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Chemiluminescence Detection of Chlorpyrifos via Luminol-H_2O_2-Ferricyanide System using Microcontroller-based Photometer

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A low-cost photometer for chemiluminescence (CL) detection of chlorpyrifos (CPF) was constructed based on Arduino microcontroller, Si photodiode, and operational amplifier. The CPF detection via CL is based on the decrease in light intensity of the sensing solution (luminol-H_2O_2-$Fe(CN)_6^{3-}$-CPF) as compared to the blank solution (luminol-H_2O_2 $Fe(CN)_6^{3-}$). The decrease in light intensity is due to the known reaction of organophosphates with H_2O_2 and luminol, thereby consuming the reactants for CL reaction. The change in response (ΔI_{CL}) was determined by the difference between the response of the blank solution (I_B) and the sensing solution (Iss). Different parameters for both blank and sensing solutions were optimized. A linearly decreasing response with increasing CPF concentration was found between 0.7 ppm and 2.45 ppm CPF (2.00–7.00 µM CPF), with a limit of detection (LOD) of 0.663 ppm (1.89 µM). The system was shown to be selective mainly toward organophosphate pesticides as non-organophosphate herbicides – such as 2,4-dichlorophenoxyacetic acid (2,4-D) and atrazine – did not show significant changes in response as compared to blank solution.

Key words: chemiluminescence, chlorpyrifos, luminol, microcontroller

INTRODUCTION

Organophosphates are a class of insecticides that primarily act by irreversibly inhibiting acetylcholinesterase (AChE), the enzyme responsible for acetylcholine hydrolysis (Lukaszewicz-Hussain 2010). Irreversible inhibition of AChE can lead to build-up of the neurotransmitter acetylcholine, which interferes with muscular response and produce serious symptoms in vital organs (Mulchandani *et al*. 2001). Despite the toxicity of organophosphates, certain organophosphates are still allowed to be used in many countries. Chlorpyrifos (CPF), an organophosphate insecticide, is widely used in agriculture and household applications. CPF and its derivative methyl-chlorpyrifos are the main active ingredients in insecticide formulations widely used to control insect and arthropod pests on agricultural crops such as grains, cotton, nuts, and fruits. Urban applications of CPF include controlling pests on lawn and ornamental plants (Mauriz *et al*. 2006). Due to the wide usage of CPF, point-source and agricultural discharges of CPF became responsible for aquatic life toxicity in urban waters (Uygun & Dilgin 2013). Due to the toxicity presented by CPF and other organophosphates, determination of organophosphate level in agricultural produce became necessary before releasing the crops to the market. This necessity gave rise to the development of several analytical methods for CPF determination, which include chromatographic methods (Guardino *et al*. 1998, Oliva *et al*. 1999), optical methods (Armenta *et al*. 2005,

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Kuswandi *et al*. 2008, Martínez Galera *et al*. 1994, Tunçeli *et al*. 2001), and electrochemical methods (Liu *et al*. 2011, Viswanathan *et al*. 2009, Zamfir *et al*. 2011). However, these methods suffer from using expensive instruments and require highly technical personnel to operate and maintain the instruments. Another drawback of the aforementioned methods is the time-consuming steps for the instrument preparation and the actual detection of analytes. Hence, there is a need for a low-cost and portable sensor for determination of organophosphates such as CPF.

Chemiluminescence (CL) is the light-emission phenomenon due to the relaxation of excited emitting species (luminophore), which are produced via chemical reaction, to its ground state. As an analytical method, CL offers relatively high sensitivity, fast response, and low background signal as compared to other methods (Gámiz-Gracia *et al*. 2005, Liu *et al*. 2010). Due to these advantages, CL has been extensively used for determination of metal ions, inorganic anions, biomolecules, carcinogens, and drugs in different environmental and clinical samples (Robards & Worsfold 1992). One of the widely used luminophores in different liquid CL systems is luminol (5-amino-2,3-dihydrophthalazine-1,4-dione), which is known to be oxidized in basic media via two-step oxidation to produce a key intermediate α-hydroxyperoxide (Merényi *et al*. 1990). Decomposition of α-hydroxyperoxide leads to the production of excited 3-aminophthalate, which undergoes radiative relaxation to its ground state (Dodeigne *et al*. 2000). Different oxidants can be employed for luminol oxidation – including ozone, halogens, singlet oxygen, persulphates, hypochlorites, H_2O_2, and $K_3[Fe(CN)_6]$ – all exhibiting CL at 425nm λ_{max} (Khan *et al*. 2014). Several studies have been conducted involving novel catalysts for luminol-H_2O_2 systems, which include Pt nanoparticles (Park *et al*. 2015), copper(II) oxide nanocubes (Kaviyarasan *et al*. 2016), and metal organic frameworks (MOFs) (Li *et al*. 2017). Yu *et al*. (2016) showed that iodophenol blue can enhance the CL emission of luminol-H_2O_2 system. Several groups have utilized luminol CL systems for organophosphate determinations. Li *et al*. (2008) utilized luminol-H_2O_2 system for direct determination of organophosphate CPF, where they showed that organophosphates such as CPF do enhance the luminol CL reaction. In their proposed mechanism, the CPF reacts with hydrogen peroxide (H_2O_2) to form a more potent oxidant that can oxidize luminol, leading to CL light emission. Hu *et al*. (2010) utilized the same principle of luminol-H_2O_2-organophosphate system, as previously mentioned, for direct determination of organophosphate quinalphos. These studies use photomultiplier tube (PMT) as photometer, which has the highest sensitivity among other light detectors such as photodiode (PD). However, PMT cannot be used in a low-cost portable photometer because of its high operating voltage, fragility, size, and cost (Yotter & Wilson 2003) – making PD a better option for fabricating portable photometer.

In this study, a low-cost photometer for CL detection was fabricated using PDs as photodetector, operational amplifier as signal amplifier, and a microcontroller. The CL detection was based on the consumption of H_2O_2 by CPF, thus leading to a decreased CL light emission. The role of the microcontroller is to process the signal coming from the PD and transmit it to a desktop computer, which serves as the readout device. It converts the analog signal from PD into a measurable digital signal that can be viewed into a computer using a program called Integrated Development Environment (Arduino IDE) that can be downloaded from the website of Arduino (https://www.arduino.cc/en/Main/Software). Arduino became a popular on-board microcontroller that can be easily programmed and incorporated in certain laboratory-relevant instruments, including digital thermometer (Kubínová & Šlégr 2015), pH meter (Kubínová & Šlégr 2015), and even a low-cost potentiostat (Li *et al*. 2018). The developed sensor showed high sensitivity and selective response toward organophosphate pesticides (CPF and fenitrothion) compared to non-organophosphate pesticides (2,4-dichlorophenoxyacetic acid and atrazine).

