Colorimetric determination of glyphosate in water using ozonolysis followed by spectrophotometric analysis of phosphate product with molybdenum blue reagent: a simple, fast and inexpensive assay of glyphosate.

Rose Criscione
Master's Thesis
Tropical Conservation Biology and Environmental Science
University of Hawai'i at Hilo
200 W. Kawili St.
Hilo, HI 96720

Thesis Advisor: Dr. Matthew Platz

Research Committee: Drs. Tracy Wiegner and Mazen Hamad

Abstract:

A simple and inexpensive assay for quantifying glyphosate in aqueous environments using ozonolysis followed by spectrophotometric analysis of phosphate product with the molybdenum blue reagent has been developed. Glyphosate was oxidized with ozone gas in the presence of sodium hydroxide ($1*10^{-3}$M) and then reacted with the molybdenum blue reagent to yield a colorimetrically quantifiable phosphate product. The absorbance of the heteropoly blue color complex was measured at 830 nm. The concentration of glyphosate was determined using a standard phosphate calibration curve concentration range from 10^{-4} to 10^{-8}M in the presence of NaOH ($1*10^{-3}$M). The reliable detection limit of glyphosate in aqueous environments when using ozonolysis and spectrophotometric methods was found to be $1*10^{-5}$M. The method developed in this study may be applicable in aquatic environments such as streams and estuaries and can be used by high school students, undergraduates, and field workers to obtain quantitative data.

Introduction:

Glyphosate is the active-ingredient in the most commonly applied herbicides including Accord®, Aquamaster®, Glyfos®, Roundup®, Rangerpro®, and Burnout®. Glyphosate-based herbicides (GBHs) are systemic, broad-spectrum, post-emergent, and non-selective (Duke et al. 2018, Gill et al. 2018, Fliss et al. 2021). Glyphosate inhibits a key enzyme found in plants, trees, fungi, algae, and some micro-organisms (Herrmann 1995, Matozzo et al.. 2019). This enzyme catalyzes the synthesis of three essential amino acids tryptophan, phenylalanine, and tyrosine. These amino acids serve as precursors for many natural products including vitamins, pigments, flavor agents and others (Duke 1990, Wang et al. 2016).

Glyphosate based herbicides (GBHs) are used to combat and control virtually all unwanted plant species, both annual and perennial, in agricultural and non-agricultural sectors (Duke et al. 2018, Gill et al. 2018, Matozzo et al. 2019). Glyphosate-based herbicides are used on crops including but not limited to corn, soybeans, cotton, pastures, orchards, rice fields (Duke et al. 2018). Glyphosate based herbicides are commonly used along non-agricultural areas including parks, forests, railways, streets, public gardens, and waterways to control aquatic weeds (van Bruggen et al. 2018, Tang et al. 2015, Duke et al. 2018, Clements et al. 2017, Gill et al. 2018, Rolando et al. 2017, Torstensson et al.. 2005, Matozzo et al. 2020).

Glyphosate is the most commonly used herbicide worldwide, spanning most countries, and indiscriminatory with regards to industrialization status. Currently, glyphosate is the most commonly used herbicide in the USA with reportedly more than 750 glyphosate formulations available (IARC 2017, Matozzo et al. 2019). GBHs are also used throughout Australia, China, India and South Africa (Matozzo et al. 2019).

Intensive worldwide use of glyphosate has led to the continuous use and seepage of glyphosate into water systems (Brovini et al. 2021). Glyphosate has been found to reach nearly all aquatic environments including nearshores, rivers, streams, watersheds and wetlands (Scribner et al. 2007, Ramierez et al. 2014, Spengler et al. 2019, Spengler et al. 2021). The glyphosate concentrations used in this study are representative of the highest concentrations observed in aquatic environments.

Glyphosate-based herbicides (GBHs) have adverse effects on virtually all animals defining the aquatic food web (Table 2). Reported studies have analyzed the effects of various GBHs (i.e., Roundup® and AquaPro®) or technical grade glyphosate acid (> 97%). Effects of GBHs impact virtually all life stages of aquatic species including embryo, larvae, juvenile and adult. Aquatic species effected by GBHs exposure inhabit aqueous environments including freshwater, saltwater, estuarian and brackish water.

