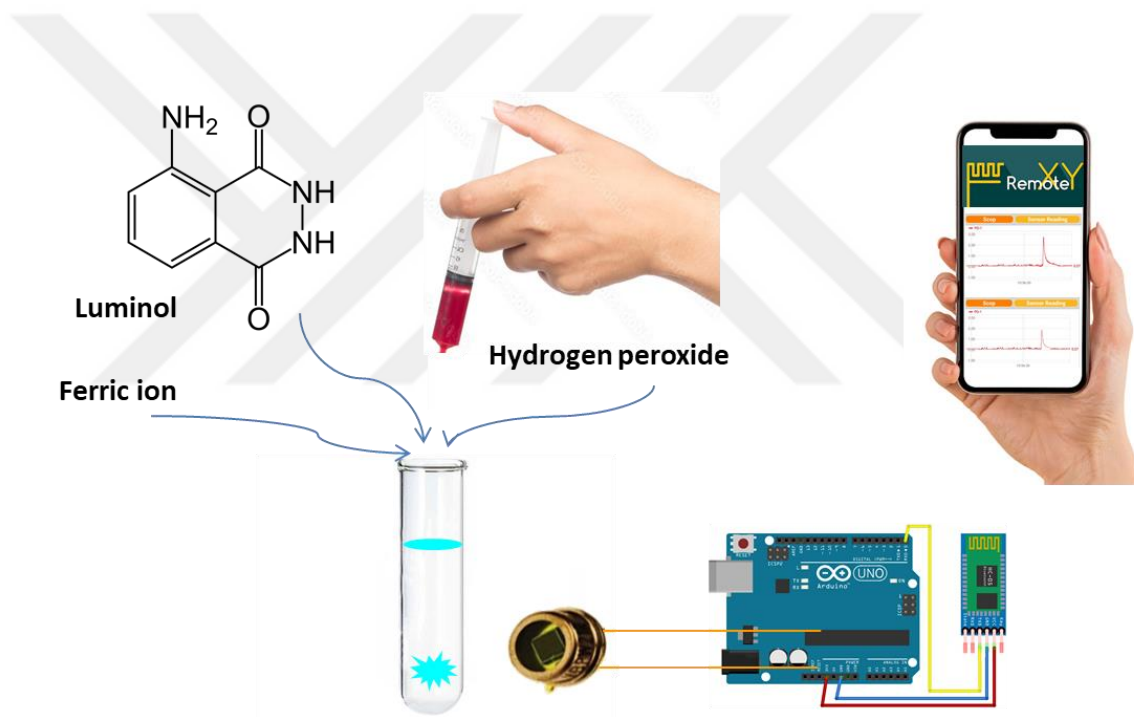


Figure 3.1 A home-made CL sensing system

3.2.2 Controlling arduino board with smartphone and remoteXYFree application via internet

This study is the first to examine the RemoteXYFree application as a data receiver from an Arduino board. RemoteXYFree enables us to develop programs that may be used to operate Arduino boards linked to internet-connected PCs from any location in the globe,

such as controlling servos, receiving data, etc., using a smartphone. It is among the most fascinating actions. When there is no internet access and close proximity, the connection can alternatively be made using a Bluetooth-enabled smartphone and Arduino board. RemoteXYFree, which offers us the dashboard and connectivity to Arduino, may be downloaded from the Google Play Store (for Android) or the App Store (for Apple) (it is virtual connectivity). Push and drag widgets to create programs in RemoteXYFree; an standard Arduino board without an internet shield linked to a smartphone and a computer with an internet connection can be used for this project. The PC's function is to make sure the Arduino board is connected to the internet and to upload the Arduino code. Installation of the RemoteXYFree library and the necessary configurations on the computer are required for this purpose. The Arduino scripts used by RemoteXYFree have a similar structure to regular Arduino codes but include particular components to interact with Android smartphones. The researcher provides a straightforward example that was slightly modified and borrowed from the internet. It can be found that writing new codes is practically identical to writing code for a standard Arduino.

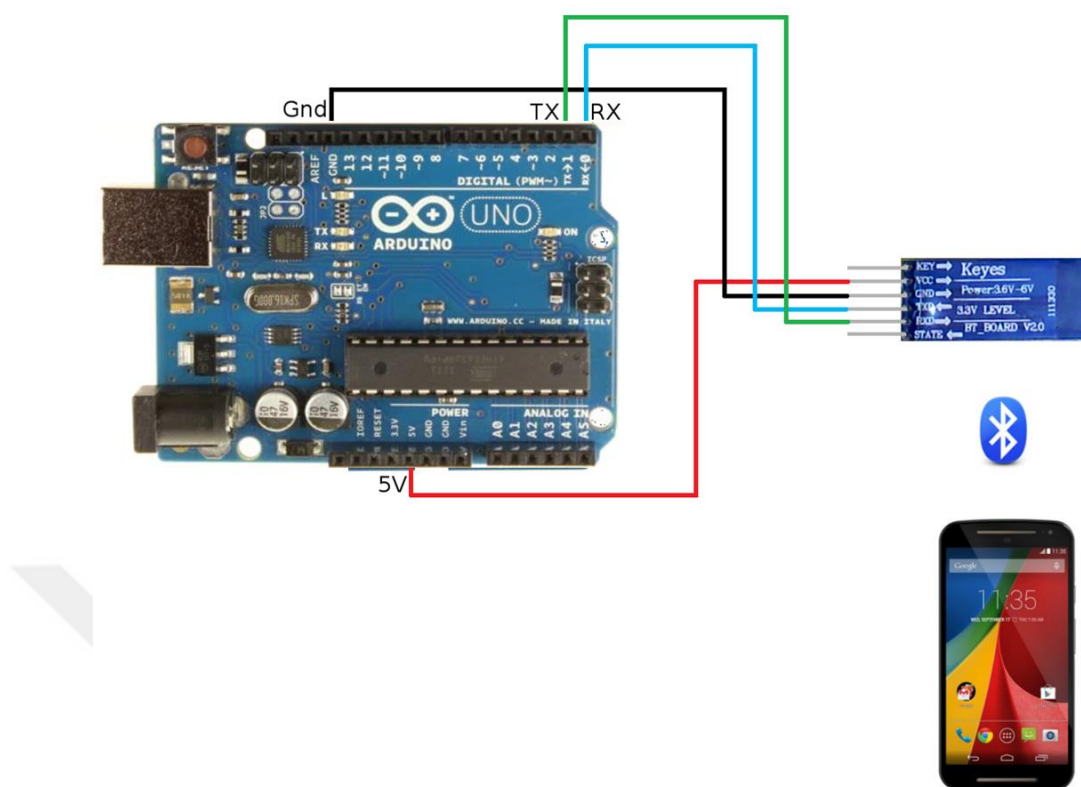


Figure 3.2 Schematic diagram of the Bluetooth device

Additionally, it can utilize a Bluetooth module with Arduino and RemoteXYFree for our project to communicate data between devices using Bluetooth (between smart phone and an Arduino board).

3.2.3 The optimization of experimental conditions of methods

The chemical variables, such as the concentration of reagents and oxidant employed for the CL reaction and some other factors, were studied in a series of tests to determine the conditions for the creation of maximal CL emission. Physical factors such as reagent and sample volume were investigated.

3.2.4 Calibration curve of methods

Working and standard solutions were created by serially diluting the stock solution with varied ranges to discover the standard curve and define the detection limit. Additional

factors for the measurement of potassium ferric cyanide were repeatability, relative standard deviation, accuracy, recoveries, and regression coefficient.

3.2.5 Determination of Ferric(III) Ba-CL methods

Standard ferric solutions were prepared by being weighted and transferred its amount to a beaker after dissolved transfer to a 250 mL graduated flask as stock solution, making up to the mark.

3.2.6 Thalassemia tests

The homemade device was used to determine Iron inpatient with thalassemia. Blood samples were taken from patients with Thalassemia (25 samples) and it was compared the results obtained from the device manufactured by us with the method adopted in the laboratories (photometric methods).

4. RESULTS AND DISCUSSION

4.1 Design of Device CL System Using a Mobile Phone as a Detector

The most crucial and novel part of this study is to use the mobile phone as a detector for CL with a combination direct reaction vessel. This system not existed in our advanced analytical laboratory. Therefore it can consider it as a construct for a semi-automated mobile phone as a detector for a batch-CL system to be used as a simple, easy-to-work, inexpensive spectrophotometer and reliable for the result described in this study. Figure 4.1 shows the homemade semi-automated mobile phone for batch- CL. All parts of this system were equipped from the local market. The box Figure 4.1, which was made manually, is containing all parts system which is contributing to CL sensing, conserves all sections of the device, and prevents external light to enter the box so that no noise through made inside it is black.



Figure 4.1 The image of the Arduino-based CL measuring device

More of these homemade instruments are currently in use throughout the world than any other single Spectrophotometer model. It's adapted by researchers to be a CL detector for many assessments through some changes such as removing the source, monochromatic, and can change the sensitivity by operational amplifiers or other variations as in this work Figure 4.2. A systematic connection begins with a photocell to end with a mobile as a detector.

