Biomedicine and Chemical Sciences 2(2) (2023) 139-142

11-BCS-1773-410

Content lists available at:
www.journals.irapa.org/index.php/BCS/issue/view/23

Biomedicine and Chemical Sciences

Journal homepage: https://www.journals.irapa.org/index.php/BCS

Design of a New Bioluminescence Sensor Using an Arduino Device Linked to Bluetooth Mobile Phone and USB Computer to Measure Triglycerides

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ARTICLEINFO

Article history:
Received on: December 25, 2022
Revised on: February 01, 2023
Accepted on: February 10, 2023
Published on: April 01, 2023

Keywords:
Bioluminescence
Chemiluminescence
Hydrogen peroxide
Luminol
Triglyceride

ABSTRACT

In this work, a new device is designed for measuring the light intensity of the Bioluminescence reaction between Luminol with Hydrogen peroxide that is produced as an activity of Triglycerides Phosphate Oxidase. The project includes the use of a homemade and semi-automated device for the determination of Chemiluminescence (CL) and Bioluminescence light by direct reaction analysis of Luminol with oxidant and using a Mobile –phone as a recorder which is employed in chemistry with Arduino and applied this device into the enzymatic reaction for determine Triglyceride by this new methods. The method is easy, simple, and rapid with high sensitivity for the determination of hydrogen peroxide. The light of the Bioluminescence reaction of Luminol (5-amino-2,3-dihydro-1,4_phthalazinedione) with oxidant (H_2O_2) was sensed by photocell and the signal was sent to Mobile. The method used to determine glycerol and compare the results of the device with real value to give high accuracy, Triglyceride was determined in its kit. It gave 99.2 and ±0.1 of Recovery and RSD, respectively.

1. Introduction

Chemiluminescence is an important method for the quantification and analysis of various macromolecules. CL methods are based on the emission of light (luminescence) from a sample being investigated as a result of a chemical reaction (Delafresnaye, et al., 2020). The luminescence reagent is continuously added to the sample and mixed with the column elute in the mixer. The resulting luminescence from the chemical reaction is then measured using a photomultiplier tube when the luminescence raises to its highest intensity (Vinegoni, et al., 2017). CL is produced as a result of a chemical reaction usually involving an oxidation-reduction process. The most common mechanism for such emission is the conversion of chemical energy, released in a highly exothermic reaction, into light energy in the visible region. In some chemical reactions, energy can be transferred to electrons in the chemical bonds (Calabretta, et al., 2021). Chemiluminescence, which is generated by a chemical reaction to give emission of light (luminescence), is one method of enzyme-based optical biosensing using this property. Luminol oxidized by H_2O_2 by peroxidase-mediated oxidation, Seitz's group built up an H_2O_2 biosensor in an alkaline medium. Generally, the CL reactions were expressed as equations 1 and 2 (Song, et al., 2019):

$$A + B \longrightarrow C^*\ldots\ldots\ldots\ldots(1)$$

$$C^* \dashrightarrow C + h\nu\ldots\ldots\ldots\ldots(2)$$

Bioluminescence is associated with the emission of light by living microorganisms. The bacterial luminescence lux gene has been widely applied as a reporter either in an

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How to cite:

Ali, A. M., & Hussein, M. A. (2023). Design of a New Bioluminescence Sensor Using an Arduino Device Linked to Bluetooth Mobile Phone and USB Computer to Measure Triglycerides. *Biomedicine and Chemical Sciences, 2*(2), 139–142.

