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A colorimetric assay with leuco crystal violet for the detection of inorganic phosphate in water

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Abstract. Phosphate enters the aquatic system through runoff from pastures, croplands, urban areas, and sewage treatment systems and fosters algal bloom causing eutrophication at higher concentrations in water. Therefore, controlling its concentration through routine water quality monitoring in aquatic ecosystems is essential. Using a smartphone camera and colour analysis app, a simple colourimetric technique was developed to quantitatively detect inorganic phosphate in natural and treated drinking water samples using colourless leuco crystal violet (LCV). Upon reaction with phosphate (PO_4^{3-}), the LCV forms leuco crystal violet phosphate (LCV-P) complex with a violet colour, showing maximum absorbance at 583 nm. A light-sensitive colourimetric box with in-built LED light was constructed to maintain the consistency of the image quality for precise and accurate measurement. The red, green and blue (RGB) analyses of the digital images were done to determine the linear response of the intensity of G against the concentration of the LCV-P complex. A microplate reader was also used to analyse the UV-Vis absorbance of the samples to validate the results further. Under optimum conditions, the colourimetric assay achieved a detection limit of 0.25 μM with a linear range between 0.1 and 1 μM. Thus, the proposed colourimetric assay was highly sensitive and selective towards phosphate in natural and drinking water samples.

Keywords: Colourimetry, leuco-crystal violet, phosphate, water, digital imaging.

Classification numbers: 3.4.2, 3.3.1, 2.1.3.

1. INTRODUCTION

Phosphate is the source of an essential plant nutrient, phosphorus and is a necessary compound for the growth of plants and animals and is also a key water quality parameter. According to the World Health Organization (WHO) [1], the minimum allowable limit of phosphorus in drinking water is 2 mg/L, and limits for natural and wastewater are 0.2 and 10 mg/L, respectively [2]. While the presence of phosphate in water is essential, it becomes an environmental hazard when the over-enrichment of aquatic ecosystems occurs by phosphate through runoff from croplands, pastures, sewage treatment systems, surface erosion, and urban centres. This causes algal bloom, which significantly decreases the dissolved oxygen level in

water [3] and ultimately results in eutrophication. Eutrophication causes a decline in water quality and poses a grave threat to the aquatic ecosystem and organisms. Hence, maintaining the optimal amount of phosphate in the aquatic environment is necessary. Phosphate in the human system is obtained either from the consumption of food or as phosphate salts from oral medication, which is safe for short-term consumption in controlled amounts (< 1250 mg daily) [3]. Though phosphate does not cause major detrimental effects on a normal healthy human being, it is still important to regulate phosphate levels in potable water as a precautionary measure. Regulating phosphate requires an easy but reliable quantification protocol.

Table 1. Summary of various analyses based on colourimetric phosphate detection using different materials and techniques.

Method	Novelties of experiment	Linear Range (mM)	LOD (mM)	Ref.
UV-Vis spectroscopy	Hydrazine sulfate replaced ascorbic acid	3.7×10^{-3} - 3.7×10^{-2}	9.7×10^{-4}	[5]
Continuous flow analysis	Colourimetry coupled with continuous flow analysis	$< 10^{5} \times 10^{-6}$	1.1 10^{-6}	[6]
UV-Vis spectroscopy	Gold nanoparticles (AuNPs) reacted with europium ions	5×10^{-4} - 3×10^{-2}	76×10^{-6}	[7]
Digital imaging with Image J software	Utilising a scanner as the detection method	1.67×10^{-3} - 8.34×10^{-2}	4.17×10^{-4}	[8]
Digital imaging with Image J software	RGB analyses (via ImageJ)	0.1 - 10 ppm	0.28 ppm	[9]
UV-Vis spectroscopy	Molybdenum blue colorimetric method	Up to 10 µM	0.07 µM	[10]
UV-Vis spectroscopy	Using C18-functionalized silica coated magnetite	1.0 - 30.0 µg P L^{-1}	0.3 µg P L^{-1}	[11]
UV-Vis spectroscopy	Novel colorimetric probe (TNT@MB)	1 - 40 µM	0.59 µM	[12]
Colorimetric detection using ImageJ software	Dip strip assay with wet Chemistry	0.1 - 25 ppm	0.134 ppm	[13]
Digital imaging analysis	Utilises LCV in inorganic phosphate	1×10^{-4} – 1×10^{-3}	2.5×10^{-4}	This work

The available protocols for quantitatively determining phosphate, as summarised in Table 1, have extensively used colourimetric techniques. The target analytes are converted to a suitable colour compound or complex in colourimetry. The intensity of colour and the analyte

concentration is then obtained using electromagnetic radiation in the visible region of the electromagnetic spectrum. The analysis normally involves comparing an unknown complex with a known standard to determine whether they have similar colour intensity [4], using a colourimeter or a spectrophotometer to measure the absorbance. However, there are situations when such instruments may not be available, and an alternative may be required.

Smartphones with high-resolution cameras and digital photography apps may be an attractive alternative to expensive spectrophotometric or colourimetric devices for quantifying colour intensity in colourimetric assays. This low-cost, on-site diagnostic test concept has been exploited in many areas of food analysis [14], forensic science [15], and environmental monitoring [16]. Apps with different functionalities have turned smartphones into portable handheld analytical instruments. Colour Grab is a readily available Android application in Google Play Store and can enhance the image captured by the camera and quantitatively express any colours in RGB-256 colour system or Hexadecimal (Hex) values through mathematical algorithms. Similarly, SI ColourPicker, a Java-based, open-source RGB analysing software, gives colour values in captured images in real time without being connected to an external computer [17].