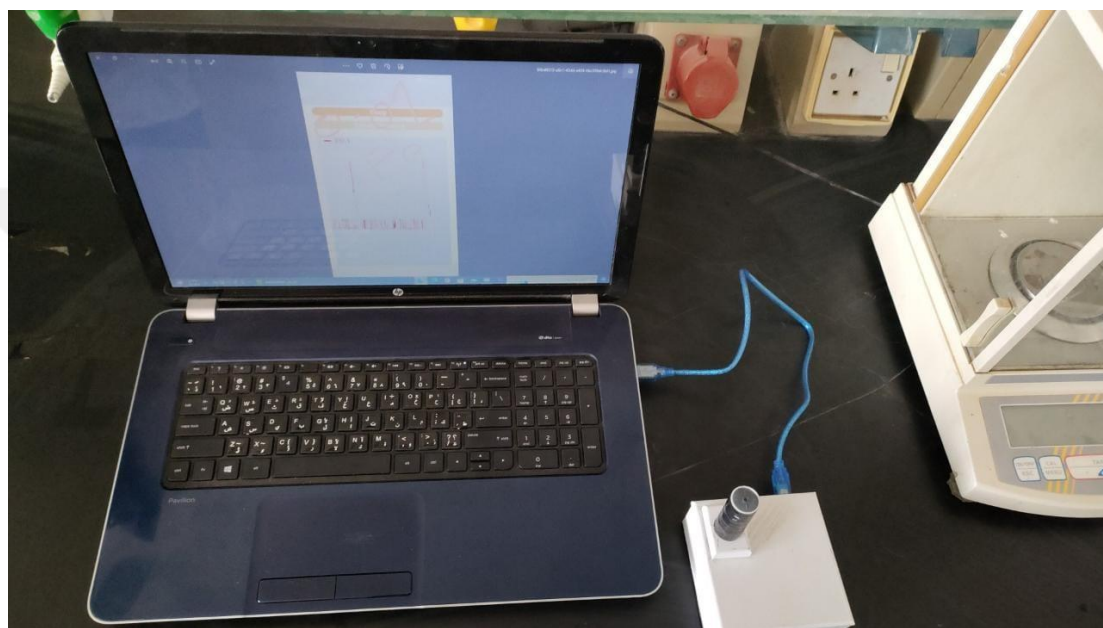


Figure 4.2 The general scheme of CL system fabricated in this study

It can be explained the detailed design of the system using the captured pictures of each part which it is starting in the holder of the reaction vessel to end in the mobile phone with high sensitivity and really stable baseline. In this system, a syringe was used to propel 10⁻² M luminol and then the oxidant into reacting tube, which it held in its vessel holder.

One kind of light sensor is an LDR (light-dependent resistor), a resistor whose resistance varies with the intensity of the light shining on it. To function, an LDR takes use of the semiconductor's photoconductivity, which allows electrons to move more freely when struck by light (energy of photons). The Advanced Photonix PDV-P5002 illustrated in is a good example of a light-dependent resistor Figure 4.2. This photocell's

resistance ranges from about 500 k in the dark to around 10 k in high light. Figure 4.3 shows a simple circuit to LDR illustrating how the PDV-P5002 can be used as an ambient light sensor that feeds either a digital or analog input to the PIC32. This is because the PDV-P5002 is sensitive to light in the wavelength range of 370 to 700 nm, which is roughly the same range to which the human eye is responsive.

An LDR is connected to an amplifier with one rode and to the Arduino with the second rode of another side. The role of the amplifier is to magnitude the signal produced as a result of light emission from the reaction holder Figure 4.3.

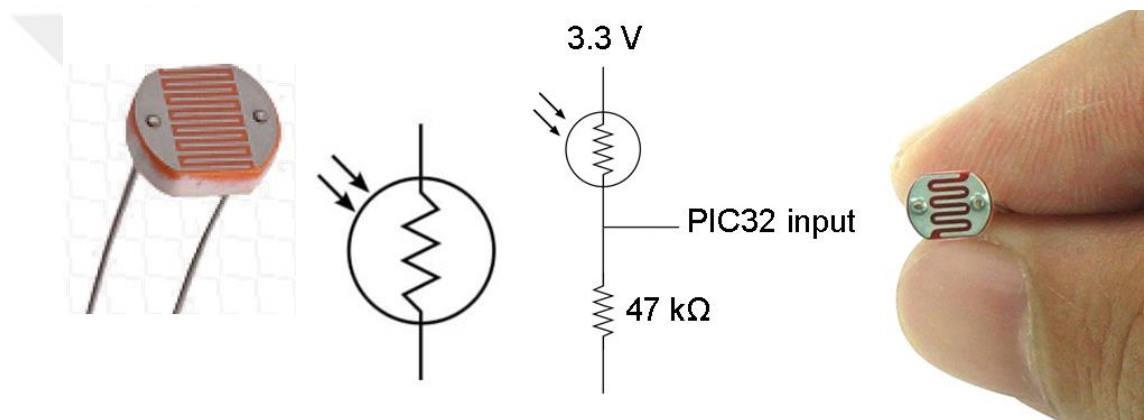


Figure 4.3 LDR and its sample circuit

The amplifier acts to amplify the analytical signal for light that emits from the CL vessel, and glass tubing, Figure 4.4 and convert this signal to Arduino. Arduino device Figure 4.5 has a role to transfer the signal (as wireless signal) to mobile through Bluetooth device Figure 4.6 which attach to it.

