Portable two-color photometer based on paired light emitter detector diodes and its application to the determination of paraquat and diquat

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Abstract

Here we describe a methodology for the determination of paraquat and diquat using a newly developed portable photometer equipped with two colors of paired light emitter detector diodes (PEDD). The colorimetric measurements employed in this work include the redox reactions between 1) dithiothreitol and diquat to produce the red color characteristic of a diquat radical and 2) between sodium dithionite and either diquat or paraquat that results in the green and blue colors of diquat and paraquat radicals, respectively. The addition of sodium dithionite or dithiothreitol in a solid-state provides reproducible absorbance of the radicals, prevents decomposition of the reagents in a solution, and simplifies handling of the reagents. The diquat radical produced by dithiothreitol (λ_{max} = 495 nm) was successfully detected by using a pair of blue LEDs with a maximum emission wavelength at 472 nm while the radicals of paraquat (λ_{max} = 603 nm) and diquat (λ_{max} = 771 nm) reduced by sodium dithionite were measured by a pair of orange LEDs with a maximum emission wavelength of 609 nm. The proposed method consists of measuring diquat radicals at 472 nm, estimating the absorbance of diquat radicals at 609 nm, and subtracting the estimated absorbance of diquat radicals from the total absorbance at 609 nm to determine paraquat radicals. The developed method yielded examples of excellent linear regression (r^2) of more than 0.99 in three calibration curves of the radicals measured at 472 nm for diquat radicals and measured at 609 nm for both diquat and paraquat radicals. The intra-day (n = 3) and inter-day (n = 3) precision of three calibration curves were less than or equal to 5%. By comparison with the standard method of high-performance liquid chromatography, the reliability of the proposed method was proven via the analysis of paraquat and diquat radicals in a commercially available herbicide.

1. Introduction

In 1973, Flaschka et al. first reported the development of a photometric detection device using a light-emitting diode (LED) as a light source and a phototransistor as a light detector [1]. Since then, studies on the development of the devices and systems utilizing LEDs as a light source and a detector have increased due to several advantages of LEDs such as a wide range of emitting wavelengths, long lifetime, low operation voltage, small size, inexpensiveness, and quick response. Researchers have created LED-based photometric detection devices in several designs and have applied them to a broader range of fields. For example, Chuntib et al. combined a flow injection system with a red light-emitting diode-light dependent resistor (LED-LDR) colorimeter for paraquat analysis in water [2]. Lau et al. employed a paired emitter detector diode (PEDD) using LEDs emitting at 621 nm for measuring the pH levels of buffer solutions with bromocresol green as an indicator [3]. Reis group developed LED-based photometer using various path lengths of flow cell to achieve ethanol determination in beverages [4].

Multi-color detection is one of the challenges of using LEDs in photometric detectors for absorbance measurement in the determination of multiple analytes. For several decades, scientists have reported the use of LEDs in detection. For instance, Catarino et al. fabricated a photometric system equipped with an LED array with six different emission wavelengths as light emitters and photodiodes as light sensors to quantify hemozoin in blood samples [5]. Sorouraddin et al. used a red-blue-green LED as a light source and LDR as a light sensor to analyze a five-color mixture of food dyes [6]. Some photometric detection devices required a flow system to accomplish simultaneous determination, in which the devices would need a plug-in power supply for the flow system [7]. Furthermore, the detection system had to employ three power supplies for the light source LEDs, photodetectors, and amplifier. Conversely, LED as the light detector simplifies the device since no power supply is necessary to generate photovoltaic output. To our best knowledge, there has been no report of a miniaturized PEDD photometric detector that permits multi-color measurements without the flow system and plug-in power supplies for light detectors to achieve a fully portable device with low power consumption.