MATERIALS AND METHODS

Reagents and Chemicals

Luminol; potassium hexacyanoferrate(III); H_2O_2; CPF; atrazine; and 2,4-dichlorophenoxyacetic acid (2,4-D) were all purchased from Sigma-Aldrich. Sodium hydroxide (NaOH) was purchased from Merck. Ethanol was used as the solvent for stock solutions of pesticides/ herbicides. All other solutions were prepared using deionized water.

Apparatus

Low-cost photometer was fabricated using Si photodiode, single-supply operational amplifier (Op-Amp), 1 MΩ resistor, 10 nF capacitor, and Arduino® Uno microcontroller board. The Si PD (Centronic OSD15-E Visible Light Si Photodiode, Through Hole TO-5), Op-Amp (LTC1050 Zero-Drift operational amplifier), and Arduino® Uno were purchased from RS Components Philippines. All other electronic components (e.g., resistor, capacitor, etc.) were purchased from a local electronics shop. A 9V battery was used as the power supply for the Op-Amp. Figure 1 shows the circuit diagram and actual image for the fabricated photometer. A plastic cuvette was used as the reaction cell. The cuvette is contained in a machine-fabricated plastic enclosure, which will serve as the black box. UV-Vis measurements and CL emission spectrum measurements were done using ALS SEC2000 UV-VIS Spectrometer (ALS-Japan).

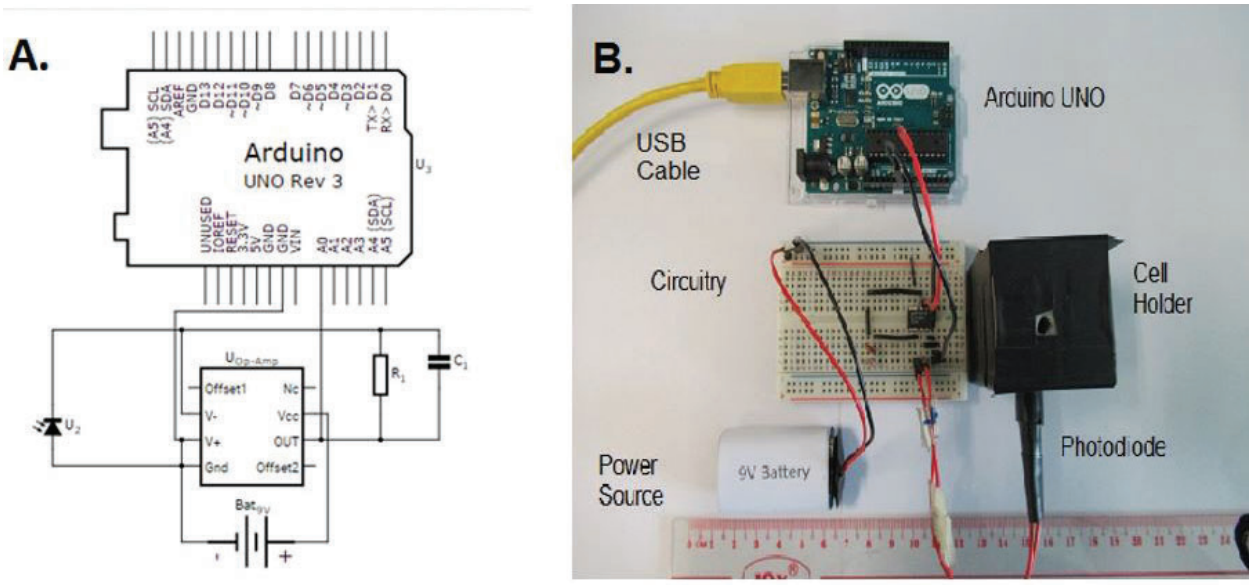

Figure 1. Circuit diagram (A) and actual image (B) of the fabricated low-cost photometer for CL detection.

Procedure for CPF Determination

A blank solution was prepared containing luminol and H_2O_2 (in 1% ethanol), while the sensing solution was prepared containing luminol, H_2O_2, and CPF. Two milliliters (2.0 mL) of either blank or sensing solution is transferred to the reaction cell, after which 1.0 mL of $K_3[Fe(CN)_6]$ solution is then added. The CL intensity was recorded via Arduino IDE. The change in CL intensity (ΔI_{CL}) is then calculated as the difference between CL intensity of blank (I_{Blank}) and sensing solution (I_{ss}).

Optimization of Parameters

Different parameters – specifically pH, luminol concentration, H_2O_2 concentration, and $K_3[Fe(CN)_6]$ concentration – were optimized. The ΔI_{CL} values were recorded and obtained at each solution system with varying concentrations of components. The highest average ΔI_{CL} value was chosen to be the optimized for each parameter in order to have the highest sensitivity for the resulting sensor. Final optimized parameters were used in actual measurement of CPF – in both blank and sensing solutions.

RESULTS AND DISCUSSION

Proof of Indirect CL Detection: Role of H_2O_2

H_2O_2 is a strong oxidant with a standard reduction potential of 1.8V. Under right conditions, H_2O_2 can oxidize a wide variety of chemicals, including organic compounds with oxidizable functional groups. As presented by the groups of Hu and Li, organophosphates such as CPF can be cleaved oxidatively with H_2O_2 (Hu *et al*. 2010, Li *et al*. 2008) The reaction between CPF and H_2O_2 is depicted in Appendix I. In the reaction, peroxophosphonate and 3,5,6-trichloropyridin-2-olate are formed from the oxidative cleavage of CPF. The formed peroxophosphonate can then oxidize luminol to form excited 3-aminopthalate leading to CL emission, though this emission is too weak to be detected by the used PD at low concentrations of organophosphate. These reactions – once completed – will result to a decrease in H_2O_2 and luminol species in sensing solution, eventually leading to an increase in ΔI_{CL} ($\Delta I_{CL} = I_{Blank} - I_{SS}$). In order to prove the occurrence of these reactions, the CL intensities of different systems were measured.