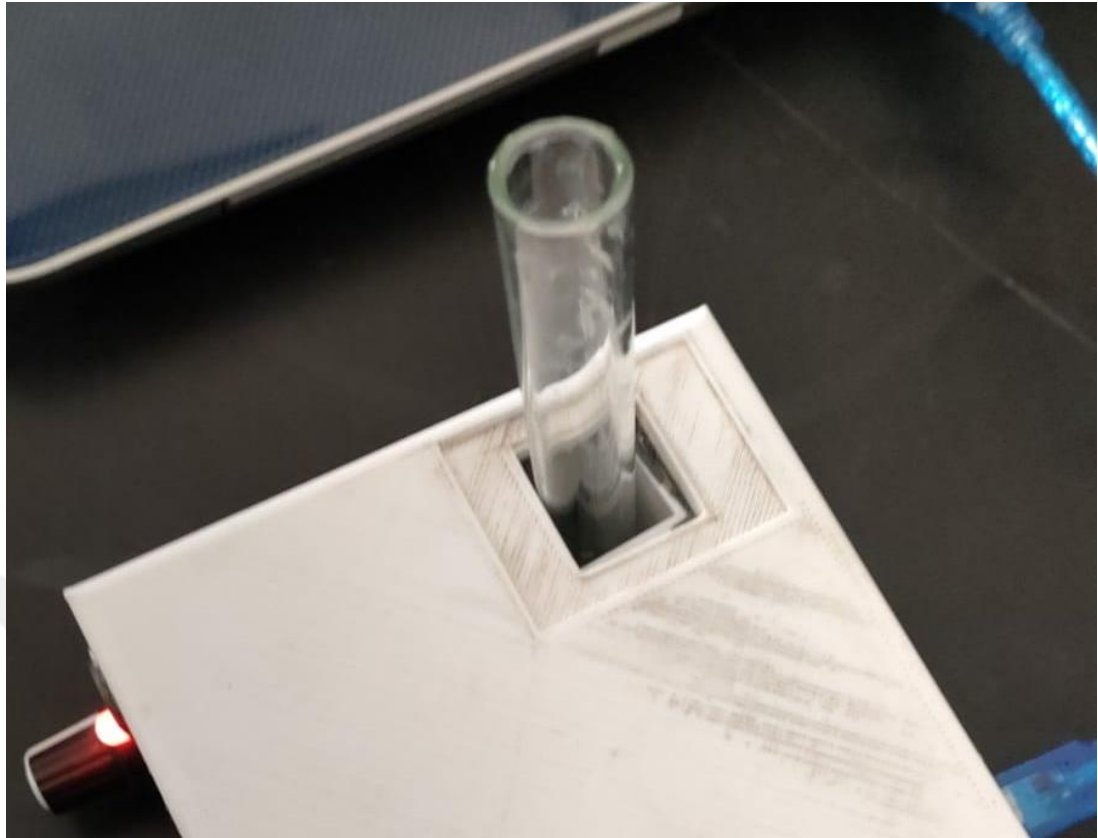


Figure 4.4 Loading of measurement tube on CL measurement device



Figure 4.5 The image of Arduino board used in this study

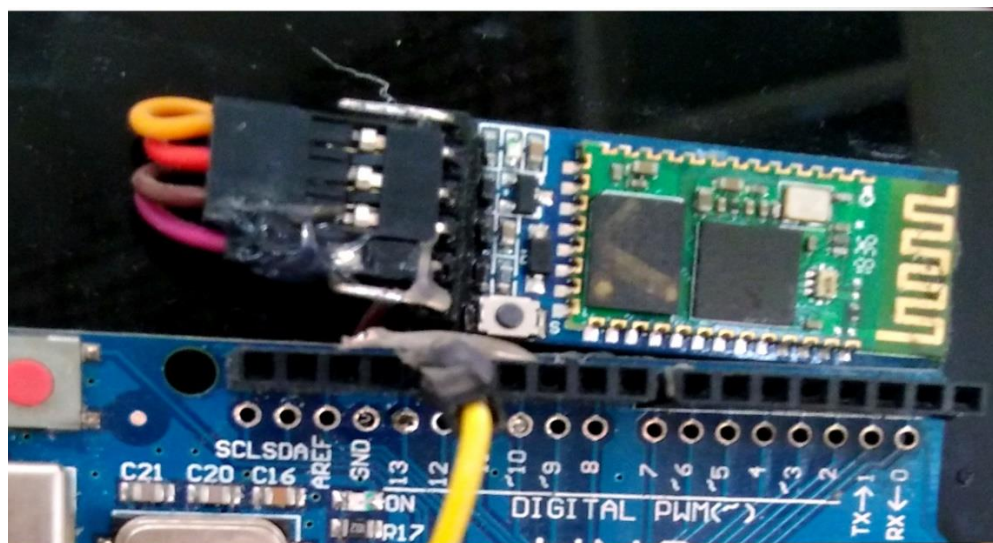


Figure 4.6 Photograph of the Bluetooth device connected to the Arduino board

Emitted light by LDR for CL only and it is considered an important part of homemade devices which is designed in the laboratory Figure 4.7. The signal received by the mobile phone through Bluetooth and displayed by the Remote XY application, which is set on the phone, and drawn as a chart between analytical signal and time as a function of a certain concentration for luminol, hydrogen peroxide, and ferric ion, the sensor response (LDR) converts light either to an analog signal by Arduino and send it through Bluetooth to mobile or digital data and send it to the computer through cable data. In all experiments, the rubber tip is used at the top of the home device (Figure 4.7 and Figure 4.8) through it the reactant injected to prevent surrounding light to enter the reaction tube during the injection process which it is caused noise in the signal recording.



Figure 4.7 Setup of the Arduino-based CL system which is designed in laboratory

The signal height was recorded with (5 mV) input sensitivity Figure 4.8, and its height was related to the concentration of injected samples. Increasing the sensitivity by using an amplifier gives us the option to detect small amounts of substance and this option has been obtained by the variable switch to raise the analytical signal when needed. The chart of the Signal that estimates the hypochlorite concentration gets it by using Mobile type i-mobile Huawei Y7 prime –LDN-L21(Chinas).

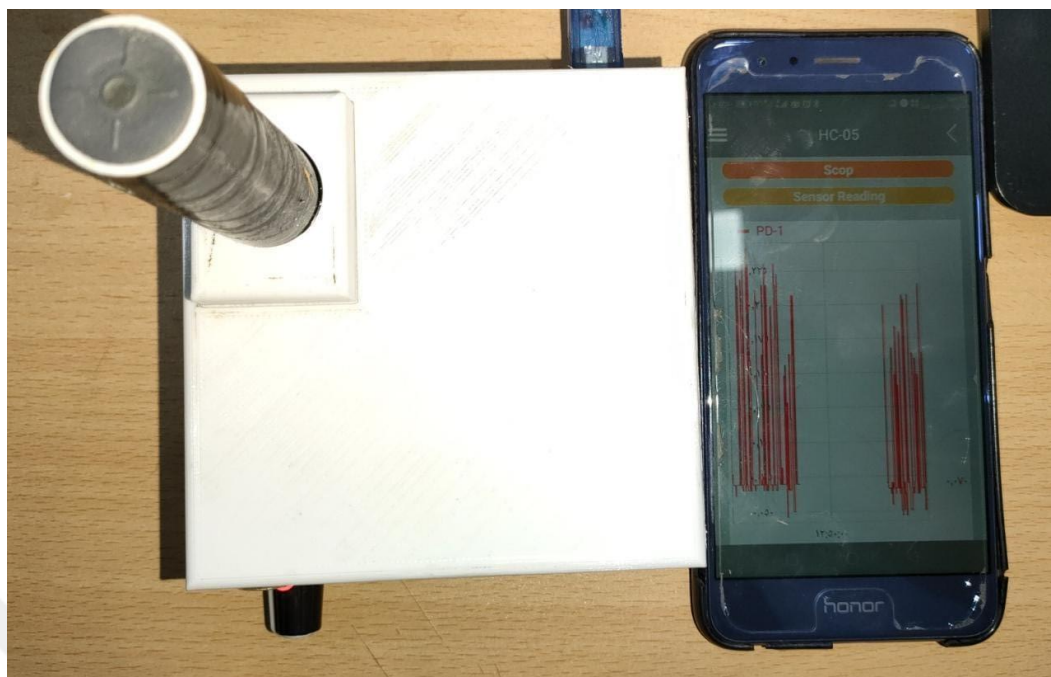


Figure 4.8 The photograph of rubber tip for materials injection

It is noted that the measurements display by the graph of light Intensity versus Time, using a direct injection valve, and these charts display by Remote XY. Also has been possible to connect the device by computer and send data as a digital number to Excel through a USB cable by using (NI LabView 2018 v18.0 x86) program, it is worth noting that a USB cable is considered a power supply for the device or by using a 5-volt power supply.

For all that mentioned above will observe there is no need for a power supply, monochromator, regulator of current intensity, photomultiplier tubes (PMT), or high voltage, all of these factors are reduced to the simple device, easy to use, small, portable, no high voltage and stable current, and cheap as well as with simple practice can make it classroom by programming the Arduino.

4.2 Batch Chemiluminescence System (BCL)

Through a series of tests, the BCL reaction was fine-tuned for fast measurements of ferric ions. The main peak height was the average of three replicates. The laboratory

temperature was between 25 and 35 °C in general. The following optimum conditions have been studied to determine Ferric ions by the homemade BCL system.

4.2.1 Effect of luminol concentration

The concentration of Luminol, at pH 10 as recommended, plays an important role in the CL reaction. Different concentrations of Luminol solution were used as a direct reaction in the range $1 - 1 \times 10^{-5}$ M. The optimum concentration was chosen in a way that yielded a maximum and reproducible peak height with oxidant. Figure 4.9 shows the effect of luminol concentration on peak intensity. The maximum signal levels obtained from these graphs were presented in Table 4.1. Figure 4.10 shows the variation graph of the maximum signal values with luminol concentration. Figure 4.10 show that when luminol concentration is 1.0×10^{-1} , the signal intensity is highest, it leads to more intense CL light. So 1.0×10^{-2} Luminol concentration was chosen as the optimum concentration due to a good CL yield and reproducibility peak height. This concentration is higher than other concentrations of Luminol and causes poor reproducibility.