One of the most sensitive colourimetric determinations of inorganic phosphate was based on forming heteropolyacids [5], such as phosphomolybdate and vanadomolybdate complexes. This assay employed a mixture of ascorbic acid, ammonium molybdate and antimony (II) reagents to produce a blue-coloured complex which exhibited a shift in the absorption spectrum in direct proportion to phosphate concentration in the solution [18]. On the other hand, a protocol for colourimetric detection of organic phosphate in double-stranded DNA (dsDNA) was based on the incorporation of crystal violet (CV) and sodium sulfite (Na_2SO_3) [19]. CV is a triphenylmethane dye violet in colour with a chromophore in an aqueous solution that forms a LCV compound when added to Na_2SO_3. The LCV, in the presence of phosphorus, reverts to violet colour due to the formation of the LCV-P complex. The CV is commonly used for staining in biological applications, as a tooth whitening agent, to detect other analytes in the micro determination of iodine [20], and in forensic sciences to recover footwear and body impressions in blood at crime scenes [21]. The principle of detecting phosphorus in dsDNA using LCV was adapted to detect inorganic phosphate, which will also cause a reversion colour in LCV solution, from colourless solution back to violet due to the formation of LCV-P complex.

In this work, we developed a simple colourimetric assay to quantify inorganic phosphate in water samples using the LCV in combination with the SI ColourPicker Android App. to determine the RGB distribution of the colourimetric results. The research aimed to establish a rapid, cost-effective and portable quantitative colourimetric assay to determine inorganic phosphate in water samples. The assay was developed as a single reagent detection technique and successfully applied to quantify the concentration of inorganic phosphate in real water samples from natural and treated sources.

2. MATERIALS AND METHODS

2.1. Reagents and materials

All chemicals were analytical reagent grade and were purchased from Sigma Aldrich. All solutions were prepared in deionised water from the Millipore water purification system (Direct-Q® 8 UV Remote Water Purification System). LCV was prepared from a mixture of 0.05 mM

CV and 50 mM sodium sulfite solutions at a 1:15 ratio. Due to the photo-sensitivity of CV, the solution was kept light-shielded prior to analysis [22]. Potassium dihydrogen phosphate was used to prepare all the standard solutions of inorganic phosphate. Stock-solution of phosphate was prepared by serial dilution to make 10 mM to 0.0001 mM of phosphate standards. Prepared phosphate standards were added to the LCV and placed in a dry bath (MyBlock™ Mini DryBath, Benchmark Scientific, US) at 75 ℃ for 40 minutes. The mixture was allowed to cool at room temperature and pressure for at least 30 seconds before analysis.

2.2. Spectrophotometer and digital imaging

Digital images were captured using a Sony Xperia Z3 (20.7 Megapixels camera) using a custom-designed colourimetric box. The box was fabricated using a hard black cardboard (15cm × 9cm × 8.5cm) wrapped with black construction paper to block ambient environmental light (Fig. 1). The colourimetric box was illuminated by an inbuilt LED flashlight located just below the phone camera to avoid the capture of reflected light on the surface of the reaction tubes [23]. Clear reaction tubes allowed white light to pass through the sample for selective absorption (or reflection). Plain office paper was used as the background while capturing the images to reduce the reflection and enhance the image quality. The mobile phone was placed on the top side over the opening of the box with a focal distance to ensure better focus for image acquisition. Once the visual analysis was done from the reaction tubes, the solution was added to a 96-well plate reader (Thermo Scientific™ Multiskan™ FC Microplate Photometer) for spectrophotometric analysis at 620 nm. The double-beam UV-Vis Spectrophotometer (Shimadzu, UV-1601PC) was used to analyse the UV-Vis spectra of CV colourimetric assays.

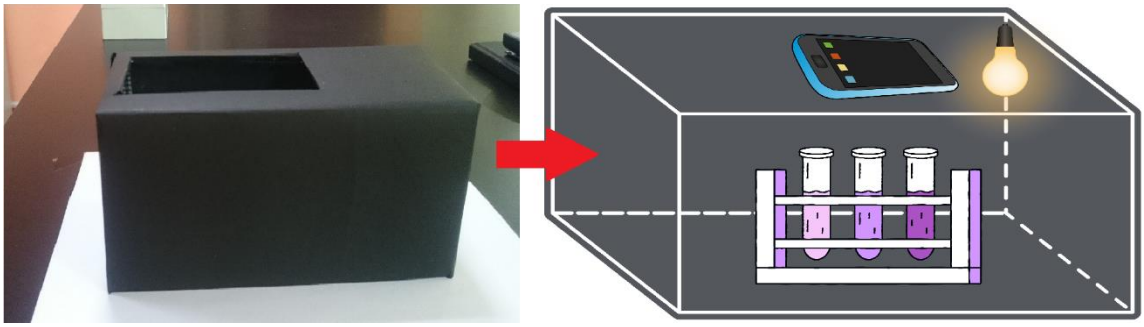

Figure 1. Colourimetric box fabricated for capturing digital images

2.3. Sample preparations

2.3.1. Artificial samples

Artificial samples A and B were prepared using potassium dihydrogen phosphate, which gave phosphate concentrations of 2.5×10^{-4} mM and 3.5 mM, respectively. Samples A and B were added to tap water samples, heated, and then analysed both spectrophotometrically in a 96-well plate reader and visually via SI ColourPicker software.