To study the effect of the presence of H_2O_2 and CPF on CL light intensity, the light intensities of different CL systems were measured using the fabricated photometer. Figure 2 shows the response-time curves as well as average responses for different CL systems. The Luminol-$Fe(CN)_6^{3-}$ system (–□–) (Figure 2A) showed weak CL light intensity (~100mV) since $Fe(CN)_6^{3-}$ can oxidize luminol to its excited state, thereby releasing light upon relaxation (Shevlin & Neufeld 1970) . However, the aforementioned CL system did not show significant difference in CL light intensity as compared to luminol-$Fe(CN)_6^{3-}$-CPF system (–△–). On the other hand, luminol-H_2O_2-$Fe(CN)_6^{3-}$-CPF CL system (–▽–) showed a significant decrease in CL light intensity as compared to luminol-H_2O_2-$Fe(CN)_6^{3-}$ (–○–) (Figure 2B). This decrease in CL intensity of the luminol-H_2O_2-$Fe(CN)_6^{3-}$ CL system in the presence of CPF can be attributed to the reaction between CPF and H_2O_2, as well as between peroxophosphonate and luminol. Aside from that, comparing the blank solutions of luminol-H_2O_2-$Fe(CN)_6^{3-}$ CL system and luminol-$Fe(CN)_6^{3-}$ system, the CL system with H_2O_2 has

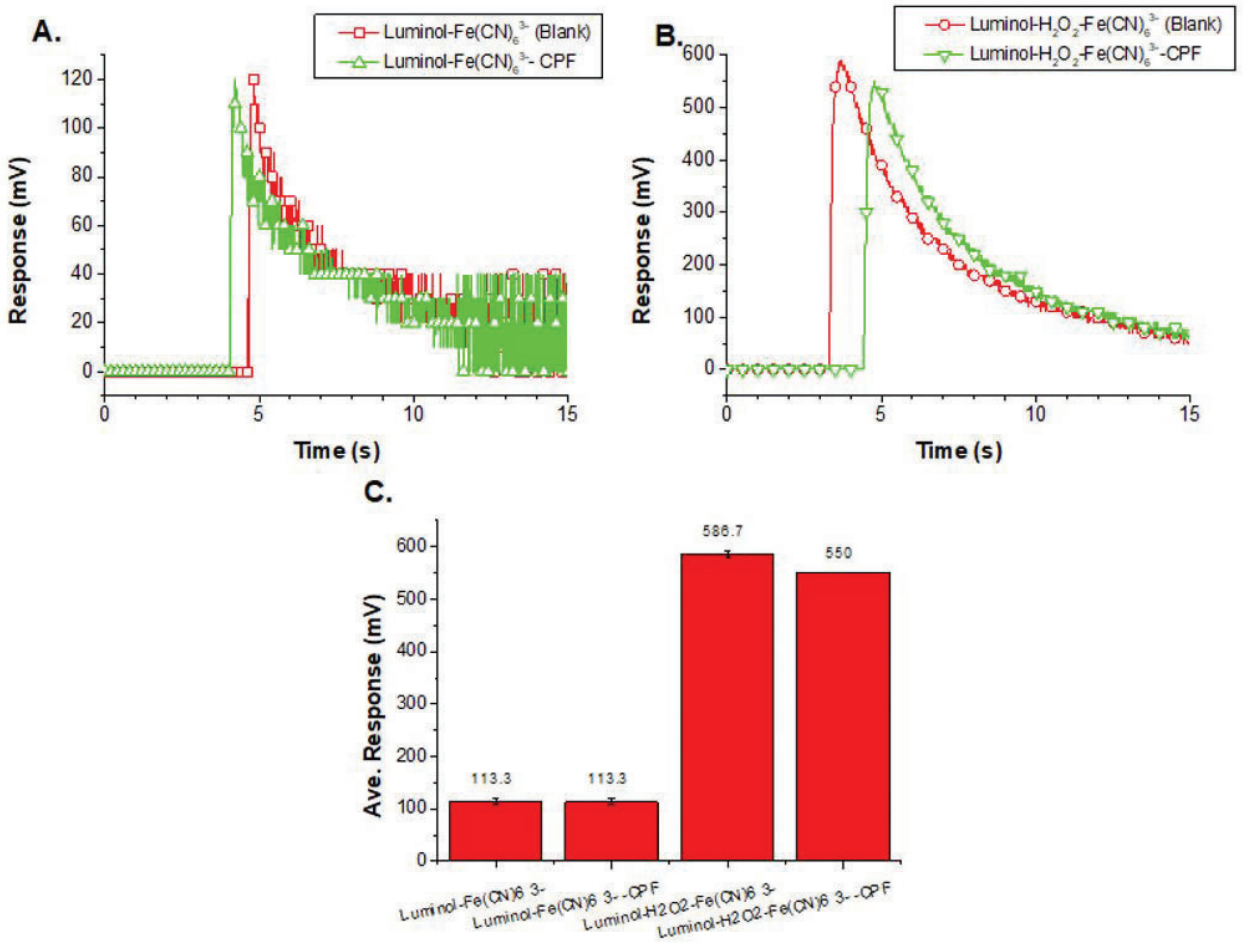

Figure 2. Response-time curves (A–B) and average responses (C) of different CL systems. All CL systems above contain 1 mM luminol and were oxidized by 5 mM $Fe(CN)_6^{3-}$. Concentration of H_2O_2 and CPF are 2 mM and 3 µM, respectively. Three measurements were done for each system.

significantly larger CL intensity – leading to higher signal to noise ratio (S/N). This is due to the fact that $Fe(CN)_6^{3-}$ can oxidize H_2O_2 to form hydroperoxyl radicals ($HO_2\cdot$) (Girdhar & Jain 1965) – which can be an oxidant in the second oxidation step of luminol – to form α-hydroxyperoxide, leading eventually to CL emission (Merényi *et al*. 1990).