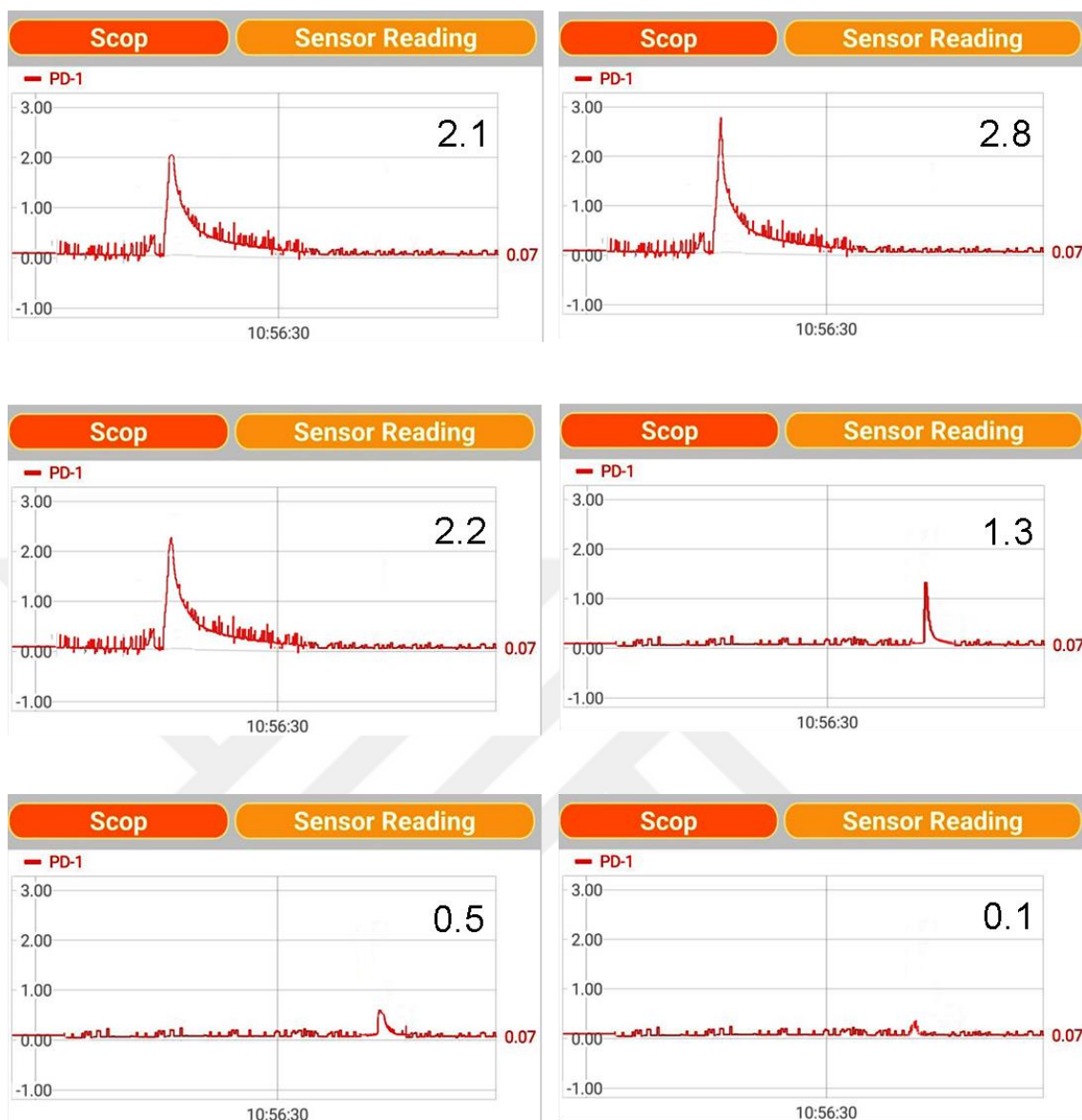


Figure 4.9 Effect of lumol concentration on peak intensity

Table 4.1 Signal values measured against luminol concentration

Concentration (M) of luminol	Signal (v)
1×10^{-5}	0.1
1×10^{-4}	0.492
1×10^{-3}	1.3
1×10^{-2}	2.2
1×10^{-1}	2.8
1	2.1

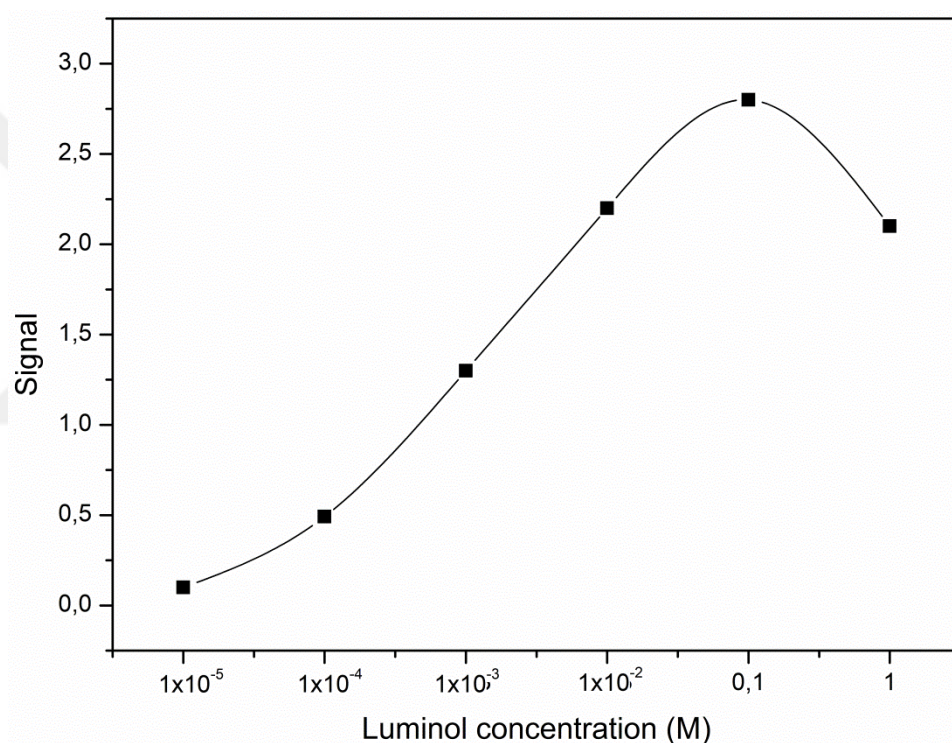


Figure 4.4 The variation graph of the maximum signal values with luminol concentration

4.2.2 Effect of luminol volume

The luminol volume had a strong influence on the sensitivity and dynamic range of the methods, the variation in the CL intensity with the luminol volume was studied in the range of 0.25 - 2 mL. This can be achieved with a syringe. Figure 4.11 shows the effect of luminol volume on peak intensity. The maximum signal levels obtained from these

graphs were presented in Table 4.2. The data obtained were plotted as shown in Figure 4.12, which indicated that the CL signal increased with increasing sample volume up to 1 mL. The peak fall down at 1.25 mL and 1mL of Luminol was selected for a subsequent experiment. 5 mL and 1mL of Luminol was selected for subsequent experiment.

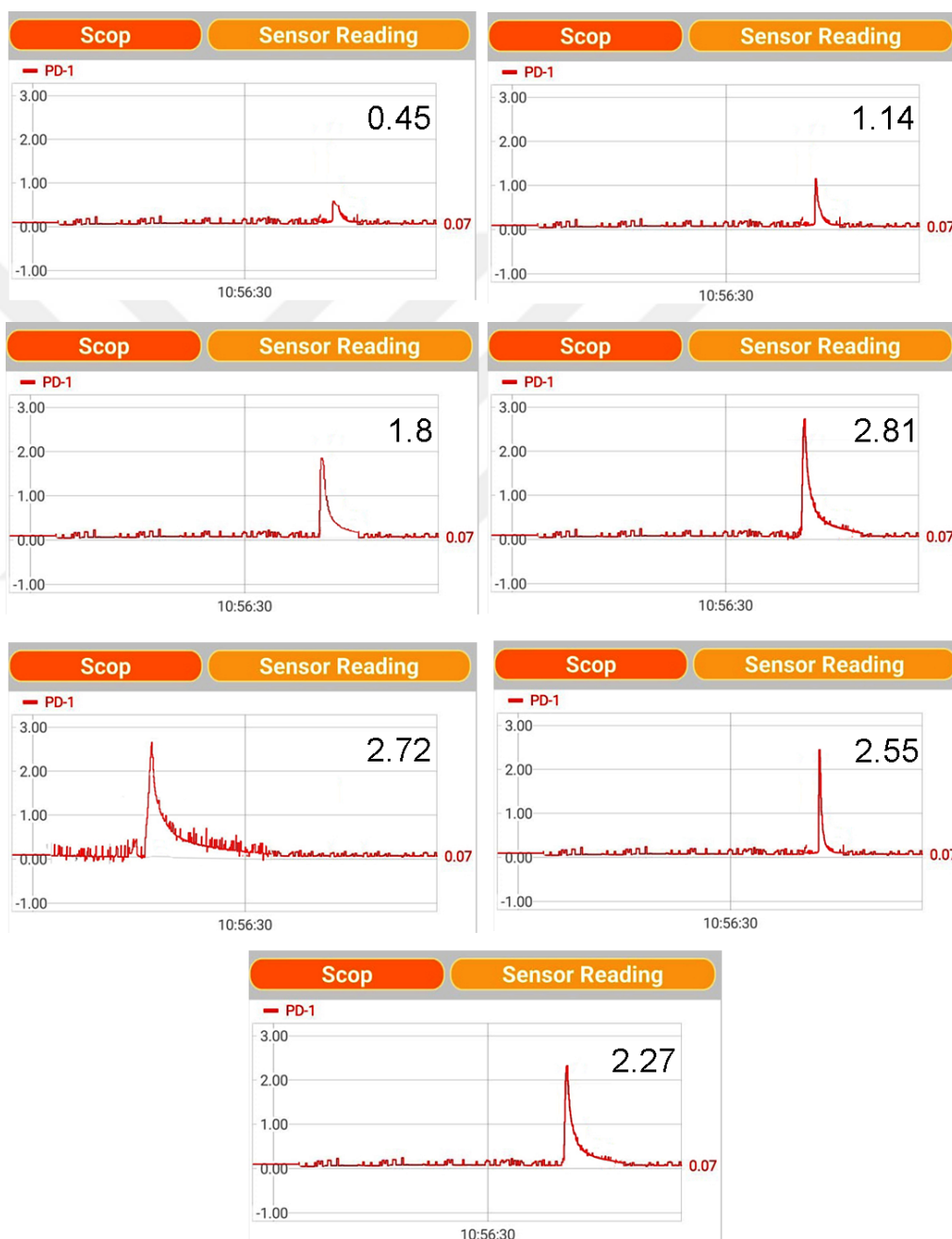


Figure 4.5 The measurement signals obtained against the volume of increased luminol