Chromatographic methods used to quantify glyphosate in aqueous solutions include various forms of liquid chromatography which differ according to performance level (i.e., HPLC, LC) and detector (i.e., FLD, UV). The limit of detection for quantifying glyphosate in aqueous media using chromatographic methods ranges from $5.91E^{-11}$M to $2.37E^{-7}$M (Peruzzo et al. 2008, Gunarathna et al. 2018). The chromatographic methods documented for quantification of glyphosate with high accuracy in aqueous solutions are time-demanding, expensive, and require specialized equipment and personnel training.

The enzyme-linked immunosorbent assay (ELISA) can also be utilized to quantify glyphosate in aqueous solutions and has a limit of detection ranging from $4.43E^{-10}$ to $2.96E^{-10}$M (Spengler et al..2018, John and Liu et al. 2018, Spengler et al. 2019, Spengler et al. 2021). The ELISA method includes purchasing a costly test-kit (Abraxis LLC), vortexing, magnetic separation, and analysis performed with an SDI RPA-II photometric analyzer.

Spectrophotometric methods reported for quantifying glyphosate in aqueous solutions include using ninhydrin in the presence of sodium molybdate to yield the Ruhemann's purple product with absorption maximum at 570nm and the oxidation of glyphosate with hydrogen peroxide followed by the addition of the molybdenum blue reagent to yield heteropoly blue complex with absorption maximum at 830nm (Glass 1981, Bhaskara and Nagaria 2006, Tzaskos et al. 2012). The limit of detection for spectrophotometric methods ranges from 2.37E-07M to 5.91E-06M glyphosate. The spectrophotometric methods documented for quantifying glyphosate in aqueous media are simple, efficient, and cost-effective. However, one of the simplest spectrophotometric methods quantifying glyphosate includes oxidation with peroxide, and has been reported to be plagued by explosions and is potentially hazardous (Glass, 1981).

Due to the absence of a simple, fast and safe spectrophotometric method for determination of glyphosate, the oxidation of glyphosate using ozonolysis followed by spectrophotometric analysis of the phosphate product with the MBR solution has been described. The present method is based on the reaction of glyphosate with a base (i.e., NaOH, $NaHCO_3$) to deprotonate the ammonium ion into an ozone sensitive amine group. Ozone reacts with the amine group through a series of intramolecular reactions to yield a colorimetrically quantifiable phosphate product. The resultant heteropoly blue color complex was measured at 830 nm.

Materials and Methods

Instruments:

The absorption spectra and absorbance of solutions were measured with Genesys UV-visible spectrophotometer (Genesys 50) at 830nm using a plastic cell with an optical path length of 1 cm. An ENALY instrument (Oxidation technologies, LLC) was used to generate ozone from

pure oxygen. Ozonolysis set-up diagram is demonstrated in Appendix 1. All reagents were weighed on an electronic balance (Ohaus Explorer) with 0.001g resolution.

Reagents:

All reagents used were of analytical reagent-grade purity or similar grade. Sodium phosphate monobasic (Sigma-Aldrich), ammonium molybdate (VWR Analytical), sulfuric acid, L-(+)-ascorbic acid (Avantor Performance Materials), and sodium chloride (Fisher Scientific) were used during this work. Sodium bicarbonate (Fischer Scientific) and sodium hydroxide (Fisher Scientific) were used as base. Ozone will be generated from pure oxygen using an ENALY instrument. Standard reference glyphosate was purchased from Acros organics part of Thermo Fisher Scientific.

Solutions:

Phosphate and sodium bicarb stock solutions

A standard stock solution containing phosphate ($1*10^{-4}$M) and sodium bicarbonate ($1*10^{-1}$M) was prepared by dissolving 0.012g of analytical grade NaH_2PO_4 and 8.4g of analytical grade $NaHCO_3$ in 1L of reagent-grade deionized water.

A sodium bicarbonate ($1*10^{-1}$M) stock solution was prepared by dissolving 8.4g of analytical grade NaHCo3 in 1L of reagent-grade deionized water.

A second standard stock solution containing phosphate ($1*10^{-5}$M) and sodium bicarbonate ($1*10^{-1}$M) was prepared by diluting 10mL of the initial phosphate stock solution to 100mL with sodium bicarbonate stock solution.