The light absorption in the UV and visible regions of the CL components and mixtures were measured in order to determine possible cross-reactions between CL reactants. Figure 3 shows the UV-Vis spectra of individual and mixture of CL components. Figure 3A shows the UV-Vis spectra of CPF solution, H_2O_2 solution, and CPF with H_2O_2 solution. The UV-Vis spectrum of CPF with H_2O_2 solution did not show significant change in the absorbance peak of CPF at around 320 nm. This is because the absorbance at 320 nm arises from the aromatic moiety of CPF and is not affected by H_2O_2. Figure 3B shows the UV-Vis spectra of luminol and CL mixtures with luminol. Two absorbance peaks have been observed for luminol located at around 300 nm and 350 nm. Solution of luminol with H_2O_2 shows no significant increase for both absorbance peaks of luminol. On the other hand, solution of luminol with H_2O_2 and CPF showed an increase in absorbance at 300 nm peak, while still no significant change was seen at 350 nm peak. This is due to the contribution of CPF in the absorption at 320 nm peak, as shown in Figure 3A. Thus, from the presented spectra, it can be deduced that there are no side reactions with the presence of CPF in the CL mixture aside from its reaction with H_2O_2. The CL emission spectra of the luminol-H_2O_2-$Fe(CN)_6^{3-}$ CL system with and without CPF were measured in order to determine the effect of CPF in the CL emission of luminol. Figure 4 shows the CL emission spectra of the CL blank solution and CL sensing solution (*i.e*., with CPF). Both spectra showed a characteristic emission peak for luminol systems at around 450 nm. However, the solution with CPF showed a significant decrease in light intensity, which confirms the observed decrease in

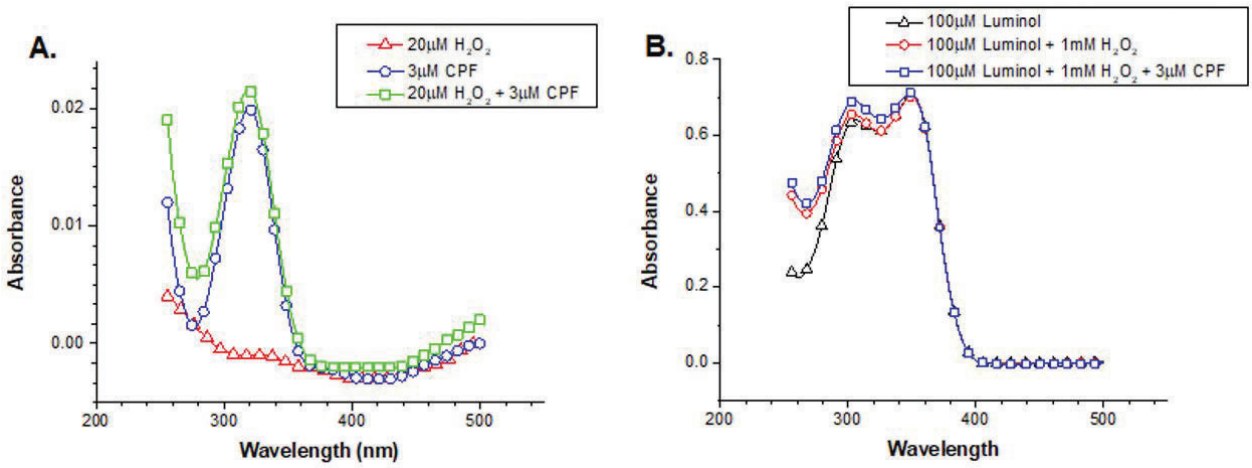

Figure 3. UV-Vis spectra of individual and mixtures of CL components.

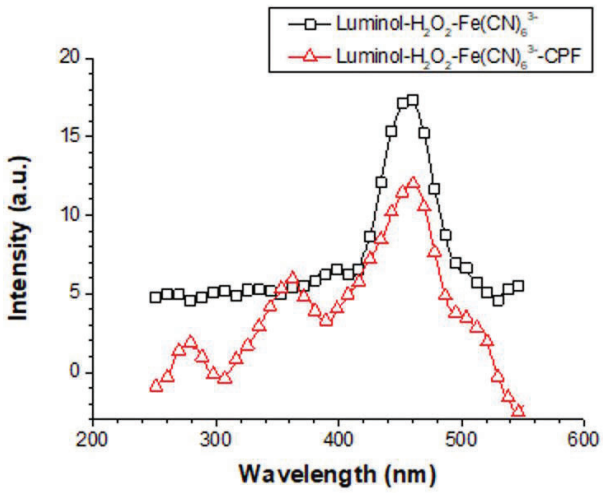

Figure 4. CL emission spectra of blank solution (luminol-H_2O_2-$Fe(CN)_6^{3-}$) and sensing solution (luminol-H_2O_2-$Fe(CN)_6^{3-}$-CPF). The sensing solution contains 3 μM CPF. Both solutions contain 1 mM luminol, 1 mM H_2O_2, and 0.1 M NaOH. Both are oxidized with 5 mM $K_3[Fe(CN)_6]$.

CL intensity using the fabricated photometer. It can also be observed that the emission spectrum of blank solution have steady light intensity from 250 nm to 400 nm, while significant decrease in light intensity at around 300 nm can be observed in the spectrum of sensing solution. The decrease in light intensity can be attributed to the light absorption of CPF species at this region, as shown in the UV-Vis spectrum of CPF at Figure 3A.

Optimization of Solution Parameters

To achieve the highest sensitivity for CPF determination using CL system under study, the concentration of various components in the CL solution (blank and sensing) were optimized using univariate optimization. For the optimization of H_2O_2 concentration, ΔI_{CL} of luminol-H_2O_2-$Fe(CN)_6^{3-}$ CL system was analyzed for different concentrations of H_2O_2 ranging 0.5–5.0 mM. Appendix II shows the optimization of H_2O_2 concentration. From the figure, increasing the H_2O_2 concentration from 0.5 mM to 2 mM increases the value of ΔI_{CL}. Further increase in concentration results to a sudden decrease of ΔI_{CL} value. It is possible that the sudden decrease in ΔI_{CL} value could be due to the fact that at large H_2O_2 concentrations, both blank and sensing solutions will emit significantly large amount of light – where the consumption of H_2O_2 in the sensing solution will be very small as compared to remaining H_2O_2 – thus leading to small ΔI_{CL} value (small difference between I_{Blank} and I_{SS}). Hence, the optimized concentration of H_2O_2 is 2mM. The ΔI_{CL} value for 1 mM and 1.5 mM H_2O_2 were not statistically different, but 2 mM was still chosen as the optimized value in order to achieve the highest sensitivity of the resulting sensor.