Diquat and paraquat are well-known herbicides that are frequently employed in agricultural fields. Diquat has less toxicity while paraquat leads to serious problems for human health because it accumulates in the lung tissues of mammalians [8, 9]. Because of the similarity in their chemical structures [10], diquat interferes with paraquat analysis, which we encountered in previous work [11]. Therefore, a spectrophotometer equipped with a scanning mode or a photometer with a multi-color detection mode is required to achieve simultaneous determinations of these chemicals [12]. On the other hand, separation techniques such as high-performance liquid chromatography (HPLC) [13], gas chromatography (GC) [14], and capillary electrophoresis (CE) [15] have also been employed for simultaneous analysis. Nevertheless,

some of the disadvantages are unavoidable, and these include the high cost of a lamp or a column, complicated operations, and the large sizes of instruments, which prohibit use in poorly equipped laboratories and in the field.

The determination between paraquat and diquat is a challenge that is an important issue because these compounds are extensively used as herbicides in agricultural countries. Paraquat is more popular than diquat but they are often mixed to prevent a fatal poisoning from a concentrated paraquat solution [10]. The Japanese association of rural medicine began promoting the co-formulation of these chemicals in 1985 in order to prevent intentional uses such as self-annihilation/murder or accidental exposure during agricultural activity [16]. Surveys to determine the rates of death from these herbicides have established that most of the cases occur in rural areas of Japan [16] where there is less facility of scientific instruments for monitoring contamination from herbicides. Therefore, a simple, inexpensive, and portable device that could analyze both of these dangerous quaternary ammonium compounds is required in both advanced and developing countries.

In this work, we demonstrated the miniaturization of a photometer using two pairs of LEDs as light emitters and light sensors for two-color measurements. The system is operated by rechargeable batteries, which are enclosed in a small box. The proposed photometer gave promising results when using two reducing reagents for the colorimetric reactions of paraquat and diquat. Diquat was reduced by dithiothreitol to produce a red-colored radical that was selectively measured at 472 nm without the interference of paraquat. Conversely, sodium dithionite reduced both paraquat and diquat to produce blue and green radicals that were measured at 609 nm. Therefore, paraquat was measured by subtracting the absorbance of diquat from the total absorbance at 609 nm. Good analytical characteristics in terms of linearity, reproducibility, and sensitivity were obtained using the developed two-color photometer. Finally, our methodology was validated by the determinations of paraquat and diquat in a commercially available herbicide, which agreed with the results of high-performance liquid chromatography.

2. Materials and methods

2.1 PEDD dual detection system setup and instrumentation

The portable system equipped with two-color paired light emitter detector diodes (PEDD) is displayed in Figure 1. A homemade aluminum cell holder was designed and fabricated with four windows that featured two pairs of LEDs placed perpendicular to one another. Blue LEDs (472 nm) and orange LEDs (609 nm) with 5 mm diameters were acquired from Elpa Asahi Electric Co., Ltd. (Osaka, Japan) and DiCUNO JP Direct (Tokyo, Japan), respectively. Each pair of LEDs faced one another to play the roles of a light source and detector. Plastic lenses (SODIAL lenses, 2.2×1.4 cm, 95% transmittance) were attached to each window in order to focus light at the center of the sample cuvette. The system requires six Li-Po

rechargeable dry cell batteries (~9 V, 800 mAh, Keenstone Ltd., CA, USA); two batteries were connected with voltage regulators (Drok, Hong Kong) to maintain a constant output voltage for the LED lights. Four batteries power two amplification units to enhance output signals from the LED light detectors. The circuit of the amplification unit was reported in our previous publication [17]. A multimeter in DC voltage mode (TDE-14, Trusco Nakayama Co., Tokyo, Japan) served as a signal readout device to measure the photovoltaic power produced by the LED light detectors. The entire system was placed in an aluminum box (18×20×9 cm), which made it suitable for portability and on-site applications. The developed device fulfills the ASSURES criteria (Affordable, Sensitive, Specific, User-friendly, Rapid and robust, Equipment-free, and Deliverable to end-users) that are suggested by the World Health Organization (WHO) [18], particularly in terms of deliverability to end-users and affordability with a total fabrication price of approximately 10,000 JPY, which corresponds to roughly 85 USD. The absorption spectra of paraquat and diquat radicals were measured via a UV-Vis spectrophotometer (UV-2400PC, Shimadzu, Kyoto, Japan) to confirm their absorption maxima.