DOI: **https://doi.org/10.48112/bcs.v2i2.410**

inducible or constitutive manner. In an inducible manner, the reporter lux gene is fused to a promoter regulated by the concentration of a compound of interest. As a result, the concentration of the compound can be quantitatively analyzed by detecting the bioluminescence intensity (Zhu, et al., 2019). In a biosensing method, the CL reaction results from a reaction of Luminol and peroxidase. The type of peroxidase, Horseradish Peroxidase (HRP) could typically catalyze the reaction of H_2O_2 with the reagent of Chemiluminescent to obtain light. In addition, G-quadruplex-hemin DNA zyme with hemin in a definite position could also be done as a peroxidase in enzyme-based Chemiluminescence biosensors. As well as, oxidases were also introduced as exogenous H_2O_2 producers, or direct transducers, for further Chemiluminescence detection. The mechanism of coupled enzyme reaction systems is introduced as equations 3 and 4 (Fahliyani, et al., 2020):

$$\text{Substrate}+O_2 \xrightarrow{\textit{oxidase}} \text{product} + H_2O_2 \quad \ldots\ldots\ldots\ldots(3)$$

$$H_2O_2+\text{luminol} \xrightarrow{\textit{peroxidase}} \text{products} + h\nu \quad \ldots\ldots\ldots\ldots\ldots(4)$$

This study aimed to use a homemade semi-automation device to measure Bioluminescence light. The device is characteristic of sensitivity, low cost, ease to use, and recallable result, and use it to determine hydrogen peroxide concentration as a function of light intensity.

2. Materials and Methods

Materials

The chemicals used were analytical-grade reagents. Also, Deionized water was used, and its conductivity was less than 0.2 µS and the measurements were done at 25C° throughout our work.

- 0.1M Na2CO3 (Merck) buffer solution: was prepared by dissolving 10.50 g of Na2CO3 and 0.5M NaOH (20 g) to exactly 1liter (Alizadeh, et al., 2017).
- 0.02 M Luminol solution: (5-amino-2,3-dihydro 1,4_phthalazinedione) (Sigma-Aldrich, St. Louis, MO, USA) was prepared by dissolving 0.3544 g of Luminol (97%) in 100 mL of 0.1M Na2CO3 solution the buffer (pH 10) (Abow, 2017).
- 30 % hydrogen peroxide, (d= 1.45 g/cm³) (Sigma, St. Louis, MO, USA) dilute in deionized water (Rahmawati, et al., 2022).
- Lipase ≥ 1000 IU/L , Peroxidase (POD) ≥ 1700 IU/L, Glycerol 3 Phosphate oxidase(GPO) ≥3000 IU/L, Glycerol Kinase (GK) ≥ 660 IU/L, 4-Amino-antipyrine(PAP) 0.5 mmol/L, Adenosine triphosphate Na(ATP) 1.3 mmol/L, Glycerol 2.28 mmol/L (Lee, et al., 2019).

Methods

In this study, measurement techniques were used. The first is the Direct system as novel CL sensing by computer and the second is a mobile –phone as a novel detector for direct CL system. The two systems were designed and constructed in the Ministry of science and technology laboratory of the chemistry department.

Direct CL System (Di-CI

Figure 1 shows a homemade CL sensing system and Figure 2 shows an overview of how homemade CL parts connected in the device and detector system which were used in this work. Arduino and Bluetooth devices were used to send the analytical signal to the mobile. The sample was introduced to the reaction tube through injection by a syringe, the photocell was sensing the visible light that emits from the reaction vessel and converting this light to a signal by Arduino and transferring it to mobile through Bluetooth with the Blynk program to give signal data. The CL intensity was recorded as the high peak by mobile (Huawei Y7 prim). A homemade system was made in the laboratory and a test tube (10 cm) was a reaction vessel.