Table 4.2 Signal values measured against luminol volume

Volume (mL) of luminol (0.1M)	Signal
0.25	0.45
0.5	1.14
0.75	1.8
1	2.81
1.25	2.72
1.5	2.55
2	2.27

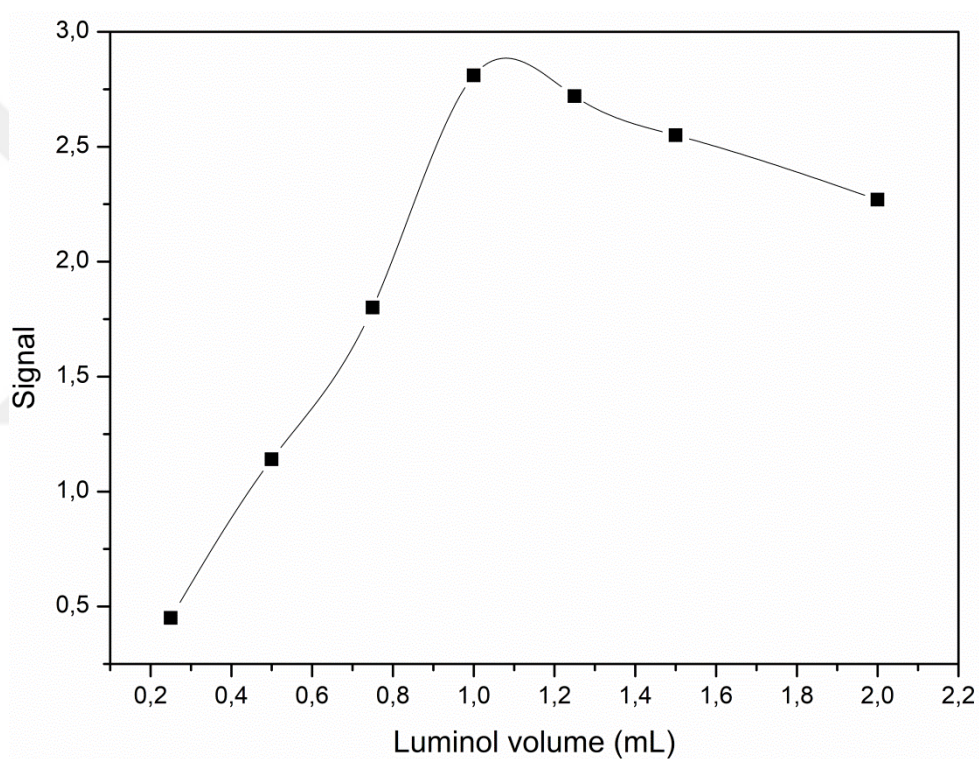


Figure 4.6 The variation graph of the maximum signal values with luminol volume

4.2.3 Effect of hydrogen peroxide volume

Hydrogen peroxide volume had a strong influence on the sensitivity and dynamic range of the methods, the variation in the CL intensity with the hydrogen peroxide volume was studied in the range of 0.5 - 2 mL. Figure 4.13 shows the effect of hydrogen peroxide volume on peak intensity. The maximum signal levels obtained from these graphs were presented in Table 4.3. The data obtained were plotted as shown in Figure

4.14, which indicated that the CL signal increased with increasing sample volume up to (1 mL). The peak fall down at 1.5 mL and 1 mL of Hydrogen peroxide was for the subsequent experiment.



Figure 4.7 The measurement signals obtained against the volume of increased hydrogen peroxide

Table 4.3 Signal values measured against hydrogen peroxide volume

Volume of hydrogen peroxide. 15%	Volume (mL) of 0.1 M Luminol	Signal
0.5	1	2.2
1	1	2.81
1.5	1	1.92
2	1	1.6

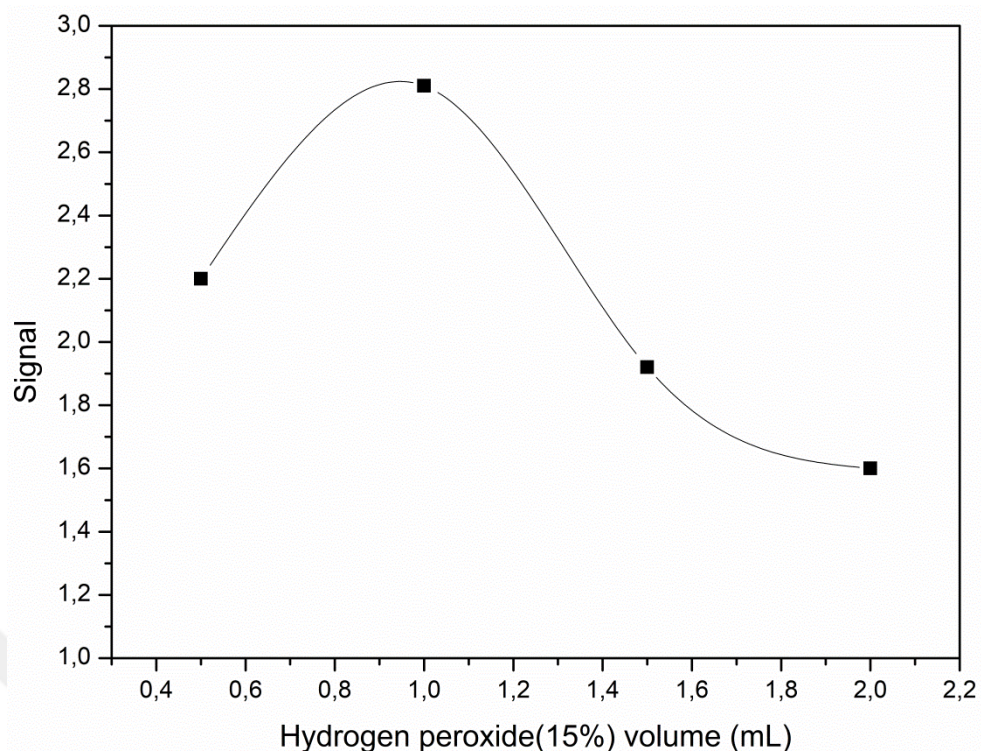


Figure 4.84 The variation graph of the maximum signal values with hydrogen peroxide volume

4.2.4 Effect of hydrogen peroxide concentration

The concentration of hydrogen peroxide plays an important role in the CL reaction. Different concentrations of hydrogen peroxide solution were used as a direct reaction in the range (30%-1%). Figure 4.15 shows the effect of hydrogen peroxide concentration on peak intensity and the maximum signal levels were presented in Table 4.4. Figure 4.16 show the effect of hydrogen peroxide concentration on the intense CL light. So this situation continues until reaches (1%) of hydrogen peroxide to give high intensity at (15%), after that decreasing hydrogen peroxide concentration, leads to less intense CL light. So this situation continues until reach 1% of hydrogen peroxide gives a high intensity at 1.2 which considers the minimum intensity that can get it.



Figure 4.9 The measurement signals obtained against the increased concentration of hydrogen peroxide

Table 4.4 Signal values measured against hydrogen peroxide concentration

Concentration of hydrogen peroxide (%)	Signal
30.00	2.65
20.00	2.77
15.00	2.81
7.50	2.70
3.75	2.60
3.00	1.60
2.00	1.40
1.00	1.20

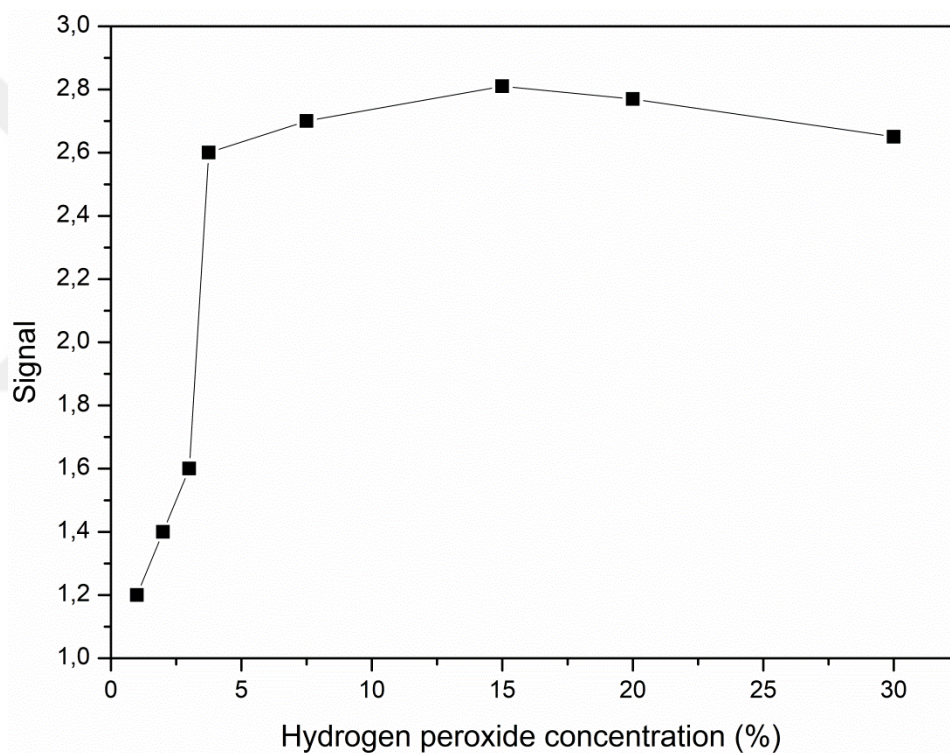


Figure 4.10 The variation graph of the maximum signal values with concentration of hydrogen peroxide

4.2.5 Effect of ferric ion concentration

The concentration of ferric ions plays an important role in the CL reaction. Different concentrations of ferric ion solution were used as batch (direct) reactions in the range $1-1 \times 10^{-6}$ M. Figure 4.17 shows the effect of ferric ion concentration on peak intensity.