2.2 Chemicals and reagents

Chemicals and reagents that were either of analytical grade or certified reference materials were utilized in this work. Paraquat, diquat, sodium dithionite (SDT), and dithiothreitol (DTT) were obtained from Sigma-Aldrich (MO, USA). Sodium hydroxide (NaOH) was purchased from Waka Pure Chemical Industries (Osaka, Japan). Purified water was supplied by a Milipore Direct-Q system (Milipore Co. Ltd., Molsheim, France). Commercial herbicide was obtained from a local market.

Appropriate amounts of paraquat and diquat were weighed and dissolved in 25 mL of water to reach a stock concentration at 500.0 mg L^{-1}. The solutions were kept at 4 °C until use. A stock solution of NaOH was prepared at 5.0 mol L^{-1} in water. A stock solution of DTT (0.2 mol L^{-1}) was freshly prepared by dissolving DTT in water.

2.3 Validation

Analytical features of the developed PEDD-based photometer including dynamic range, limits of detection (LOD) and quantification (LOQ), accuracy, and intra- and inter-day precision were studied under selected conditions. Working solutions of paraquat and diquat were prepared in 100 mmol L^{-1} NaOH (pH 13). Paraquat was prepared at 2.0, 5.0, 10.0, 20.0 and 40.0 mg L^{-1} and diquat was diluted to 5.0, 20.0, 40.0, 80.0, and 100.0 mg L^{-1}. The calibration curves of paraquat and diquat radicals using the orange LED (λ_{max} = 609 nm) were constructed by adding SDT powder into each solution. The calibration curve of diquat using the blue LED (λ_{max} = 472 nm) was constructed by adding DTT powder as a reducing agent. The

calibration curves were obtained from the linear fitting between the concentration of the analyte (x-axis) and the absorbance (y-axis), which was calculated by $absorbance = -log\frac{I}{I_0}$, where I is the potential (V) when the cuvette contains an analyte solution and I_0 is the potential (V) when the cuvette contains water. The LOD and LOQ are assigned by $\frac{3.3\,S_{y/x}}{m}\sqrt{1+h_0+\frac{1}{N}}$ and $\frac{10\,S_{y/x}}{m}\sqrt{1+h_0+\frac{1}{N}}$, where $S_{y/x}$ is the residual standard deviation, m is the slope of the univariate calibration graph, h_0 is the leverage for a blank sample, and N is the number of the samples employed for constructing the calibration curve, as reported by Olivieri [18]. The relative standard deviations (%RSD) of the slopes in the calibration curves obtained from the same day (n = 3) and on different days (n = 3) were used to report intra- and inter-day precision, respectively. Accuracy of the developed system was defined in terms of percentage recovery (%Recovery), which was calculated using the following equation: $\%\text{Recovery} = \frac{C_2 - C_1}{C_0} \times 100\%$, where C_0 is the concentration of the spiked standard (10 mg L^{-1} of each analyte), C_1 is the concentration of the analyte found in a non-spiked sample, and C_2 is the concentration of the analyte found in a spiked sample.

2.4 Determination of paraquat and diquat in a herbicide sample

To prove the applicability of our system for the discriminable measurements of paraquat and diquat, a commercial herbicide containing both paraquat and diquat was purchased from a local market and employed as a practical sample. The herbicide solution was diluted 500 times by water and then passed through a C18 Cartridge (Thermo Scientific™ HyperSep ™) to remove a blue dye contained in the sample. For analysis, the solution was diluted 10 times with 100 mmol L^{-1} NaOH before adding the reducing agent (SDT or DTT). Therefore, the total dilution factor of the sample was 5,000-fold.