2.3.2. Mineral water samples

A total of nine different bottled mineral water samples were analysed. The samples were produced in Brunei, Malaysia and Europe. The mineral water samples were pre-treated with

$AgNO_3$. In case of the formation of precipitates, the solutions were filtered using a Whatman 41-grade filter paper before adding LCV, followed by heating. The samples were then analysed spectrophotometrically in a 96-well plate reader and visually via SI colour picker software.

2.3.3. Sea water samples

The natural seawater samples were obtained from different beaches in Brunei Darussalam. All the samples were analysed on the same day of sample collection to avoid discrepancies [24]. The samples were initially filtered through a Whatman 42-grade filter paper to remove any impurities or sand (Fig. 2). Some samples may require successive purification processes due to interfering analytes in the seawater samples. The pre-treatment involved the addition of an equivalent volume of $AgNO_3$ [25]. This was done to ensure the removal of any traces of pollutants present in water, including interfering ions, microbes, etc [26]. If precipitate forms during the pre-treatment, another filtration process may require using Whatman 41-grade filter paper. The treated samples were then added to LCV and heated at 85 ℃ for 40 minutes before being analysed both spectrophotometrically in a 96-well plate reader and visually via SI ColourPicker software.

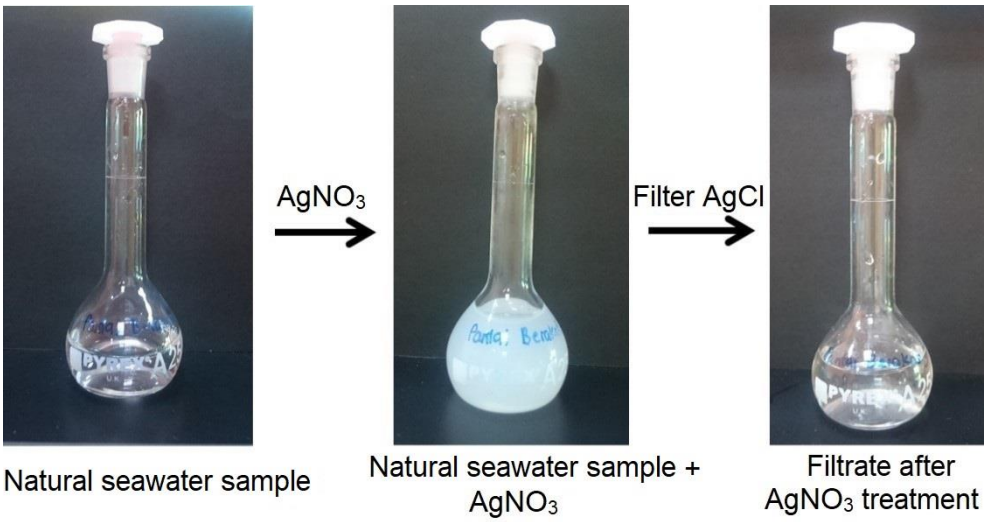

Figure 2. Pre-treatment and filtration process with $AgNO_3$ for removal of Cl^-.

3. RESULTS AND DISCUSSION

3.1. Optimisation of leuco-crystal violet

The reagent conditions described by Miyamoto and Sano [19] were used as an approximate starting point to determine the optimal reagent conditions of CV (0.05 mM) and sodium sulfite (30 mM) for the analysis. The ratio of CV to sodium sulfite that yielded a colourless LCV solution was 1:20. The concentration of sodium sulfite was increased by 20 mM to study the formation of LCV. For this combination, the CV to sodium sulfite mixing ratio that yielded a colourless LCV solution was 1:15.

3.1.1. Effect of temperature and pH

The working temperature for the assay was studied between 25 to 105 ℃. At this temperature range, the phosphate concentration was taken as 1 mM. The spectrophotometric analysis was recorded at wavelengths of 405 nm, 450 nm, 620 nm and 650 nm (Fig. 3A). The maximum absorbance was obtained at 85 ℃ for 620 nm and 650 nm, while it was lowest at 95 ℃ for 405 nm and 450 nm. The optimum temperature for heating the colourimetric assay was therefore set at 85 ^{o}C. The wavelength of violet was recorded around 590 nm [27], and the closest wavelength available based on this study was 620 nm. Therefore, absorption at 620 nm (A_{620}) was used for this study. The sensitivity of the system to pH changes was also studied. Under highly acidic conditions, at pH ~1, the CV changes to give yellow colour at 420 nm. Green colour was also observed with maximum absorbance between 420 - 620 nm. This was found to be consistent with previously published literature as discussed by Rosenstbin *et al.* [28]. However, the CV was noted to be highly unstable under acidic conditions, thus giving green and yellow-coloured products. Therefore, the effect of basic pH conditions was also studied. A stable violet colour CV solution was formed under basic conditions (pH > 1) at 590 nm. Hence, this assay was concluded to be more suitable under basic conditions to ensure the formation of a stable violet-coloured solution of the LCV-P complex.