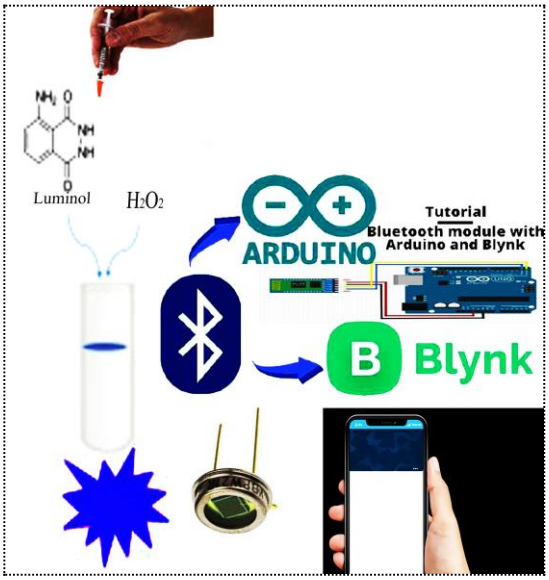

Fig. 1. Home-mad CL sensing system

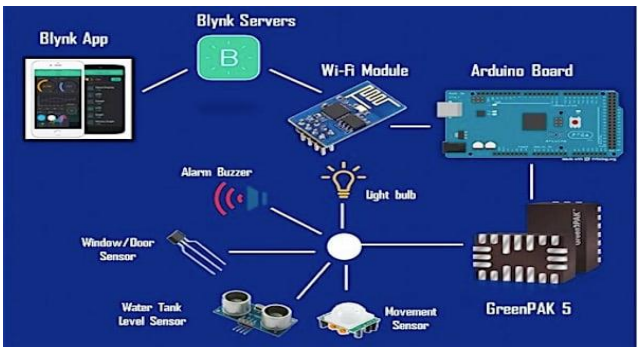

Fig. 2. Overview of connection device

Procedure

In this technique, 0.01M Luminol solution was put in a test tube (1.5 mL). The sample was injected into the injection valve and into the test tube. A 1 cm reaction test tube was inserted in the specific holder in our device before injecting the hypochlorite (the oxidant) and next step the hypochlorite sample was injected after that the light emitting was measured by the Mobile-Phone Di-CL system. Amplifier

(100) using variable resistor connected with Arduino plug of mobile to read as light intensity and determination by Blynk program. Results were treated statistically and read with an Excel program. The recorder height peak on the mobile screen can be related to the concentration. The light intensity is the CL resulting from the interaction of Luminol with Hypochlorite. Figure 1 shows the homemade Di-CL system using Mobile –Phones as a detector.

3. Result and Discussion

The device is used to measure CL intensity in biological systems that are composed of enzymatic reactions which are released by Hydrogen Peroxide as a side product of these enzymatic reactions. So the hydrogen peroxide contributes to CL light as an oxidant for Luminol for that CL light intensity enhancement when hypochlorite and hydrogen peroxide make oxidants for Luminol together. The method was successfully applied to the analysis of five replicate of measurements. The recoveries results of (98%) were in good agreement. Therefore, the enzyme chosen for this test is Triglycerides Phosphate Oxidase (GPO). The triglycerides (GPO) method is based on the enzymatic determination of glycerol using the enzyme glycerol phosphate oxidase (GPO) after hydrolysis by lipoprotein lipase. The principle of this method was described by Fossati who coupled the reaction with the classical Trinder reaction sequence. This single reagent procedure quantitates the total glycerides in serum including the mono and diglycerides, and the free glycerol fractions. This approach is the basis for this method (equations 5-7).

$$\text{Triglycerides} \xrightarrow{\text{Lipase}} \text{Glycerol + Fatty Acids} \quad \text{.....(5)}$$

$$\text{Glycerol + AT} \xrightarrow{\text{GK}} \text{Glycerol-1-phosphate + ADP} \quad \text{.....(6)}$$

$$\text{Glycerol-1- phosphate + } O_2 \xrightarrow{\text{GPO}} \text{DAP + } H_2O_2 \quad \text{.....(7)}$$

Serum triglycerides are hydrolyzed to glycerol and free fatty acids by lipase. In the presence of ATP and glycerol kinase (GK), the glycerol is converted to glycerol-1-phosphate. The glycerol-1-phosphate is then oxidized by glycerol phosphate oxidase (GPO) to yield hydrogen peroxide. The condensation of hydrogen peroxide with 4-chlorophenol and 4-aminophenazone (4-AA) in the presence of peroxidase (POD) produces a red-colored quinonimine dye which absorbs at, or near 500nm. The intensity of the colored complex formed is directly proportional to the triglyceride concentration of the sample. Table 2 explain the result of testing for two experiments, so these resulting can be calculated Triglycerides concentration by a Homemade device through compare the signal of certain concentration from hydrogen peroxide with the signal produced by hydrogen peroxide as a result of enzymatic reaction which it can determine the concentration of triglycerides.