High-performance liquid chromatography (HPLC) with UV-Vis detection (EXTREMA, JASCO Corporation, Tokyo, Japan) was employed as a standard method to verify the concentrations of paraquat and diquat in the commercial herbicide that were determined by our device. Mixtures of paraquat and diquat were injected into the sample loop (20 µL) and introduced into a reversed-phase column (Inertsil™, ODS-2.5 µm, 4.6×150 mm, GL Sciences, Tokyo, Japan) by a mobile phase consisting of 200 mmol L^{-1} phosphoric acid, 0.1 mol L^{-1} dimethylamine, 12 mmol L^{-1} sodium 1-heptanesulfonate, and 20% MeOH, as reported by Hara et al. [11]. The separation was performed via the isocratic mode at room temperature with a flow rate of 0.5 mL min^{-1} and a detection wavelength of 290 nm.

3. Results and discussion

3.1 Fabrication of the portable dual PEDD system

The PEDD system consisted of two voltage regulators for LED light sources, a homemade aluminum plate holder equipped with four windows and lenses, and two amplification units. Two LEDs with the same emission wavelength were placed facing each other as illustrated in Figure 1. Six rechargeable batteries (~9 V) operated the entire system, because the LEDs and amplification units require only small operation voltages. The device is completely portable with all compartments housed in an aluminum box (size 18.0×19.8 cm) without a need for the external power supplies that are generally required for conventional instruments. It should be noted that rechargeable batteries are more apt to provide reproducible results than non-chargeable batteries because of their stable output, as mentioned in our prior publication [17].

The selection of the proper LEDs for the determinations of paraquat and diquat was accomplished by matching the emission maxima of LEDs with the absorption bands of paraquat and diquat radicals. A paraquat radical has a maximum wavelength at 603 nm, which meant that the 609 nm maximum emission wavelength of an orange LED was suitable for paraquat analysis. The absorption maximum of a diquat radical is 495 nm when using DTT as a reducing agent so that a blue LED with a maximum emission wavelength of 472 nm was chosen for diquat analysis. The operating voltages for both of these LEDs were selected based on the characteristics (dynamic range, sensitivity, and r^2) of the calibration curves for paraquat and diquat. Applied voltages of 2.50 V and 2.45 V were suitable when using orange and blue LEDs for paraquat and diquat analyses, respectively, because of wider linear ranges, better sensitivities, and good correlation coefficients ($r^2 > 0.99$). Study of the applied voltage for paraquat analysis was reported in our previous study [11] whereas the results for diquat analysis are shown in Table S1 (Supplementary 1).

3.2 Study of reaction conditions

In this research, the reaction for diquat analysis was investigated in detail while that for paraquat analysis were reported in our previous paper [11]. Briefly, paraquat analysis was achieved by using SDT powder to reduce paraquat to its radical under alkaline conditions of 100 mmol L^{-1} NaOH (pH 13). For the quantitative analysis of diquat, another redox reaction was employed to produce colored diquat radicals. The selection of reducing agents, the NaOH concentration, the molar ratio of the reducing reagent, and the effect of the state of DTT on the production of a diquat radical was investigated in the preliminary study.

First, we examined three reducing agents mentioned in the literature: 2-mercaptoethanol (2-ME), L-cysteine (L-Cys), and dithiothreitol (DTT) [20]. We found that at a pH of 13, the 2-ME successfully reduced diquat to its radical, but 25 min was required to complete the reaction. Conversely, L-Cys was dissolved in 1.0 mol L^{-1} HCl, and this prevented its decomposition. However, L-Cys was inappropriate for the dual analysis of paraquat and diquat because the strong acidic conditions affected the stability of the

paraquat radical, which was apparent by the decrease in absorbance. Even when using L-Cys hydrochloride monohydrate, the stability of the paraquat radical was decreased, although it was soluble in water. Consequently, DTT was the most suitable as a reducing reagent, because paraquat had no influence on the diquat analysis.