3.1.2. Effect of time on colour stability

The colour stability for the formed LCV-P complex was studied by heating at 10 minutes intervals from 0 to 80 minutes (Fig. 3B). At wavelengths of 620 nm and 650 nm, a general increase in absorbance was observed between 10 to 40 minutes of heating. The absorbance remained constant beyond 40 minutes. At wavelengths 450 nm and 405 nm, the absorbance started to plateau at about 70 minutes of heating. Therefore, for maximum colour stability at 620 nm, a minimum heating time of 40 minutes is required to produce quantifiable colourimetric results. Once the mixture was cooled, the spectrophotometric analysis was carried out immediately to ensure measurement before the degradation of the LCV-P complex. For this reason, a time window of 30 to 60 seconds was adopted in this study to enhance the sensitivity and permit reliable quantification.

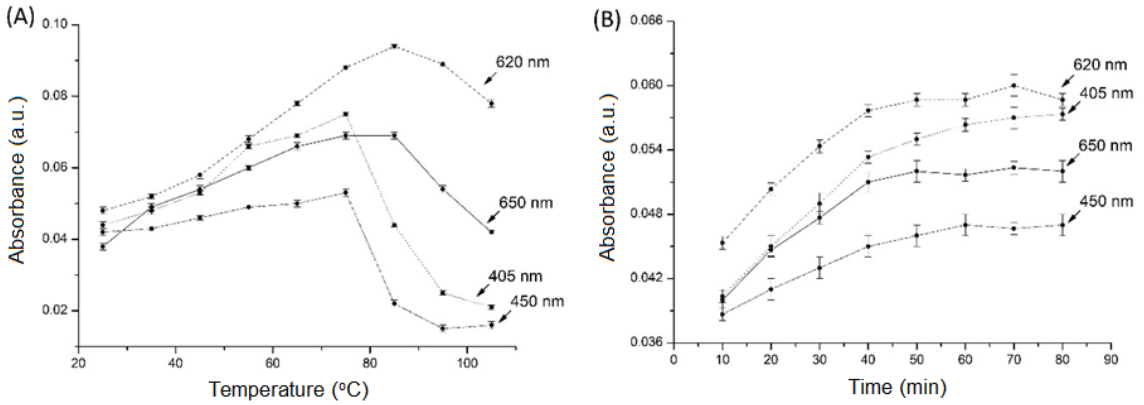

Figure 3. Effects of (A) temperature (B) heating time on the absorbance (at wavelengths 650 nm, 620 nm, 450 nm and 405 nm). All data were an average of three replicate measurements.

3.2. Colourimetric analysis

3.2.1. UV-Vis spectral analysis

In UV-Vis spectral analysis, the maximum absorbance at 583 nm (A_{583}) for 0.05 mM of CV was 0.172 compared to 0.025 for the colourless LCV solution. The formation of LCV from CV upon adding sodium sulfite reduced absorbance. This could be due to a chromophoric conjugated system failure that causes the binding of the sulfite group to the central carbon of CV [19]. In comparison, the A_{583} for the LCV-P complex was 0.12, which is about 4.8 times higher than the LCV solution (Fig. 4). In addition, the optimum absorption wavelength obtained for the LCV-P solution was 578 nm, with a slight hypsochromic shift (displacement of absorption maximum from the red to ultraviolet) [29]. Since 96 well-plate reader was used for the colourimetric assay in this study and the wavelength closest to 583 nm was 620 nm, absorbance was recorded at A_{620}.

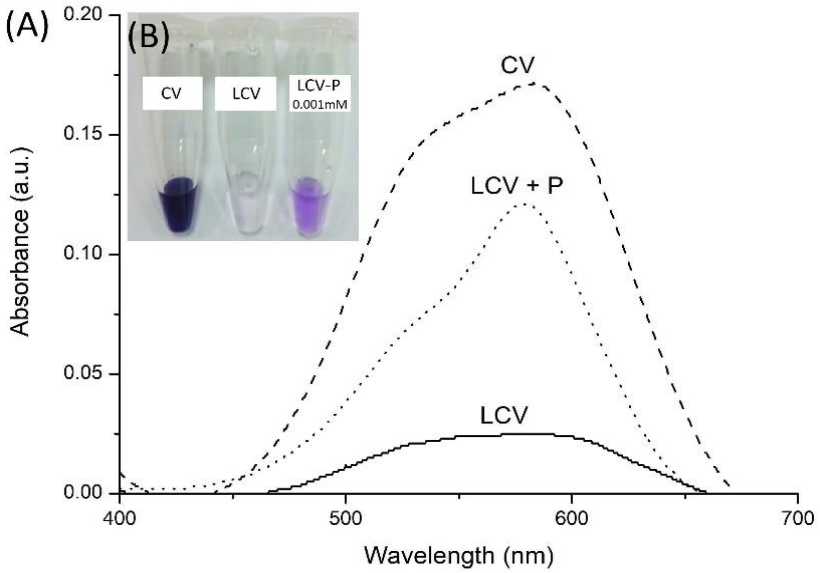

Figure 4. The colourimetric response assay (A) UV-Vis absorption spectra of CV (crystal violet), LCV-P (leuco - crystal violet - phosphate complex) and LCV (leuco - crystal violet) (B) Digital image of CV, LCV and LCV-P complex formed with LCV in the presence of 0.001 mM phosphate. All data were an average of three replicate measurements.