Phosphate and sodium hydroxide stock solutions

A standard stock solution containing phosphate ($1*10^{-4}$M) and sodium hydroxide ($1*10^{-3}$M) was prepared by dissolving 0.012g of analytical grade NaH_2PO_4 and 0.04g of analytical grade NaOH in 1L of reagent-grade deionized water.

A sodium hydroxide ($1*10^{-3}$M) stock solution was prepared by dissolving 0.04g of analytical grade NaOH in 1L of reagent-grade deionized water.

A series of standard stock solutions containing phosphate ($1*10^{-8}$-$1*10^{-5}$M) and sodium hydroxide ($1*10^{-3}$M) were prepared by serial dilution.

Glyphosate stock solutions

A standard stock solution containing glyphosate ($1*10^{-4}$M) and sodium hydroxide ($1*10^{-3}$M) was prepared by dissolving 0.017g of analytical grade glyphosate and 0.04g of analytical grade sodium hydroxide in 1L of reagent-grade deionized water.

A second stock solution containing glyphosate ($1*10^{-5}$M) and sodium hydroxide ($1*10^{-3}$M) was prepared by diluting 10mL of the initial glyphosate stock solution to 100mL with the standard stock solution of sodium hydroxide.

Molybdenum Blue Reagent (MBR) stock solutions

Ammonium molybdate ($4.71*10^{-2}$M) solutions were prepared by dissolving 0.900 g of analytical grade $(NH_4)_6Mo_7O_{24}*4H_2O$ in 22.5mL of reagent-grade deionized water, followed by the addition of 75mL of H_2SO_4 (2.5M). Ascorbic acid ($1.80*10^{-1}$M) solutions were prepared by dissolving 0.795g of analytical grade $C_6H_8O_6$ in 45mL of reagent grade deionized water. Molybdenum blue reagent (MBR) solutions were prepared by adding the ammonium molybdate solution to the ascorbic acid solution. Due to the exothermic reaction between H_2SO_4 and water, the ammonium molybdate solution was poured *into* the ascorbic acid solution. Additionally, due to the rate of decomposition of ascorbic acid in water, the molybdenum blue reagent solution was used immediately after preparation.

Procedure for base comparison

Phosphate calibration curves ranging from 0.01-$1*10^{-5}$M PO_4 in the presence of $1*10^{-3}$M NaOH were prepared by adding aliquots of 0-10mL NaH_2PO_4($1*10^{-5}$M) stock solution containing NaOH ($1*10^{-3}$M) to 0-10mL aliquots of NaOH ($1*10^{-3}$M) stock solution. Phosphate calibration curves ranging from 0.01-$1*10^{-5}$ PO_4 in the presence of $1*10^{-1}$M $NaHCO_3$ were prepared by adding aliquots of 0-10mL NaH_2PO_4 ($1*10^{-5}$M) stock solution containing $NaHCO_3$ ($1*10^{-1}$M) to 0-10mL aliquots of $NaHCO_3$ ($1*10^{-1}$M) stock solution. Phosphate was measured colorimetrically as the phosphomolybdate heteropoly blue complex, which was formed by adding 4mL aliquots of the MBR solution to all test tubes. All samples were stored under a ventilation hood for 20-24 hours to allow for color development. The absorbance of the phosphomolybdate heteropoly blue complex was measured against the MBR solution containing $1*10^{-3}$M NaOH on a Genesys 50 spectrophotometer at 830nm. From this data, two separate calibration curves were constructed with the absorbances at 830nm as a function of phosphate concentration in the range from 0.01 to $1*10^{-5}$M PO_4. A calibration curve representing the mean absorbances of phosphate in the presence of sodium hydroxide was constructed by plotting the mean absorbances of phosphate solutions containing sodium hydroxide ($1*10^{-3}$M) as a function of phosphate concentrations. A calibration curve representing the mean absorbance of phosphate in presence of sodium bicarbonate was constructed by plotting the mean absorbances of phosphate solutions containing sodium bicarbonate ($1*10^{-1}$M) as a function of phosphate concentrations. A line-of-best-fit was obtained for both the plots of phosphate in the presence of sodium hydroxide and sodium bicarbonate against absorbance. Using Beer-Lambert Law the slope (absorbance over concentration) of the best-fit line for each plot was used to calculate the molar absorptivity of phosphate in the presence of either sodium hydroxide or sodium bicarbonate. Molar absorptivity was used to compare calibration curve plots and identify if phosphate solutions containing sodium hydroxide or sodium bicarbonate produced a preferred light absorbing blue color complex.