Second, 10 mg L^{-1} (~ 0.03 mmol L^{-1}) of diquat solution was reacted with 3 mmol L^{-1} DTT in various concentrations of NaOH: 50, 100, 200, and 300 mmol L^{-1}. The reaction time was decreased from 30 min to 15 min when the concentration of NaOH was changed from 50 mmol L^{-1} to 100 mmol L^{-1}. However, the absorbance of the diquat radical decayed at concentrations of 200 and 300 mmol L^{-1}. Therefore, 100 mmol L^{-1} NaOH was chosen as best for diquat analysis. It was also advantageous that both reactions of STD and DTT worked under the same alkaline conditions as that of paraquat with STD because this simplified the adjustment of the pH for the analysis reactions of paraquat and diquat.

Third, a 10 mg L^{-1} (~ 0.03 mmol L^{-1}) solution of diquat was reacted with four different concentrations of DTT in 100 mmol L^{-1} NaOH. The DTT concentrations were 3, 9, 15, and 30 mmol L^{-1}, which were 100, 300, 500, and 1,000-fold the concentration of diquat. Figure 2 shows how higher ratios reduced the analysis time and enhanced the absorbance of a diquat radical—with the exception of the 1,000-fold concentration where the absorbance was decreased. This decrease could have been the result of a decomposition of the diquat radical via further reduction that resulted in a colorless solution. This hypothesis was based on the mechanism that decreased the size of the paraquat radical in the presence of a high concentration of reductant, as mentioned by Minakata et al. [21].

Finally, we attempted the use of DTT powder to analyze diquat radicals in a manner similar to using STD powder in paraquat analysis. One spoon of DTT powder was estimated to be ~5±2 mg (n = 8), which corresponds to a ratio of approximately 550±200-fold that of diquat. No difference was observed in the absorbance of diquat radicals (%RSD < 5%) by comparison with the results obtained when a DTT solution was added. Hence, we concluded that it is unnecessary to establish the exact amount of DTT in order to obtain a reproducible absorbance of a diquat radical when the ratio is maintained within a range of from 350 to 750-fold. Therefore, we employed the DTT in its solid state, because the solid state of DTT is more stable than a solution with a lifetime of several hours [22], and there is no need to dilute the sample solutions.

3.3 Dual analysis of paraquat and diquat

As demonstrated in our previous paper, the reaction of diquat with STD is similar to that of paraquat, and this similarity causes diquat to be an interfering agent. Therefore, a method is needed that will allow for the discrimination of paraquat from diquat without separation. We examined two different ways to determine paraquat and diquat concentrations using a two-color photometer that was developed in

this work: 1) using simultaneous equations; and, 2) using three independent equations, as shown in Table 1.

Simultaneous equations were obtained by combining two linear equations for paraquat and diquat for two detection wavelengths (blue LED = 472 nm and orange LED = 609 nm) using a reaction with SDT wherein paraquat and diquat were assumed to not interfere with the absorbance of the other. The results obtained by solving the simultaneous equations are displayed in Table S2 (Supplementary 2). Table S2 shows that higher concentrations of diquat provide larger positive errors for paraquat concentrations. A weak transmittance of light was obtained under higher concentrations, and this led to inaccurate results, which is a limitation of this system. Therefore, the simultaneous equations could not independently determine paraquat and diquat in a mixture when using the developed photometer. It should be noted that the simultaneous equations worked well when using a conventional spectrophotometer, which indicates that the reaction with SDT could be used for the simultaneous equation method.