3.2.2. Beer Lambert's Law analysis

The mathematical expression of Beer Lambert's law is $A = c l \varepsilon$ (A = absorbance, c = concentration of the substance in $molL^{-1}$, and ε = constant molar extinction in $L\ mol^{-1}cm^{-1}$) [30], which shows higher conformity only for diluted solutions. The absorbance obtained for solutions at higher concentrations tends to deviate from linearity due to the interactions between solute and solvent molecules [31]. Using optimum conditions, a series of standard phosphate (0.0001 - 10 mM) were added to LCV solutions to determine the sensitivity of this study with Beer Lambert's law. The solutions were analysed by UV-Vis spectroscopy at 620 nm with three replicates. A calibration curve was plotted for 0.0001 - 10 mM of phosphate, which gave a working range for the assay as 0.0001 - 0.001 mM (Fig. 5). Accordingly, to plot the calibration curve during sample analysis the phosphate concentration in this range was used.

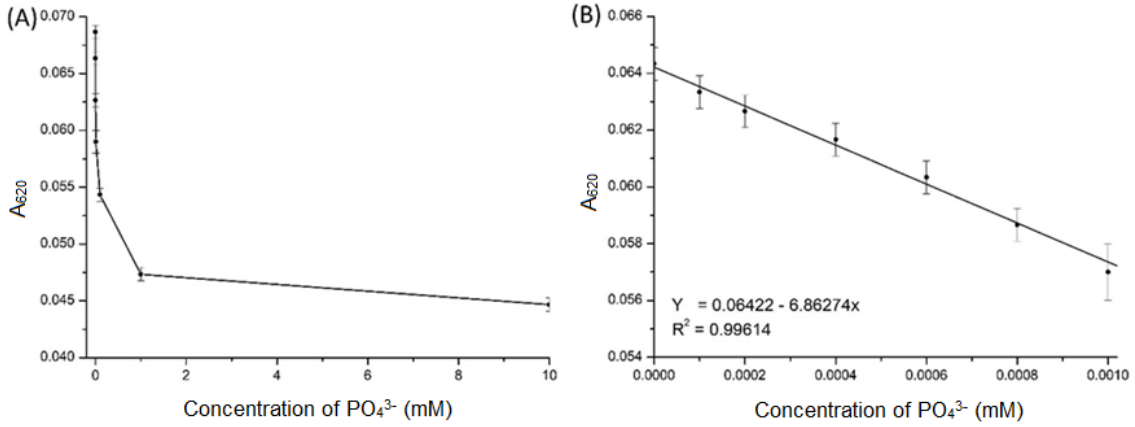

Figure 5. The UV-Vis absorbance at A_{620} of (A) phosphate concentration ranging from 0 - 10 mM (B) linear relationship between absorbance and phosphate concentration between 0.0001 - 0.001 mM. All data were an average of three replicate measurements.

3.2.3. Colourimetric analysis with SI ColourPicker software

The formation of the LCV-P complex was captured using the custom set-up for a series of standard phosphate (0 - 0.001 mM) concentrations. The RGB of the formation was determined with SI ColourPicker software. The RGB intensities were plotted against corresponding phosphate concentrations (Fig. 6A). Results showed increased RGB intensities with increasing phosphate concentrations per Beer Lambert's law. In the RGB plot, the intensity of G showed a better slope and R^2 value than the intensities of B and R. Hence, the intensity of G was used in subsequent experiments for the analysis. The limit of detection was 8.22×10^{-5} mM based on 3σ was obtained for determining phosphate.

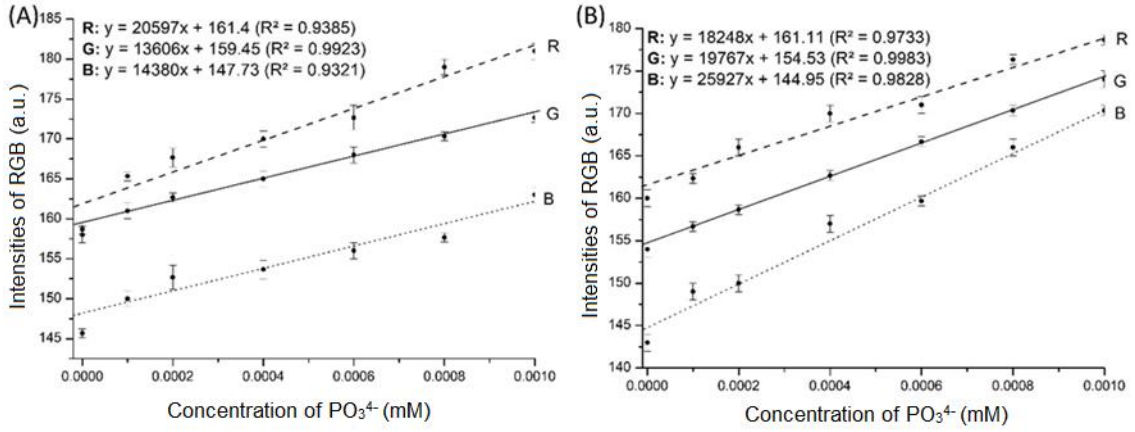

Figure 6. The intensities of RGB of standard phosphate extracted using (A) SI Colourpicker (B) ColourGrab. All data were an average of three replicate measurements.