The maximum signal levels obtained from these graphs were presented in Table 4.5. Figure 4.18 shows the variation graph of the maximum signal values with ferric ion concentration. So this situation continues to decrease until reaches 1×10^{-5} M of ferric ion to give high intensity at this concentration (0.2), after that no signal at 1×10^{-6} M.



Figure 4.11 The variations in signal intensity with ferric ion concentration

Table 4.5 Signal values measured against ferric ion concentration

Ferric ion concentration (M)	Signal
0.000001	0.00
0.000010	0.20
0.000100	0.50
0.001000	1.10
0.010000	1.70
0.100000	2.79
1.00	2.50

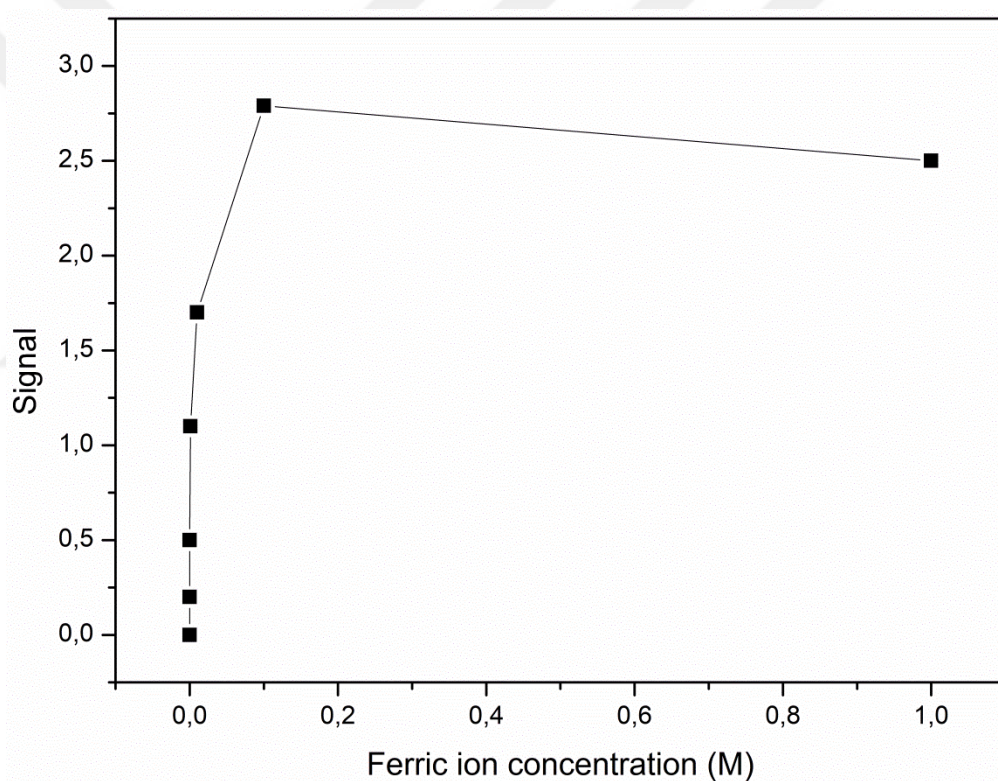


Figure 4.12 The variation graph of the maximum signal values with ferric ion concentration

4.2.6 Effect of ferric ion volume

Ferric ion volume had an influence on the sensitivity and dynamic range of the methods, the variation in the CL intensity with the ferric ion volume was studied in the range of

(0.5-2) mL. Figure 4.19 shows the effect of ferric ion volume on peak intensity and the maximum signal levels obtained from these graphs were presented in Table 4.6. Figure 4.20 shows the variation graph of the maximum signal values with ferric ion volume. Figure 4.20 indicated that the CL signal increased with increasing sample volume up to (1 mL). The peak fall down at 1.5 mL and 1 mL of ferric ion was for a subsequent experiment.