To achieve a determination of paraquat and diquat in a mixture, we developed a method using three separate equations to eliminate the interference of a diquat radical in the reaction with SDT. The equations are linear equations of 1) diquat at 472 nm to obtain the diquat concentration, 2) diquat at 609 nm to convert the diquat concentration to absorbance at 609 nm, and 3) paraquat at 609 nm to obtain the paraquat concentration after subtracting the calculated absorbance of diquat at 609 nm from the total absorbance of the mixture. With this method, the reaction of diquat with DTT required 10 minutes in order to obtain stable absorbance whereas the reactions of paraquat and diquat with SDT were completed immediately. Table S3 (Supplementary 2) shows the calculated concentrations of both paraquat and diquat using the proposed method. Paraquat showed no influence on the reaction of diquat with DTT so that diquat could be determined selectively without the interference of paraquat (see Supplementary 3 for more information on the effect of paraquat on diquat analysis when using DTT as a reducing agent). The calculated concentrations agreed well with the prepared concentrations of the mixtures, so there was no error when the absorbance contributed by diquat was subtracted from the absorbance of the mixture at 609 nm. Namely, we confirmed that the method using three equations accurately determined the concentrations of paraquat and diquat. Therefore, the analytical characteristics for the detection of diquat reduced by DTT at 472 nm, as well as for diquat reduced by SDT at 609 nm, and paraquat reduced by SDT at 609 nm were studied using the developed two-color photometer.

3.4 Analytical characteristics

The performance of our developed photometer was evaluated in terms of linearity, LOD, LOQ, and precision (intra- and inter-day), as shown in Table 2. The calculations of the LOD and LOQ followed the recommendations of the International Union of Pure and Applied Chemistry (IUPAC) [19]. Although the

formula for the estimation of LOD and LOQ involves a residual standard deviation ($S_{y/x}$), it was zero in the measurements of diquat using SDT in this study. Therefore, we estimated the LOD and LOQ values as the lowest concentrations that gave a discriminable signal when using our device. Moreover, the linearities of the calibration curves were proven not only by using the correlation coefficients (r^2) but also by comparing the experimental *F* values with the tabulated critical *F* value, which also is a recommendation of the IUPAC. The calculation confirmed that our calibration curves have linearity because the experimental *F* values (see in Table 2) were lower than the tabulated *F* value ($F_{\text{tubulated}} = 3.71$) at a 95% confidence level. However, the Experimental F value for the calibration graph of diquat with SDT could not be calculated because the standard deviation was zero.

3.5 Herbicide sample analysis

The reliability of our developed methodology was confirmed by the determination of paraquat and diquat in a commercial herbicide. As mentioned in Section 2.5, the herbicide was passed through a C-18 column to remove a blue dye in the solution prior to the reactions. The analytical results proved that the developed method was appropriate for use in actual sample analyses since the concentrations of paraquat ($(1.2\pm0.0)\times10^5$ mg L^{-1}) and diquat ($(1.5\pm0.0))\times10^5$ mg L^{-1}) were in good agreement with analysis using the HPLC method: paraquat ($(1.2\pm0.0)\times10^5$ mg L^{-1}) and diquat ($(1.5\pm0.0)\times10^5$ mg L^{-1}). In addition, when 10 mg L^{-1} of each analyte was spiked into the samples to assess accuracy, the portable photometer provided 99.43±4.91% for paraquat and 86.89±2.38% for diquat, which confirms the practical applicability to actual sample analysis.

4. Conclusions

We accomplished the development of a portable two-color photometer that uses LEDs. Two PEDDs for measuring the absorbance at 472 nm and 609 nm were configured perpendicularly in a cuvette holder. The developed device is completely portable since it requires only small rechargeable batteries for operation. The dual detection system was applied to the determinations of paraquat and diquat without cross-talk between two PEDDs. When DTT was employed as a reducing reagent, diquat produced a colored radical that was detected at 472 nm without interference from paraquat. Conversely, reduction with SDT produced diquat and paraquat radicals that were detected at 609 nm. Therefore, the concentration of paraquat was determined by subtracting the absorbance of diquat from the total absorbance at 609 nm. This methodology permitted accurate analyses of paraquat and diquat by eliminating the interference of diquat from paraquat analysis that we encountered in our previous work [11]. The developed method was validated by investigating the concentrations of paraquat and diquat contained in a commercial herbicide and