For the optimization of $Fe(CN)_6^{3-}$ concentration, the ΔI_{CL} values for luminol-H_2O_2-$Fe(CN)_6^{3-}$ CL system for different concentrations of $Fe(CN)_6^{3-}$ were compared. Appendix III shows the optimization of $Fe(CN)_6^{3-}$ concentration. An increase in ΔI_{CL} value can be observed upon increase of $Fe(CN)_6^{3-}$ concentration from 1 mM to 10 mM. However, there is a sudden decrease in ΔI_{CL} value upon increasing the $Fe(CN)_6^{3-}$ concentration to 12 mM. The ΔI_{CL} value then remains significantly the same with further increase of $Fe(CN)_6^{3-}$ concentration. Hence, the optimized concentration of $Fe(CN)_6^{3-}$ is 10 mM.

For the optimization of luminol concentration, the ΔI_{CL} values were compared for luminol-H_2O_2-$Fe(CN)_6^{3-}$ CL systems – with varying luminol concentration ranging 1.0–9.0 mM, as shown in Appendix IV. From the figure,

there were no significant changes in ΔI_{CL} values for CL system with 1.0–4.0 mM luminol concentration. The ΔI_{CL} values then increase upon raising the luminol concentration up to 6 mM. However, a sudden decrease in ΔI_{CL} values was observed upon increasing the luminol concentration further to 7.0 mM. Hence, the optimized concentration of luminol is 6 mM. It is possible that the steady ΔI_{CL} values observed upon using 1–4mM luminol concentration shows that no CL reaction route is dominating the other on these luminol concentrations. The increase in measured signal with further increase in the luminol concentration up to 6 mM could be due to domination of the luminol-H_2O_2-$Fe(CN)_6^{3-}$ CL reaction route, leading to a more pronounced difference between blank and sensing solution. Lastly, it is possible that the sudden decrease in ΔI_{CL} values upon increasing further the luminol concentration to 7 mM could be due to domination of competing luminol-$Fe(CN)_6^{3-}$ CL reaction, thus leading to a lesser difference in CL intensity between blank and sensing solution. The ΔI_{CL} values for 5 mM and 6 mM H_2O_2 are not statistically different, but the 6 mM H_2O_2 was still chosen as the optimized value to ensure the highest sensitivity for the resulting sensor.

Lastly, the pH of both blank and sensing solution was optimized. The pH values of the solutions were adjusted using NaOH solution. For pH optimization, the ΔI_{CL} values of the luminol-H_2O_2-$Fe(CN)_6^{3-}$ with varying pH were compared. Appendix V shows the optimization of reaction solution pH. As clearly shown in the figure, increasing the pH of both blank and sensing solution increases the ΔI_{CL} values. This is due to the fact that luminol CL reaction is enhanced at more basic media in both aqueous and polar aprotic solvent such as DMSO (Khan *et al*. 2014). Hence, the optimized pH of both blank and sensing solution is pH 12.7.

To summarize, the optimized parameters for CPF determination using luminol-H_2O_2-$Fe(CN)_6^{3-}$ CL system were as follows: 2 mM H_2O_2, 10 mM $K_3[Fe(CN)_6]$, 6 mM luminol, and pH 12.7. The optimized parameters were applied to both blank and sensing solution.

CPF Determination

Using the optimized parameters, a calibration curve was done by measuring the ΔI_{CL} values at different CPF concentrations. Figure 5 shows the calibration and the response-time curves, which were recorded for different CPF concentrations. As shown in Figure 5A, a linearly increasing ΔI_{CL} value was observed with increasing CPF concentration. Figure 5B shows that the CL intensity decreases with increasing CPF concentration, which is due to consumption of H_2O_2 and luminol. The limit of detection (LOD) for CPF determination for the said system is calculated to be 0.663 ppm (1.89 μM). The calculated LOD for the proposed sensing method is much lower than the maximum residual limit (MRL) of CPF for banana (3 ppm CPF) as set by the European Union (EU) Commission (EC 2014). Table 1 summarizes the analytical merits for this method of CPF determination.

Table 1. Summary of analytical merits for method of CPF determination.

Figures of Merit	Values
Linear Range	0.7–2.45 ppm CPF
Linearity (r^2)	0.99045
Sensitivity	117 mV/ppm
LOD ($3\sigma_{Blank}$)	0.663 ppm (1.89 μM)

The concentrations in the calibration curve use ppm (mg CPF/ kg solvent). In case of using extraction solution, the measured concentration of the sensing solution – through the calibration curve – will be converted into ppm pesticide in fruit (mg CPF/ kg fruit or vegetable sample) using the equation below:

$$ppm\left(\frac{mg\ CPF}{kg\ sample}\right) = \left(\frac{mg\ CPF}{kg\ solvent}\right)\left(\frac{0.025\ kg\ solvent}{250\ x\ 10^{-6} kg\ solvent}\right) \quad (1)$$

$$(weight\ of\ solvent\ used\ in\ extraction\ in\ kg)\left(\frac{1}{weight\ of\ sample\ in\ kg}\right)$$

The use of the equation above is rather a crude method of converting from weight of fruit to weight of solvent used. Technically, the process could be idealized by considering that the mg pesticide present in a particular mass of fruit or vegetable will be transferred to the extraction solving having the same mass as the fruit or vegetable. However, it is possible that not all of the pesticide is transferred into the extraction solvent and – in many cases – extraction only uses a small amount of solvent (*i.e*., not necessary as the same mass as the fruit or vegetable) to allow for 'pre-concentration' of the extracted pesticide. At best, the LOD serves to provide a mark of the sensitivity limit of the optimized CPF-luminol detection method, which can be directly correlated to the available pesticide from the source.