Procedure for quantifying glyphosate

Phosphate formed from the oxidation of glyphosate and ozone was measured using ozonolysis followed by spectrophotometric methods utilizing the molybdenum blue reagent. Two sets of glyphosate samples ranging from 10^{-8} to 10^{-5}M were prepared by adding 0,10mL

aliquots of glyphosate ($1*10^{-4}$M) solution, 0.01,0.1,1mL aliquots of NaOH ($1*10^{-3}$M) solution, and 0,9,9.9,9.99mL aliquots of glyphosate ($1*10^{-5}$M) solution to test-tubes. One set of test tubes containing 10^{-8} to 10^{-5}M GP and 10^{-3}M NaOH were subjected to ozonolysis, the other set of test tubes were not. To oxidize glyphosate into a colorimetrically quantifiable phosphate product, each sample was subjected to 15 minutes of ozone gas. A 4mL aliquot of MBR solution was added to each test-tube after ozonolysis. All samples were stored under a ventilation hood for 20-24 hours to allow for color development. The absorbance of the phosphomolybdate heteropoly blue complex was measured on a Genesys 50 spectrophotometer at 830nm against the set of samples that were not subjected to ozone gas. It was assumed that 100% of phosphorus in glyphosate converts to phosphate. The quantity of glyphosate in each sample was calculated by using the phosphate calibration curve containing NaOH ($1*10^{-3}$M). To account for the dilution of sample by MBR solution, the concentration of glyphosate was then multiplied by 1.4. A percent difference between the known initial glyphosate stock concentration and the calculated phosphate concentration was determined for each sample and used to determine detection limit.

Procedure for quantifying glyphosate in presence of phosphate

Phosphate formed from the oxidation of glyphosate in the presence of residual phosphate concentrations was determined in a similar way. Glyphosate samples ranging from $0.9 *10^{-5}$ to 10^{-6}M in presence of 10^{-5} to 10^{-6}M PO_4 were prepared by adding 0.1,1mL aliquots of NaH_2PO_4($1*10^{-4}$M) solution, 0,8,8.9,9,9.9mL aliquots of NaOH ($1*10^{-3}$M) solution and 0,1,9,9.9mL aliquots of glyphosate ($1*10^{-5}$M) solution to test-tubes. Each sample was subjected to ozonolysis procedure as described above. The addition of MBR and color development were also performed as described above. The absorbance of the phosphomolybdate heteropoly blue complex was measured on a Genesys 50 spectrophotometer at 830nm against samples containing only PO_4. The quantity of glyphosate and percent difference for each sample was calculated as described above.

Procedure for determining ozone reactivity with phosphate

Ozone reactivity with phosphate was quantified based on a ratio of absorbance values comparing phosphate samples that were subjected to ozonolysis to phosphate samples that were not. Two separate sets of phosphate samples containing 10^{-8} to 10^{-5}M PO_4 were prepared by adding 10mL aliquots of NaH_2PO_4 ($1*10^{-5}$M) solution, NaH_2PO_4 ($1*10^{-6}$M) solution, NaH2Po4 ($1*10^{-7}$M) solution, NaH2PO4 ($1*10^{-8}$M) solution to four separate test-tubes. Only one set of test tubes containing 10^{-8} to 10^{-5}M PO_4 was subjected to ozonolysis procedure as described above. The addition of MBR solution and heteropoly blue color development were performed as described above. The absorbance of the phosphomolybdate heteropoly blue complex for each sample was measured on a Genesys 50 spectrophotometer at 830nm against a 2mL aliquot of MBR solution. The ratio of absorbance values across phosphate samples containing 10^{-8} to 10^{-5}M PO_4 was calculated by dividing absorbance values of phosphate samples that were subjected to ozonolysis to the absorbance values of phosphate samples that were not.