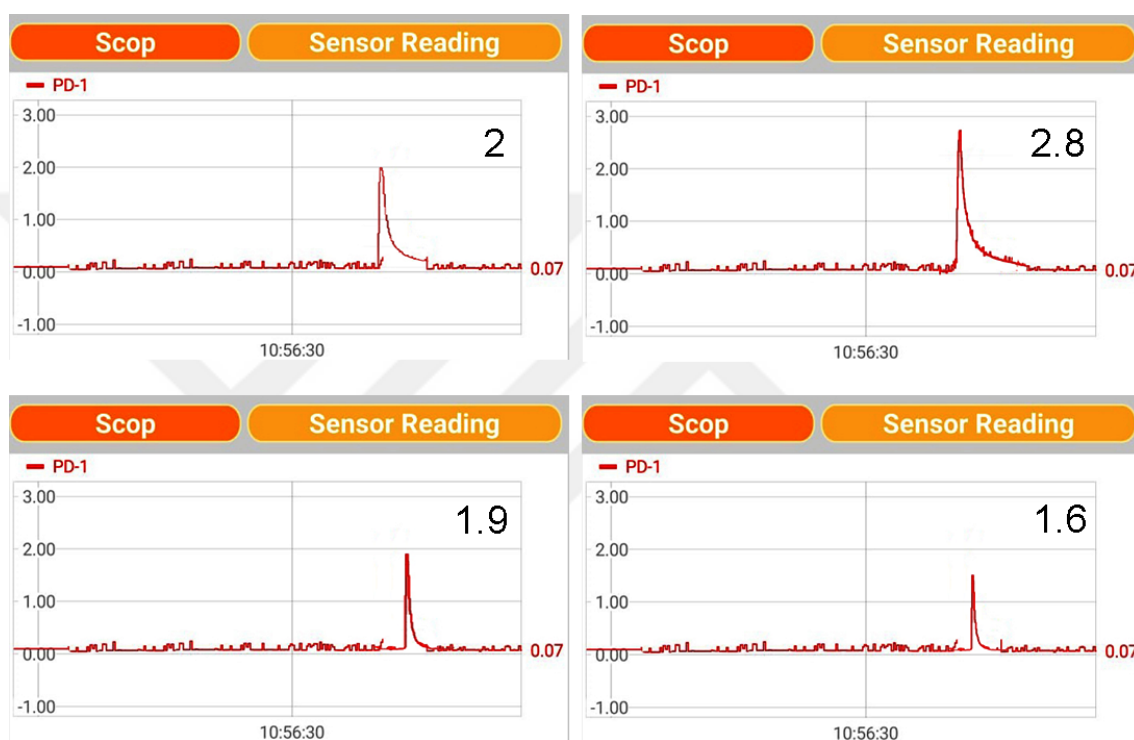


Figure 4.19 The measurement signals against the increased volume of ferric ion

Table 4.6 Signal values measured against ferric ion volume

Volume (mL) of ferric ion	Signal
0.5	2.00
1.0	2.80
1.5	1.90
2.0	1.60

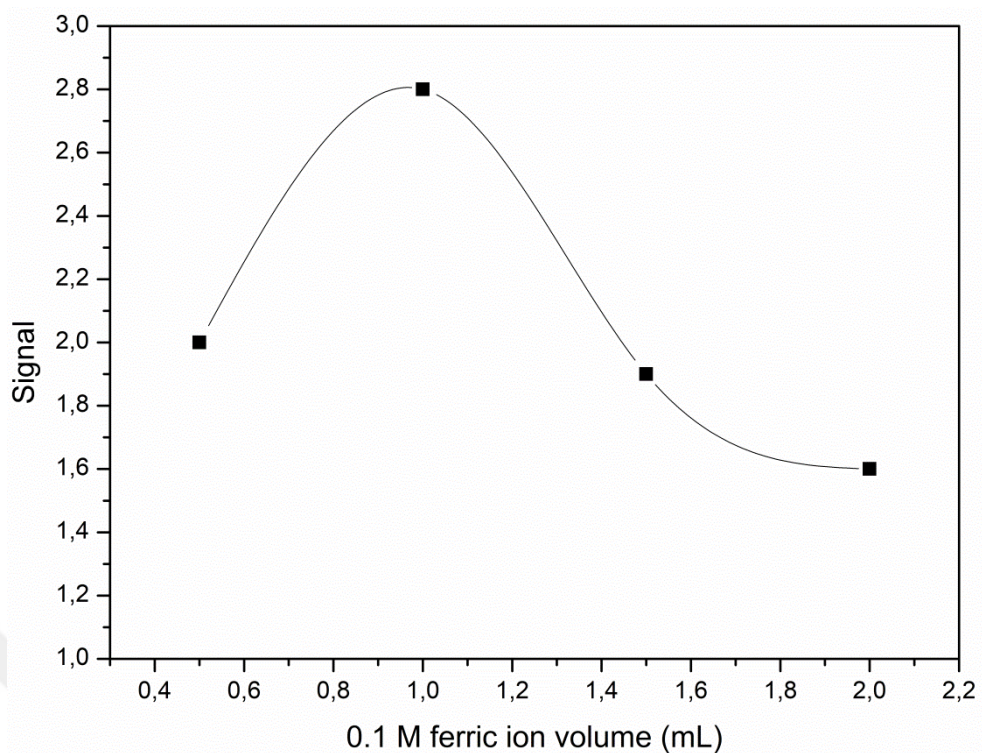


Figure 4.13 The variation graph of the maximum signal values with ferric ion volume of ferric ion

4.2.7 Detection limit of device

Figure 4.21 shows an example of the main Di-CL peak. The peak height, start/end of the peak, upper/ lower noise limits, and mean noise. Equation (4.1) (where the signal/noise is at least 3) gives a definition of how signal/noise has been measured. The limit of detection has been calculated as the point on the calibration graph. The magnitude of the mean peak-to-peak noise (0.07v) in the Figure 4.21 is caused by rising the sensitivity of the device to give background emission in the absence of the reactant.

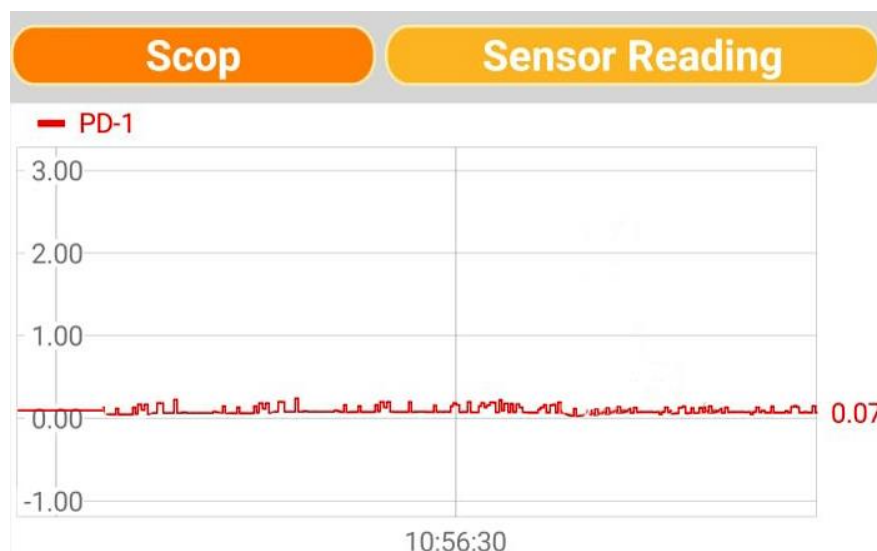


Figure 4.14 The measurement baseline of the device

$$\frac{\text{Signal}}{\text{noise}} = \frac{\text{Peak height}}{\text{Upper noise level} - \text{Lower noise level}} \quad (4.1)$$

4.3 Reproducibility

The reproducibility of peak height under optimum conditions is reported for ferric ions. Relative standard deviations (R.S.D %) were calculated for 7 times determinations of standards deviation of hypochlorite was $\sigma = 0.00534$, and R.S.D%= 0.076%, Figure 4.22 shows reproducibility graphs for ferric ions.

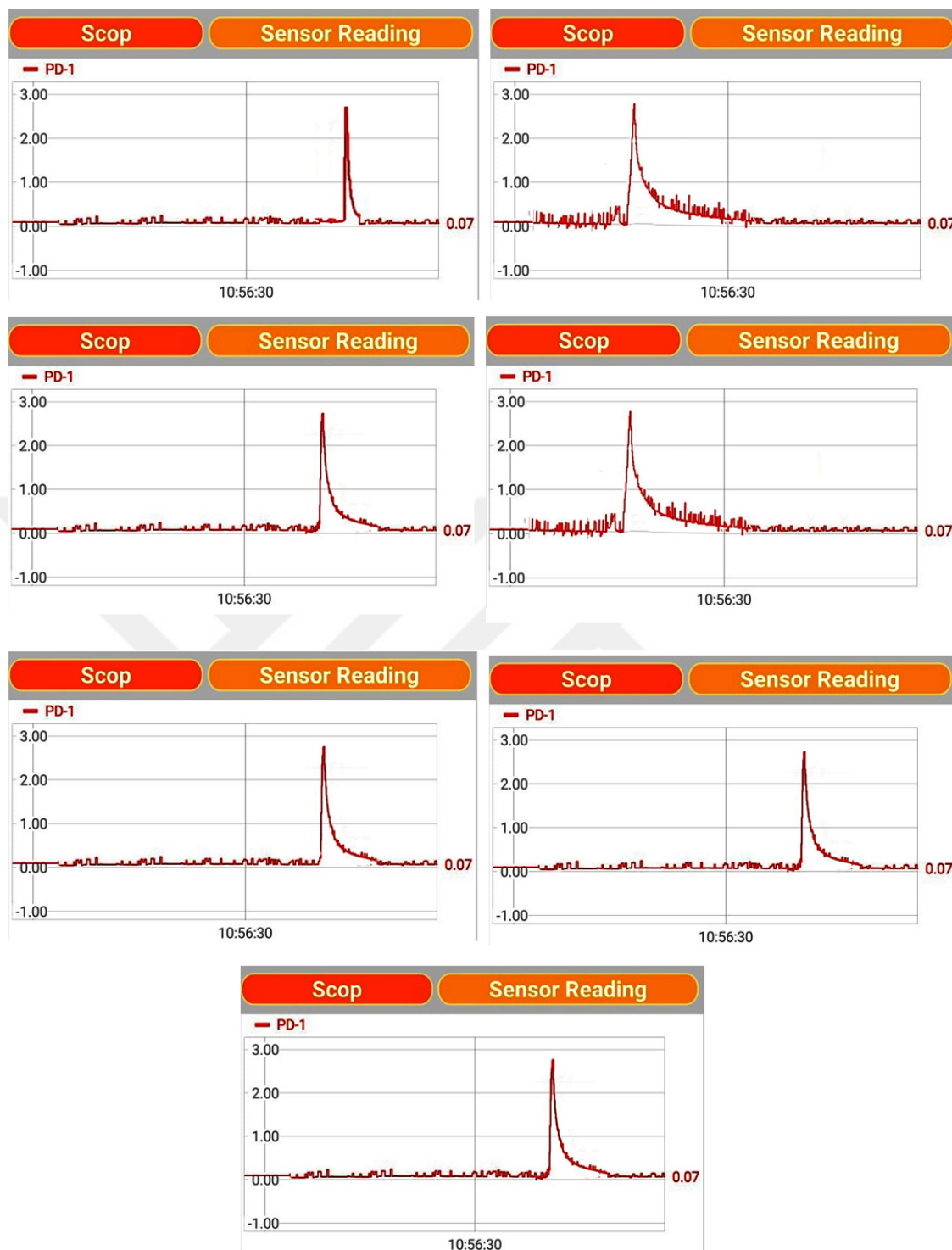


Figure 4.15 Reproducibility graphs for ferric ions

4.4 Standard Calibration for Ferric Ion

Under the conditions established, a calibration graph for ferric ions was obtained. Figure 4.23 shows signals obtained at the ferric ion concentration in the range of 0.1-0.00001 M. The maximum signal levels were given in Table 4.4. Figure 4.23 shows the calibration graph plotted between the ferric ion concentration (in the range of 0.1-0.00001 M) and the CL intensity peak height. As can be seen from the figure, the resulting graph is linear. The linear graph has a regression coefficient and correlation coefficient of ($r^2 = 0.9996$, $r = 0.9997$) for (6 points). The detection limit ($3\sigma \times \text{noise}$) was in M and the R.S.D % for six replicate analyses of Fe^{3+} standards was 0.076 %.