3.2.4. Colourimetric analysis with a smartphone app

An alternative to UV-vis spectroscopy and SI ColourPicker software was performed for the colourimetric phosphate detection using the Android colour analyser app - ColourGrab. The

intensities of RGB for the assay were determined directly using a smartphone camera and the ColourGrab app (Fig. 6B). The results were compared to SI ColourPicker by transferring the images to a desktop. ColourGrab results were comparable to SI ColourPicker. Like SI ColourPicker, the intensity of G showed better slope and R^2 value with a detection limit of 4.69×10^{-5} mM based on 3σ. The ColourGrab app was employed to analyse the prepared unknown artificial samples, A and B. The actual phosphate concentration in unknown A was 2.5×10^{-4} mM, and the intensity of the G plot measured it as 1.46×10^{-4} mM. Whereas for unknown B, the actual phosphate concentration was 3.5 mM, estimated as 3.07 mM by the intensity of G analysis by the ColourGrab app. The results indicated the ability of the ColourGrab app to quantify the unknown artificial samples with little deviation from the actual concentration. Therefore, we concluded that the proposed method is useful for the qualitative determination of phosphate and has the potential to be used in the rapid colourimetric detection of phosphate.

3.3. Interference Study

3.3.1. Interference with single analytes

Selectivity and sensitivity of the assay were carried out with potential interfering anions including SCN^-, NO_3^-, SO_4^{2-}, Cl^-, F^-, Br^-, BrO_3^- and $C_2O_4^{2-}$ and several common metallic cations including Sn^{2+}, Zn^{2+}, Al^{3+}, Ba^{2+}, Ni^{2+}, Co^{2+}, Cu^{2+} and Cr^{2+}. Anions (1 mM) and cations (0.1 mM) samples were prepared with double distilled water. Phosphate, at 0.1 mM, changed the colour intensity and absorbance with LCV. Each anion/cation was added to the LCV solutions to study the single analyte interferences. The UV-Vis results (Fig. 7A) indicated that the anions except Cl^- and $C_2O_4^{2-}$ and metallic cations had no colourimetric responses with LCV, even when their concentrations in solution were ten times greater than that of phosphate. Hence, Cl^- and $C_2O_4^{2-}$ were identified as potential interfering agents in this assay.

3.3.2. Interference in the presence of phosphate and other analytes

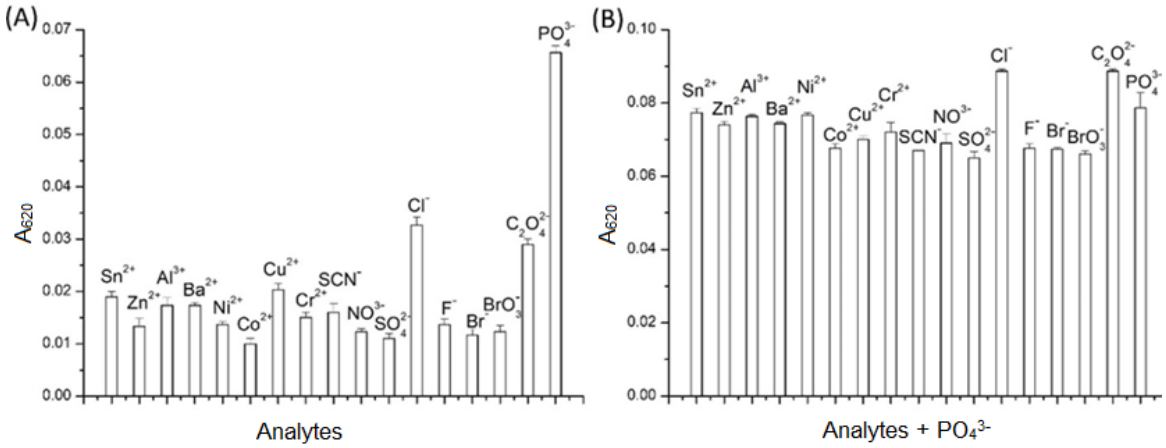

Figure 7. The UV-Vis absorbance responses of (A) anions (SCN^-, $NO3^-$, SO_4^{2-}, Cl^-, F^-, Br^-, BrO_3^- and $C_2O_4^{2-}$), metallic cations (Sn^{2+}, Zn^{2+}, Al^{3+}, Ba^{2+}, Ni^{2+}, Co^{2+}, Cu^{2+} and Cr^{2+}) and phosphate (PO_4^{3-}) (B) anions and metallic cations in the presence of phosphate (PO_4^{3-}). The concentration of anions is 1 mM, and cations are 0.1 mM. All data were an average of three replicate measurements.

Further investigation was done to see the potential interferences by adding phosphate (0.1 mM) into the anions and cations to observe for colourimetric responses. Spectrophotometric results showed that in the presence of phosphate, the absorbance increased for all analyte mixtures (Fig. 7B). Significant colourimetric changes were observed, particularly for Cl^- and $C_2O_4^{2-}$ as described in Section 3.3.1. Both interferents resulted in a high absorbance in the presence of phosphate compared to the absorbance of the solution containing phosphate only. It was concluded that the samples containing Cl^- and $C_2O_4^{2-}$ may require pre-treatment to remove these interferents prior to colourimetric phosphate detection by the proposed assay.