Linear correlation analysis of the calibration curve was performed, and the coefficient of linearity was evaluated by determining the r-squared (R^2) value. The R^2 value was determined to be near unity, hence the concentration of CPF is linearly correlated with ΔI_{CL}. Similar studies using luminol-$Fe(CN)_6^{3-}$ system has R^2 value closer to unity (Du *et al*. 2001a, 2001b, 2002; Liu *et al*. 2005). However, these studies utilized PMT as light detector, which has greater performance in light sensing as compared to a photodiode. In order to evaluate if the linear model is the best fit for the calibration curve, a residual analysis was done. Appendix VI shows the residual plot for the calibration curve of the studied CPF sensing. The residual plot

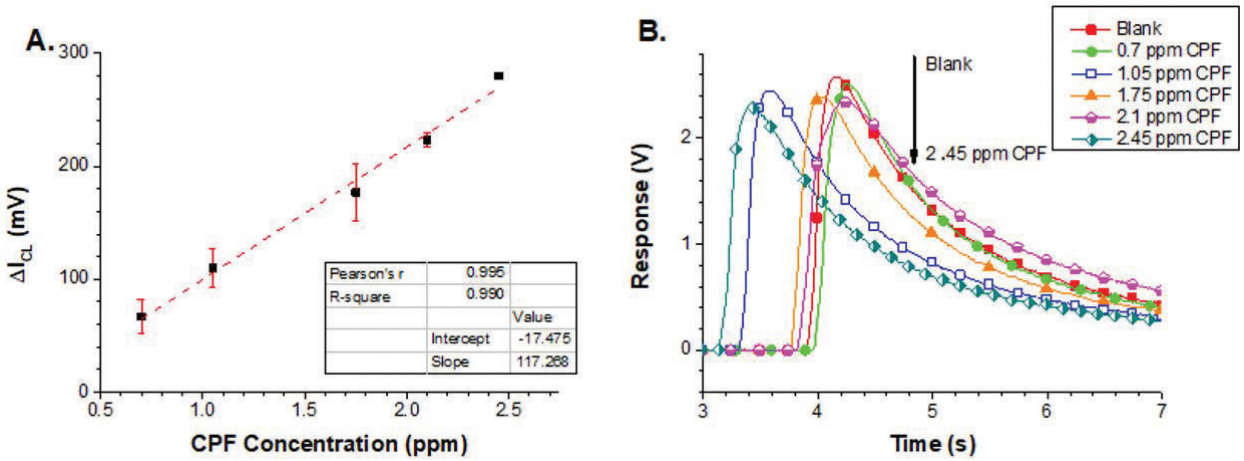

Figure 5. Calibration curve for CPF determination. Sensing solution conditions: 6 mM luminol, 2 mM H_2O_2, CPF (varying concentration), and pH 12.7. Blank solution conditions: 6 mM luminol, 2 mM H_2O_2, pH 12.7, and in 1% ethanol. Oxidant solution condition: 10 mM $K_3[Fe(CN)_6]$. Three measurements were done for each concentration.

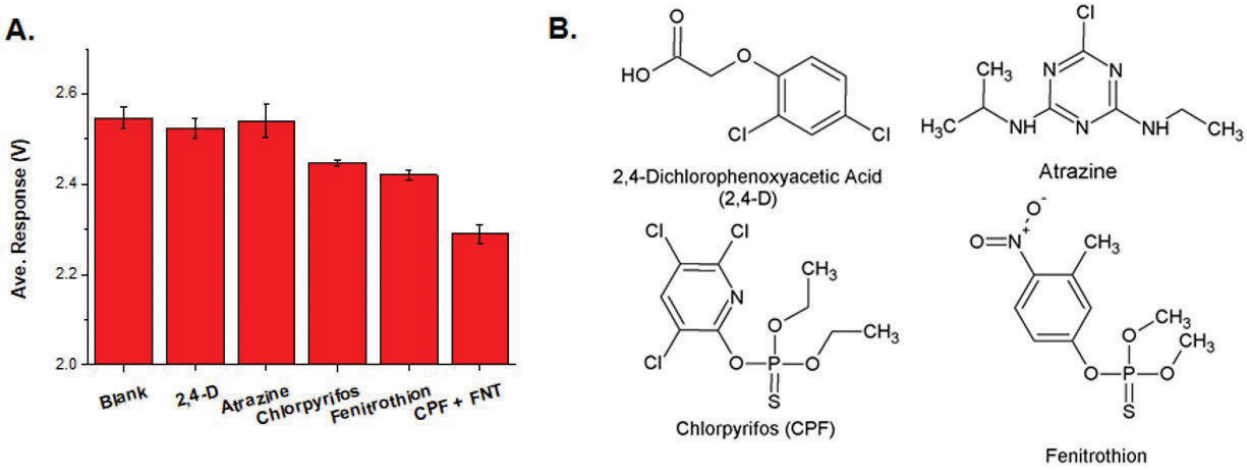

Figure 6. (A) CL response to 2,4-D and atrazine as compared to CPF, fenitrothion (FNT), 1:1 CPF-FNT, and blank solution. (B) Chemical structures of different pesticides/ herbicides. Optimized conditions are used for both blank and sensing solutions. Pesticides/ herbicides in sensing solutions are in 3 μM concentration.

showed random behavior of the measurements, indicating that the residuals do not contradict the linear assumption of the calibration curve. This means that the calculated least square regression of the calibration curve is a good predictor of CPF concentration using the measured ΔI_{CL}.

Response towards other Herbicides/Pesticides

To assess the selectivity of the luminol-H_2O_2-$Fe(CN)_6^{3-}$ CL system for organophosphate (*e.g.*, CPF) detection, the CL responses of non-organophosphate [2,4-dichlorophenoxyacetic acid (2,4-D) and atrazine] sensing solutions were recorded and compared to blank solution and organophosphate [CPF and fenitrothion (FNT)] sensing solutions. Figure 6A shows the average responses of blank and different sensing solutions. From the figure, the average responses of sensing solutions with non-organophosphate compounds (*i.e.*, 2,4-D and atrazine; structures shown in Figure 6B) showed no significant difference with that of blank solution. On the other hand, the sensing solution with CPF showed a significant decrease as compared to the blank solution. These results showed that 2,4-D and atrazine do not react with any of the species present in the solution that will lead to CL reactant consumption. Therefore, the said compounds will not be a potential interference to CPF sensing. The sensing solution with FNT, which is also an organophosphate, was observed to have similar decrease in CL intensity as compared with CPF sensing solution. This can be attributed to the fact that H_2O_2 reacts only with organophosphates and not to non-organophosphates that were studied (2,4-D and

atrazine). This indicates that the proposed sensing method is selective for organophosphate pesticides. Sensing solution with FNT and CPF showed a larger decrease due to larger concentration of organophosphate present. Thus, the proposed method can be applied for total organophosphate determination.