287 comparing the results with those measured via HPLC as a standard method and by the determination of %
288 recovery.
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Table 1 The equations used to calculate paraquat and diquat concentrations

Methods of calculation	Equations
Simultaneous equations	$A_{472} = 0.0006C_{DQ} + 0.0002C_{PQ} - 0.0063$
	$A_{609} = 0.0001C_{DQ} + 0.0025C_{PQ} - 0.0014$
Three separated equations	$A_{472} = 0.0018C_{DQ} - 0.0034$
	$A_{609} = 0.0001C_{DQ} + 0.0003$
	$A_{609} = 0.002C_{PQ} - 0.0014$

Remark: *A = absorbance, C_{PQ} = concentration of paraquat in mg L^{-1}, C_{DQ} = concentration of diquat in mg L^{-1}, working range for simultaneous equations; 10 mg $L^{-1} \leq C_{DQ\ at\ 472\ nm} \leq 100$ mg L^{-1}, 20 mg $L^{-1} \leq C_{PQ\ at\ 472\ nm} \leq 40$ mg L^{-1}, 20 mg $L^{-1} \leq C_{DQ\ at\ 609\ nm} \leq 100$ mg L^{-1}, 2 mg $L^{-1} \leq C_{PQ\ at\ 609\ nm} \leq 40$ mg L^{-1}, and working range for three separated equations; 2.5 mg $L^{-1} \leq C_{DQ\ at\ 472\ nm} \leq 40$ mg L^{-1}, 20 mg $L^{-1} \leq C_{DQ\ at\ 609\ nm} \leq 100$ mg L^{-1}, 2 mg $L^{-1} \leq C_{PQ\ at\ 609\ nm} \leq 40$ mg L^{-1}.*

Table 2 Analytical characteristics obtained using the developed device

Analytical parameters	diquat, DTT (blue LED)	diquat, SDT (orange LED)	paraquat, SDT (orange LED)
LOD	0.80 mg L^{-1}	< 5 mg L^{-1}	0.56 mg L^{-1}
LOQ	2.44 p mg L^{-1}	< 17 mg L^{-1}	1.70 mg L^{-1}
Working range	2.5-40 mg L^{-1}	20-100 mg L^{-1}	2-40 mg L^{-1}
Linear equation, r^2	A = 0.0025C – 0.0026, 0.9983	A = 0.0001C + 0.0003, 0.9978	A = 0.002C – 0.0014, 0.9975
Experiment *F* value	1.59	-	0.14
Intra-day precision	1.13 %RSD	3.87 %RSD	1.87 %RSD
Inter-day precision	3.48 %RSD	4.66 %RSD	5.19 %RSD

Remark: *A = absorbance and C = analyte concentration in mg L^{-1}*

List of figure captions

Figure 1 Three-dimensional drawing of the developed portable two-color photometer with paired light emitter detector diodes for dual analysis of paraquat and diquat.

Figure 2 Absorbance of a diquat radical using different mole ratios between diquat and dithiothreitol. The absorbance of the diquat radical was measured at 495 nm.