3.3.3. Removal of interferences

Detection of phosphate in samples containing two or more ions required pre-treatments of samples to remove the interferences [32]. As mentioned in Section 3.3.1, Cl^- and $C_2O_4^{2-}$ were found to interfere with the colourimetric detection of phosphate. Since $C_2O_4^{2-}$ is not commonly encountered in water samples; therefore this interferent is deemed negligible as the proposed assay aims to analyse natural and treated water samples [33]. Pre-treatment of samples with $AgNO_3$ resulted in the displacement of Cl^- ions through aggregation with Cl^- ions in solution from white AgCl precipitate [34]. Accordingly, Cl^- ions could be filtered out of the sample solution using AgCl using a conventional filter paper. The removal was confirmed by the significant decrease in the UV-Vis absorbance of Cl^- in the samples pre-treated with $AgNO_3$. The pre-treatment of phosphate solution with $AgNO_3^-$ reduced the effect of the interferences and allowed the detection of phosphate in solution with enhanced selectivity and sensitivity.

3.4. Analysis of phosphate in water samples

3.4.1. Artificial water samples

Unknown artificial samples were prepared as mentioned in Section 2.3.1 and analysed through visual comparison against a series of standard phosphate solutions (1 mM, 0.1 mM, 0.01 mM, 0.001 mM and 0.0001 mM of phosphate). In addition, the intensity of G analysis was performed on these samples. Visual comparison identified the unknown concentrations of phosphate in unknown A and B samples as between 0.0001 to 0.001 mM and 1 to 10 mM, respectively (Fig. 8). Though the visual comparison is a rapid qualitative method to estimate the phosphate concentrations in the samples, it is amenable to human errors or bias especially when the colour values were difficult to compare. Therefore, the proposed alternative approach analysed the results by digital imaging to extract intensities of RGB with the aid of SI ColourPicker software. The intensity of G results further confirmed the phosphate concentrations in unknown A and B to be between the concentrations mentioned above. A calibration plot was constructed with another series of standards to determine the unknown A (a series of 0.0002 mM, 0.0004 mM, 0.0006 mM and 0.0008 mM concentrations of phosphate) and unknown B (a series of 2 mM, 4 mM, 6 mM and 8 mM concentrations of phosphate). For unknown A, substituting the intensity of G values in the equation gave a phosphate concentration of about 2.21×10^{-4} mM. Similarly, the concentration was estimated to be 3.10 mM for unknown B. Close conformity between the actual and theoretical concentrations of phosphate demonstrated the suitability of this method for quantitatively detecting phosphate.

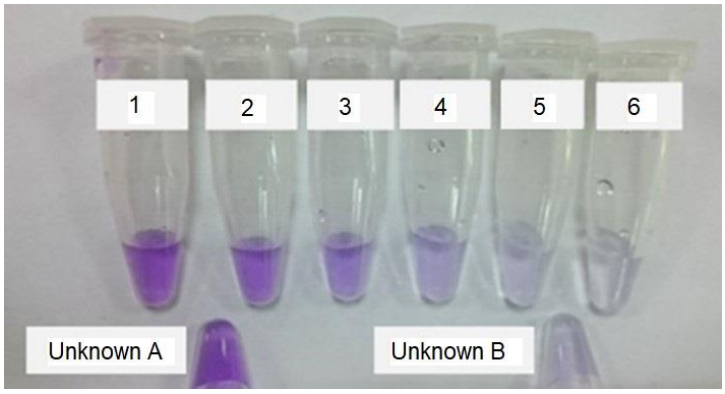

Figure 8. The visual comparison of unknown artificial samples A and B against the standard phosphate concentrations: (1) 0.0001 mM, (2) 0.001 mM, (3) 0.01 mM, (4) 0.1 mM, (5) 1 mM, (6)10 mM.

3.4.2. Tap water samples

Tap water samples were pre-treated with $AgNO_3$ to remove interference from Cl^- ions before analysis. Visual comparison indicated phosphate concentration between 0 to 0.0001 mM. The obtained concentration of phosphate in the tap water sample from the analysis of the intensity of G values was 3.27×10^{-5} mM which corresponded to 1.14 ppm (Fig. 9). The treated tap water in Brunei Darussalam is considered safe to consume directly from the tap, and hence it meets the phosphate concentration of about 2 ppm for drinking water as set by WHO [35,36]. Consequently, the calculated values of phosphate concentration in tap water through the proposed assay agreed with the aforementioned standard literature value.

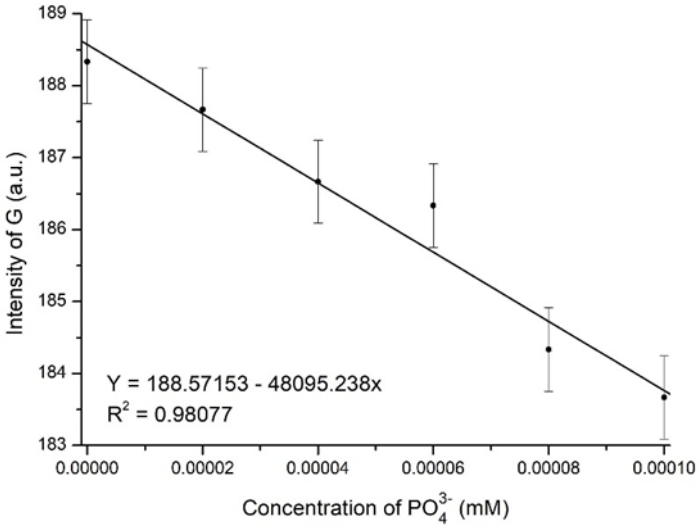

Figure 9. The calibration graph using an intensity of G intensity against phosphate concentration in the 0 - 0.0001 mM range. All data were an average of three replicate measurements.