CONCLUSION

A low-cost and portable photometer has been constructed for CL detection of organophosphates such as CPF. Due to low concentration occurrence of CPF in real samples, an indirect method of CPF determination has been employed in order for PD to be used as CL detector. In this study, the detection is based on the consumption of CL components through a known reaction with organophosphates. Different parameters such as pH and reactant concentrations were optimized for the CPF detection. Two CL reaction routes – luminol-$Fe(CN)_6^{3-}$ and luminol-H_2O_2-$Fe(CN)_6^{3-}$ – has been presented, but only the luminol-H_2O_2-$Fe(CN)_6^{3-}$ gave a significant difference between blank and sensing solution. The LOD for CPF detection was calculated to be 0.663 ppm CPF. CL responses towards non-organophosphates herbicides such as 2,4-D and atrazine showed no significant changes as compared to blank solution. Other organophosphate such as FNT showed similar response with CPF. Thus, the designed photometer-based detection system with microcontroller was proven to be effective for the sensitive detection of organophosphates such as CPF and FNT. The design may be further developed into a portable sensor employing the CL-based detection of organophosphate pesticides. Real sample analyses on a banana sample were also done by combining the developed method shown in this study and a widely used real sample preparation called the QuECheRS method (which stands for 'Quick, Easy, Cheap, Rugged, Safe'). However, initial results showed that the acetonitrile that is used in the QuECheRS method significantly quenched the CL signal, even at 1% (v/v) concentration in the final sensing solution. It is therefore recommended to either use ethanol in the QuECheRS method, or use entirely different sample preparation that will use ethanol as the final solvent for CPF.

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NOTE ON APPENDICES

The complete appendices section of the study is accessible at http://philjournsci.dost.gov.ph

REFERENCES

ARMENTA S, QUINTÁS G, GARRIGUES S, DE LA GUARDIA M. 2005. A validated and fast procedure for FTIR determination of Cypermethrin and Chlorpyrifos. Talanta 67(3): 634–639. doi: http://dx.doi.org/10.1016/j.talanta.2005.03.008

DODEIGNE C, THUNUS L, LEJEUNE R. 2000. Chemiluminescence as diagnostic tool. A review. Talanta 51(3): 415–439. doi: http://dx.doi.org/10.1016/S0039-9140(99)00294-5

DU J, LI Y, LU J. 2001a. Flow injection chemiluminescence determination of polyhydroxy phenols using luminol–ferricyanide/ferrocyanide system. Talanta 55(6): 1055–58. doi: https://doi.org/10.1016/S0039-9140(01)00452-0

DU J, LI Y, LU J. 2001b. Flow Injection Chemiluminescence Determination of Rutin based on its enhancing effect on the Luminol-Ferricyanide/Ferrocyanide system. Analytical Letters 34(10): 1741–48. doi:10.1081/AL-100105357

DU J, LI Y, LU J. 2002. Flow injection chemiluminescence determination of captopril based on its enhancing effect on the luminol–ferricyanide/ferrocyanide reaction. Luminescence 17(3): 165–167. doi:10.1002/bio.685

[EC] EU Commission. 2014. Commission Regulation (EU) 2016/60. Official Journal of the European Union 12(4): 3640. doi:10.2903/j.efsa.2014.3640

GÁMIZ-GRACIA L, GARCÍA-CAMPAÑA AM, SOTO-CHINCHILLA JJ, HUERTAS-PÉREZ JF, GONZÁLEZ-CASADO A. 2005. Analysis of pesticides by chemiluminescence detection in the liquid phase. TrAC Trends in Analytical Chemistry 24(11): 927-942. doi: http://dx.doi.org/10.1016/j.trac.2005.05.009

GIRDHAR KK, JAIN DVS. 1965. Kinetics of the decomposition of hydrogen peroxide in presence of ferricyanide. Journal of Inorganic and Nuclear Chemistry 27(12): 2653–57. doi: http://dx.doi.org/10.1016/0022-1902(65)80169-5

GUARDINO X, OBIOLS J, ROSELL MG, FARRAN A, SERRA C. 1998. Determination of chlorpyrifos in air, leaves and soil from a greenhouse by gas-chromatography with nitrogen-phosphorus detection, high-performance liquid chromatography and capillary electrophoresis. Journal of Chromatography

A 823(1–2): 91–96. doi: http://dx.doi.org/10.1016/S0021-9673(98)00272-6

HU H, LIU X, JIANG F, YAO X, CUI X. 2010. A novel chemiluminescence assay of organophosphorous pesticide quinalphos residue in vegetable with luminol detection. Chemistry Central Journal 4(1): 13. doi:10.1186/1752-153X-4-13

KAVIYARASAN K, ANANDAN S, MANGALARAJA RV, SIVASANKAR T, ASHOKKUMAR M. 2016. Sonochemical synthesis of Cu_2O nanocubes for enhanced chemiluminescence applications. Ultrasonics Sonochemistry 29: 388–393. doi: http://dx.doi.org/10.1016/j.ultsonch.2015.10.018

KHAN P, IDREES D, MOXLEY MA, CORBETT JA, AHMAD F, VON FIGURA G, SLY WS, WAHEED A, HASSAN MI. 2014. Luminol-based Chemiluminescent Signals: Clinical and Non-clinical Application and Future Uses. Applied Biochemistry and Biotechnology 173(2): 333–355. doi:10.1007/s12010-014-0850-1

KUBÍNOVÁ Š, ŠLÉGR J. 2015. ChemDuino: Adapting Arduino for Low-Cost Chemical Measurements in Lecture and Laboratory. Journal of Chemical Education 92(10): 1751–53. doi:10.1021/ed5008102

KUSWANDI B, FIKRIYAH CI, GANI AA. 2008. An optical fiber biosensor for chlorpyrifos using a single sol-gel film containing acetylcholinesterase and bromothymol blue. Talanta 74(4): 613–618. doi: http://dx.doi.org/10.1016/j.talanta.2007.06.042

LI A, LIU X, KONG J, HUANG R, WU C. 2008. Chemiluminescence Determination of Organophosphorus Pesticides Chlorpyrifos in Vegetable. Analytical Letters 41(8): 1375–86. doi:10.1080/00032710802119228