Figure 4.163 The measurement signals for the calibration graph of Fe^{3+}

Table 4.7 Signal values measured against ferric ion concentration

Ferric ion concentration (M)	Signal
0.000001	0.00
0.000010	0.5
0.000100	1.10
0.001000	1.65
0.010000	2.20
0.100000	2.79

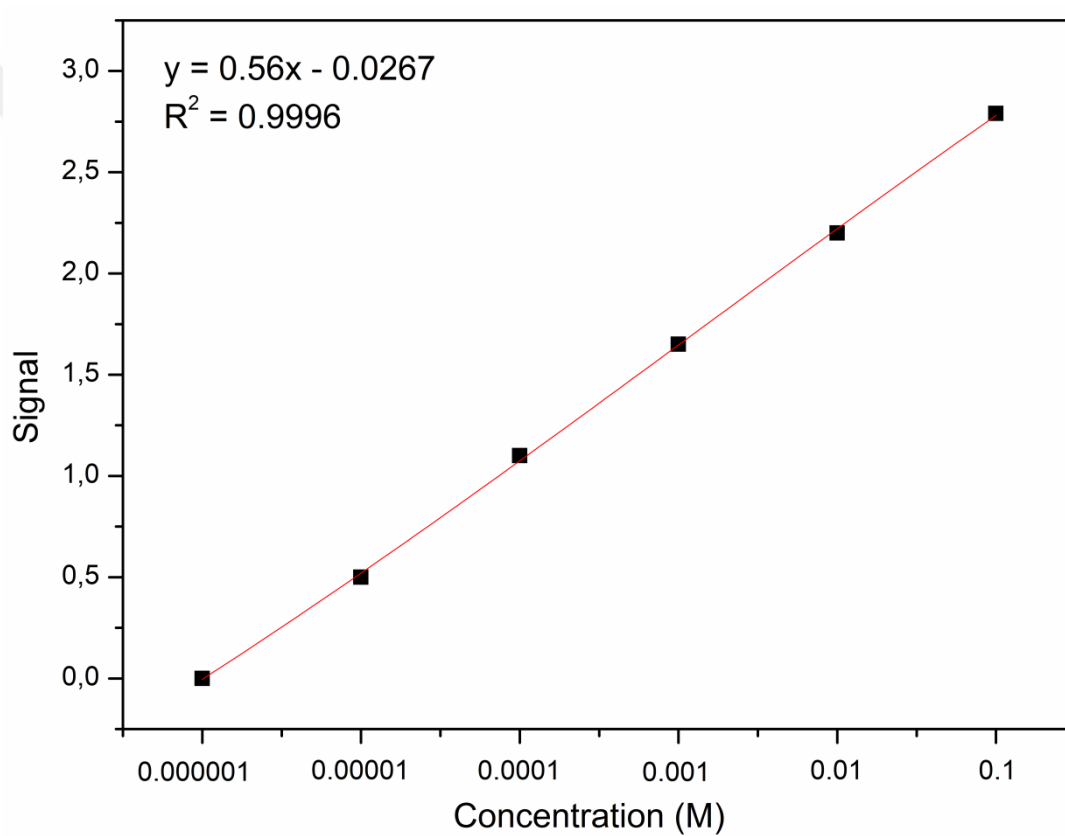


Figure 4.17 Calibration curve for ferric ion concentration

Table 4.8 Optimum conditions of determination Fe^{3+} by Di- CL system

Parameters	Value
Cons. of Luminol	0.1M
Luminol volume	1 mL
Hydrogen peroxide Conc.	15%
Hydrogen peroxide volume	1 mL
Linearity range	0.00001 – 0.1 M
Regression coefficient	$R^2 = 0.9996$
Correlation coefficient	$R = 0.9997$
Detection limit($3\sigma \times \text{Noise}$)	$2.2885 \times 10^{-8} \text{ M}$
R.S.D %	0.076%
Standard Deviation	0.00534
Equation of Calibration Curve	$y = 0.56x - 0.0267$

4.5 Accuracy

In order to establish the accuracy and validity of the homemade Batch-CL system, several representative sample analyses were used Table 4.9. The average recoveries for five measurements were in the range (98.5 – 100.13 %). Good agreements between the results were obtained as shown in Table 4.9, which clearly indicated that the homemade Ba-CL system by the proposed method is suitable for the determination of Ferric ion.

Table 4.9 Determination of Fe^{3+} in representative sample

Samples	Claimed mg L^{-1}	Determination of Fe^{3+} by homemade Ba-CL system*	
		Found (mg L^{-1})	Recovery % $\pm$ R.S.D%
1	10.00	9.85	98.50 ± 0.11
2	100.00	100.11	100.13 ± 0.13

* Average of five measurements

4.6 Applications

4.6.1 Determination iron (III) in samples

The proposed Ba-CL method was used for the analysis of iron (III) in two commercial formulations directly after being prepared it. The method was successfully applied to the analysis of five replicate of iron (III), as shown in Table 4.10. The recoveries results of 98.75 – 99.05% were in good agreement with the declared contents. In tow solutions, quantitative recoveries were obtained.

Table 4.10 Application of the proposed method for determination of Fe^{3+} in two solutions of Fe^{3+}

Solutions	Conc. of Fe^{3+} (mg L^{-1})		Recovery % RSD% $\pm$
	present	Found	
1	40	39.5	98.75 $\pm$ 0.10
2	85	84.2	99.05 $\pm$ 0.13

* Average five measurements

4.6.2 Determination of iron in serum to thalassemia patients

Serum iron was measured using a colorimetric Cromatest[®] kit supplied by Linear Chemicals, Spain. The principle of the test depends on the fact that the Fe^{3+} bound to serum transferrin will dissociate in a weak-acid medium by Teepol and guanidium chloride and then be reduced by hydroxylamine to Fe^{2+} . Ferrous ions can produce a colored complex with Ferrozine[®] that has the following chemical formula: 3-(2-pyridyl)-5,6-bis(4-phenylsulfonic acid)-1,2,4-triazine, monosodium salt to form a tris ferrozine/ iron, $\text{Fe}(\text{Fz})_3$ complex which proportional to the concentration of iron present in the sample (Carter 1971, Williams *et al.* 1977, Artiss *et al.* 1981).

It was made a homemade device to measure iron in the blood of thalassemia patients and the measurement results were compared with those of the colorimetric Cromatest kit array. The results were almost similar as shown in Table 4.11.

Reagents

Reagent 1: Buffer/Reductant. Guanidine chloride 1.0 mol/L, hydroxylamine 0.6 mol/L, acetate buffer 400 mmol/L pH 4.0, Teepol.

Reagent 2: Chromogen. FerroZine 8mmol/L, sodium acetate 400mmol/L

Reagent 3: Iron standard. Ferric ion 100 µg/dL (17.9µmol/L)

Table 4.11 The comparison of measurement results of the homemade device and test kit

Concentration of iron (µg/dL)		*Recovery % *RSD%
Present by colorimetric Cromatest kit	Found by home-made device	
251	250	100.00%, ± 0.10
322	320	99.99%, ± 0.19
284	286	100.01%, ± 0.14
263	265	100.01%, ± 0.21
390	389	100.00%, ± 0.13
377	378	100.00%, ± 0.16
362	364	100.01%, ± 0.12
296	295	100.00%, ± 0.20
266	265	100.00%, ± 0.18
302	303	100.00%, ± 0.12
287	288	100.00%, ± 0.22
279	277	99.99%, ± 0.21
263	262	100.00%, ± 0.15
348	349	100.00%, ± 0.14
367	368	100.00%, ± 0.16
293	291	99.99%, ± 0.13
271	270	100.00%, ± 0.10
286	287	100.00%, ± 0.15
333	335	100.01%, ± 0.17
359	359	100.00%, ± 0.17
319	320	100.00%, ± 0.16
325	325	100.00%, ± 0.12
364	364	100.00%, ± 0.16
339	337	99.99%, ± 0.19
342	341	100.00%, ± 0.20

5 CONCLUSIONS AND RECOMMENDATION

In this study, there is use a new homemade device as a technique for chemical illumination to determine the light intensity in the chemiluminescence and use it as an application for the determination of the level of iron in the blood of Thalassemia patients. This manufacturing of a new device is very simple and at the lowest cost. Semi-automated homemade bases on the batch reaction of CL system (Ba-CL) have been designed and constructed in the laboratory to measure CL as a function of concentration. The constructed Ba-CL system offers a simple, easy-to-work, sensitive, and reproducible means for the determination of Iron (III) in different samples and commercial products. The mobile- phone was used as a novel detector for the Ba-CL system, this detection offers inexpensive and suitable for laborites. Semi-automated home - made Direct Reaction CL system (Ba-CL) can be used with any reagents and materials that have the ability to Luminescence. It can be used for Chemiluminescen devices with flow injection system and micro flowed system. Using phones, as inexpensive detectors and available for anyone to monitor absorption, emission signals, and CL. The constructed CL and mobile phone system detectors can be joined with Ion-chromatography to achieve a sensitivity of determination. A variety of applications can be achieved with the Ba-CL system such as pharmaceutically samples and other oxidation systems like Fe (III), Hypochlorite, H_2O_2 , UV lamp, and electro-generating can be used.

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