3.4.3. Mineral water samples

Nine mineral water samples (a - i) brands were pre-treated with $AgNO_3$ before analysis. Visual comparisons indicated very low phosphate concentration, between 0 to 0.0001 mM, in all the samples. By intensity of G analysis, the mineral water samples (a – f) showed lower than

8.18×10^{-5} mM (2.85 ppm) of phosphate concentration (Table 2). This value agrees with the WHO's standard guidelines on water quality and human health for regulation and standard setting for drinking water worldwide [37]. However, for samples (g – i), the phosphate concentration was higher than WHO's quality standard. The addition of phosphate was started in the early 1980s to meet the quality standards for lead in the country manufactured the mineral waters, which resulted in a remarkable difference in phosphate concentration. Also, dosing of orthophosphate in drinking and environmental surface water showed a consequent reduction of heavy metals (lead, copper, zinc and nickel) [35]. Hence, the results obtained from the colourimetric assay successfully quantified phosphate in mineral water.

Table 2. Comparison of the obtained results from the spectrophotometric method and intensity of G of SI Colour Picker software for mineral water samples from different countries were obtained (a – i). All data were an average of three replicate measurements.

Origin	Sample	Intensity of G	Concentration of PO_4^{3-}	
			mM	ppm
Brunei Darussalam	a	172.7	2.63×10^{-5}	0.92
Brunei Darussalam	b	173.0	5.41×10^{-5}	1.88
Brunei Darussalam	c	173.3	8.18×10^{-5}	2.85
Malaysia	d	173.3	8.18×10^{-5}	2.85
Malaysia	e	172.7	2.63×10^{-5}	0.92
Malaysia	f	173.3	8.18×10^{-5}	2.85
France	g	174.7	1.94×10^{-4}	6.72
France	h	175.0	2.21×10^{-4}	7.60
Belgium	i	174.3	1.65×10^{-4}	5.75

3.4.4. Sea water samples

Table 3. Comparison of the obtained results from the spectrophotometric method and intensity of G of SI Colour Picker software for seawater samples (#1 - #5). All data were an average of three replicate measurements.

Location	Intensity of G	Concentration of PO_4^{3-}	
		mM	ppm
#1	182.7	0.00017	6.00
#2	183.3	0.00012	4.18
#3	181.7	0.00026	8.92
#4	181.3	0.00028	9.75
#5	181.7	0.00026	8.92

A similar approach, as adopted by Murphy and colleagues [18], was used to analyse seawater for phosphate concentrations by using the proposed colourimetric assay. Five seawater samples (Samples 1 - 5) were collected from five different beaches located in Brunei. All the samples were purified, diluted and treated with $AgNO_3$ before the analysis. The initial visual comparison demonstrated relatively low phosphate concentrations ranging between 0.0001 to 0.001 mM. As obtained from the samples, the intensity of G showed the highest phosphate concentration in sample 4 and the lowest phosphate concentration in sample 2 (Table 3). Seawater samples displayed a slightly higher concentration than the literature value of 0.1 ppm [38], possibly due to several environmental and sampling factors. Heavy rainfall may have contributed as an environmental factor to enhance the leaching of phosphates from surface runoff of ground soil to estuaries that finally accumulated and discharged into the sea [39]. Due to safety purposes, the sampling for this study was only performed along the shores of the beaches, unlike most of the published papers where sampling locations were beyond the shorelines of the beach [40], done by using boats or during scientific expeditions. It can be concluded, therefore, that the proposed assay could be usefully applied to examine phosphate concentration in seawater.

4. CONCLUSIONS

The primary aim of this study was to propose a cost-effective, single reagent-based rapid detection method for detecting inorganic phosphates in natural and treated water samples. The proposed detection strategy based on LCV was generally applicable with good selectivity and sensitivity. The suitable parameters (temperature and time) for the colourimetric assay were also optimised through experiments. Concentrations of phosphate were determined by visual comparison and by analysis of intensities of RGB from digital photographs captured using a mobile phone camera and image analysis app. UV-Vis absorbance at 620 nm for phosphate calibration standards decreased as the phosphate concentration increased, showing a relationship between the increasing G intensity and phosphate concentrations. The study also identified Cl^- ions as the main interferents with the detection system, and a strategy to remove interference has been established by pre-treatment of samples with $AgNO_3$ before colourimetric analysis. The LCV detection was successfully applied to analyse the concentrations of inorganic phosphate in different real natural and treated water samples. Spiking unknown phosphate samples further confirmed the sensitivity and selectivity of the detection system. In conclusion, the proposed phosphate detection assay showed remarkable advantages like inexpensive protocol, high efficiency, rapidness and portability compared to conventional methods like mass spectroscopy, chromatographic techniques, and solid phase extraction. Therefore, LCV assay has promising potential in quantitative colourimetric analysis of inorganic phosphate in various water samples, including detecting inorganic phosphate in wastewater and helping ensure drinking water quality.

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