LI Y, YOU X, SHI X. 2017. Enhanced Chemiluminescence Determination of Hydrogen Peroxide in Milk Sample Using Metal-organic Framework Fe-MIL-88NH2 as Peroxidase Mimetic. Food Analytical Methods 10(3): 626–633. doi:10.1007/s12161-016-0617-0

LI YC, MELENBRINK EL, CORDONIER GJ, BOGGS C, KHAN A, ISAAC MK, NKHONJERA LK, BAHATI D, BILLINGE SJ, HAILE SM, KREUTER RA, CRABLE RM, MALLOUK TE. 2018. An Easily Fabricated Low-Cost Potentiostat Coupled with User-Friendly Software for Introducing Students to Electrochemical Reactions and Electroanalytical Techniques. Journal of Chemical Education 95(9): 1658–61. doi:10.1021/acs.jchemed.8b00340

LIU H, ZHANG L, ZHOU J, HAO Y, HE P, FANG Y. 2005. Flow injection chemiluminescence determination of dobutamine hydrochloride injection using luminol–ferricyanide/ferrocyanide system. Analytica Chimica Acta 541(1): 123–127. doi: https://doi.org/10.1016/j.aca.2004.11.071

LIU M, LIN Z, LIN J-M. 2010. A review on applications of chemiluminescence detection in food analysis. Analytica Chimica Acta 670(1–2): 1–10. doi: http://dx.doi.org/10.1016/j.aca.2010.04.039

LIU T, XU M, YIN H, AI S, QU X, ZONG S. 2011. A glassy carbon electrode modified with graphene and tyrosinase immobilized on platinum nanoparticles for sensing organophosphorus pesticides. Microchimica Acta 175(1): 129. doi:10.1007/s00604-011-0665-5

LUKASZEWICZ-HUSSAIN A. 2010. Role of oxidative stress in organophosphate insecticide toxicity – Short review. Pesticide Biochemistry and Physiology 98(2): 145–150. doi: http://dx.doi.org/10.1016/j.pestbp.2010.07.006

MARTÍNEZ GALERA M, MARTÍNEZ VIDAL JL, FRENICH AG. 1994. Simultaneous Determination of Atrazine and Chlorpyrifos in Pesticide Formulations, in Soils and Waters by Derivative Spectrophotometry and Ratio Spectra Derivative. Analytical Letters 27(4): 807–818. doi:10.1080/00032719408000273

MAURIZ E, CALLE A, LECHUGA LM, QUINTANA J, MONTOYA A, MANCLÚS JJ. 2006. Real-time detection of chlorpyrifos at part per trillion levels in ground, surface and drinking water samples by a portable surface plasmon resonance immunosensor. Analytica Chimica Acta 561(1–2): 40–47. doi: http://dx.doi.org/10.1016/j.aca.2005.12.069

MERÉNYI G, LIND J, ERIKSEN TE. 1990. Luminol chemiluminescence: Chemistry, excitation, emitter. Journal of Bioluminescence and Chemiluminescence 5(1): 53–56. doi:10.1002/bio.1170050111

MULCHANDANI A, CHEN W, MULCHANDANI P, WANG J, ROGERS KR. 2001. Biosensors for direct determination of organophosphate pesticides. Biosensors and Bioelectronics 16(4–5): 225–230. doi: http://dx.doi.org/10.1016/S0956-5663(01)00126-9

OLIVA J, NAVARRO S, BARBA A, NAVARRO G. 1999. Determination of chlorpyrifos, penconazole, fenarimol, vinclozolin and metalaxyl in grapes, must and wine by on-line microextraction and gas chromatography. Journal of Chromatography A 833(1): 43–51. doi: http://dx.doi.org/10.1016/S0021-9673(98)00860-7

PARK J-M, JUNG H-W, CHANG YW, KIM H-S, KANG M-J, PYUN J-C. 2015. Chemiluminescence lateral flow immunoassay based on Pt nanoparticle with peroxidase activity. Analytica Chimica Acta 853: 360–367. doi: http://dx.doi.org/10.1016/j.aca.2014.10.011

ROBARDS K, WORSFOLD PJ. 1992. Analytical applications of liquid-phase chemiluminescence. Analytica Chimica Acta 266(2): 147–173. doi: http://dx.doi.org/10.1016/0003-2670(92)85040-D

SHEVLIN PB, NEUFELD HA. 1970. Mechanism of the ferricyanide-catalyzed chemiluminescence of luminol. The Journal of Organic Chemistry 35(7): 2178–82. doi:10.1021/jo00832a015

TUNÇELI A, BAĞ H, TÜRKER RA. 2001. Spectrophotometric determination of some pesticides in water samples after preconcentration with *Saccharomyces cerevisiae* immobilized on sepiolite. Fresenius' Journal of Analytical Chemistry 371(8): 1134–38. doi:10.1007/s002160101088

UYGUN ZO, DILGIN Y. 2013. A novel impedimetric sensor based on molecularly imprinted polypyrrole modified pencil graphite electrode for trace level determination of chlorpyrifos. Sensors and Actuators B: Chemical 188: 78–84. doi: http://dx.doi.org/10.1016/j.snb.2013.06.075

VISWANATHAN S, RADECKA H, RADECKI J. 2009. Electrochemical biosensor for pesticides based on acetylcholinesterase immobilized on polyaniline deposited on vertically assembled carbon nanotubes wrapped with ssDNA. Biosensors and Bioelectronics 24(9): 2772–77. doi: http://dx.doi.org/10.1016/j.bios.2009.01.044

YOTTER RA, WILSON DM. 2003. A review of photodetectors for sensing light-emitting reporters in biological systems. IEEE Sensors Journal 3(3): 288–303. doi:10.1109/JSEN.2003.814651

YU D, WANG P, ZHAO Y, FAN A. 2016. Iodophenol blue-enhanced luminol chemiluminescence and its application to hydrogen peroxide and glucose detection. Talanta 146: 655–661. doi: http://dx.doi.org/10.1016/j.talanta.2015.06.059

ZAMFIR L-G, ROTARIU L, BALA C. 2011. A novel, sensitive, reusable and low potential acetylcholinesterase biosensor for chlorpyrifos based on 1-butyl-3-methylimidazolium tetrafluoroborate/multiwalled carbon nanotubes gel. Biosensors and Bioelectronics 26(8): 3692–95. doi: http://dx.doi.org/10.1016/j.bios.2011.02.001