Table 1

The result of testing for two experiment

Pipette into well identified test tube	Experiment 1	Experiment 2
Luminol	1.5 mL	1.5 mL
Enzyme	1 mL	1 mL
Substrate	0.2 mL (2000 ppm)	0.2 mL (2000 ppm)
Catalyst Fe(III)	0.05 mL	0.05 mL
H_2O_2	1 mL (2176 ppm)	0
Signal	1.3	0.62

The CL light intensity was generated by the reaction of Luminol with hydrogen peroxide that is participate in CL reaction through two sources, the one from addition specific concentration, and the second coming from the enzymatic reaction. The analytical signal (1.3) is related to H_2O_2 from the two sources as in Figure 3a, and the analytical signal (0.62) is related to H_2O_2 from enzymatic reaction only as in Figure 3b.

Fig. 3. High peak intensity a: with H_2O_2 and b: without H_2O_2

The device also used to measure CL intensity in biological system that is composed of enzymatic reaction which is released Hydrogen Peroxide as side product of these enzymatic reaction. So the hydrogen peroxide contributing in CL light as an oxidant for Luminol for that CL light intensity enhancement when used slandered addition method of hydrogen peroxide make as oxidant for Luminol together (Rambaldi, et al., 2009; Guo, et al., 2018).

Table 2
Recovery and RSD for Triglyceride

Conc. of Triglyceride (mg L^{-1})		• Recovery %
present	Found	• RSD%±
2000	1984	99.2 % , ± 0.1

The new device is used with the two experiments above and the same steps to measure the intensity of CL in a biological system that consists of an enzymatic reaction. The method was successfully applied to the analysis of three replicate of measurements in, as shown in Table 3. The recoveries results of (99.2%) were in good agreement.

So that the signal of hydrogen peroxide that is added and produced from the enzymatic reaction is 1.3 and the signal of hydrogen peroxide that is produced from enzymatic

reaction only is 0.62 so that can calculate the signal of hydrogen peroxide that is added

Signal H_2O_2 that is added only = Signal H_2O_2 (Total) - Signal H_2O_2 (enzymatic reaction only)

$$\textit{Signal } H_2O_2 \textit{ that is added only} = 1.3 - 0.62$$

$$= 0.68$$

In addition, the signal H_2O_2 *only represented 2176 ppm by that can be calculate* H_2O_2 *concentration comes from enzymatic reaction by:*

$$\frac{\text{signal } H_2O_2 \text{ of enzymatic reaction}}{\text{concentration } H_2O_2 \text{ of enzymatic reaction}} = \frac{\text{signal } H_2O_2 \text{ of added}}{\text{concentration } H_2O_2 \text{ of added}}$$

$$\frac{0.62}{X} = \frac{0.68}{2176}$$

$$0.68\ X = 0.62\ x\ 2176$$

$X = 1984$ *ppm which it approximately equal to real concentration of Triglycerides 2000ppm.*

4. Conclusion

This study could use the semi-automation homemade device to measure Bioluminescence light by using a mobile phone as a detector through employing Arduino with a photocell as a light sensor. So the homemade device used to determine Triglycerides concentration through a measure of Hydrogen peroxide as a side product from the specific enzymatic reaction on Triglycerides and compare the signal of certain concentration from hydrogen peroxide with the signal produced from hydrogen peroxide as a result of enzymatic reaction which by it can determine the concentration of triglycerides.

Competing Interests

The authors have declared that no competing interests exist.

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