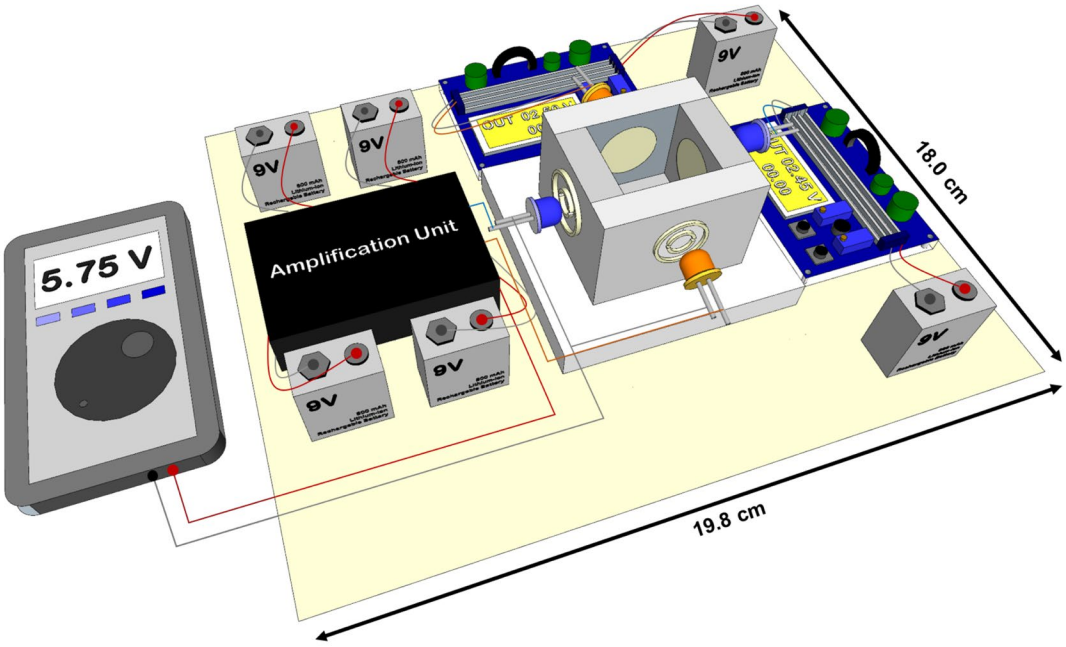

Figure 1

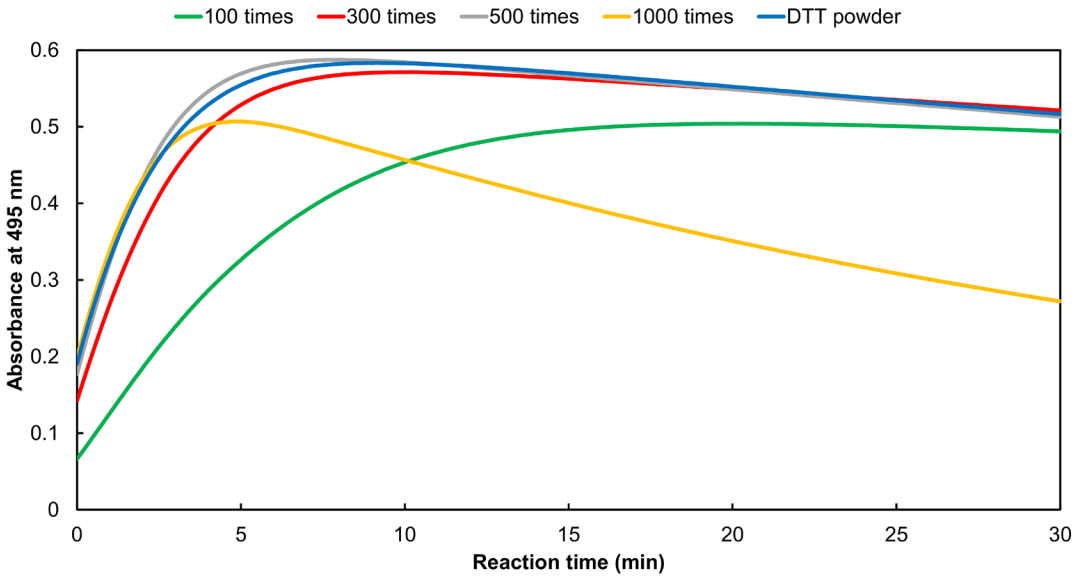
100 times
300 times
500 times
1000 times
DTT powder
Absorbance at 495 nm
0.6
0.5
0.4
0.3
0.2
0.1
0
0
5
10
15
20
25
30
Reaction time (min)

Figure 2