Results and discussion:

It has been demonstrated by ^{31}P NMR spectroscopy that ozonolysis of glyphosate in presence of a base (i.e., NaOH) completely transforms glyphosate in aqueous solution to phosphate (Platz per com, 2022). In deionized water (pka 7.0) the zwitterionic form of glyphosate that dominates consists of a protonated ammonium ion (Figure 1). In the proposed method, sodium hydroxide converts the ammonium ion to an ozone sensitive amine group. The oxidation of glyphosate fragments the molecule into a quantifiable phosphate product through a series of intramolecular reactions (Platz per com, 2022). The proposed reaction mechanism for the degradation of glyphosate into a quantifiable phosphate product is given in Figure 2. The phosphate product was further reacted with a molybdenum blue reagent solution to form a heteropoly blue complex (Figure 3-7).

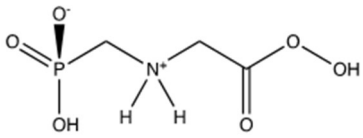

Figure 1: Molecular structure of glyphosate N-(Phosphonmethyl) Glycine in the phosphonate zwitterionic form (Pubchem, 2021)

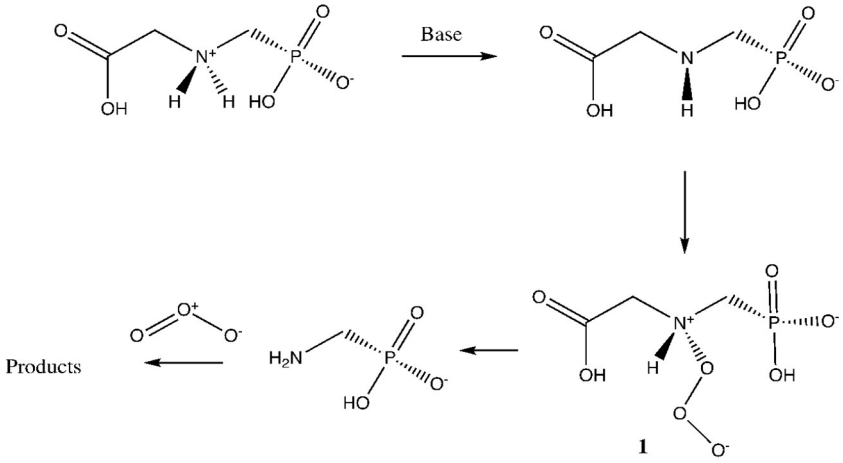

Figure 2: Mechanism of reaction of glyphosate (zwitterionic form) with base and ozone. (Platz per com, 2022)

Figure 3: Heteropoly blue color complex formation across phosphate concentrations ranging from $1*10^{-7}$M to $1*10^{-5}$M NaH_2PO_4 solutions containing NaOH ($1*10^{-3}$M).

Figure 4: Heteropoly blue color complex formation across phosphate concentrations ranging from $1*10^{-7}$M to $1*10^{-5}$M NaH_2PO_4 solutions containing $NaHCO_3$ ($1*10^{-1}$M).

Figure 5: Heteropoly blue color complex formation across glyphosate ($1*10^{-8}$ to $1*10^{-4}$M) samples that were subjected to 15min of ozone gas. Samples also contained $1*10^{-3}$M NaOH.

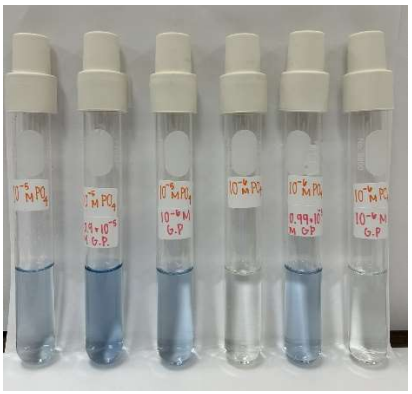

Figure 6: Heteropoly blue color complex formation across glyphosate ($1*10^{-8}$ to $1*10^{-4}$M) samples containing various concentrations of phosphate ($1*10^{-5}$ to $1*10^{-6}$M NaH_2PO_4). All samples were subjected to 15min of ozone gas and contained $1*10^{-3}$M NaOH.

Figure 7: Heteropoly blue color complex formation across phosphate concentrations ranging from $1*10^{-8}$ to $1*10^{-5}$M NaH_2PO_4 comparing samples subjected to ozonolysis to samples not subjected to ozonolysis. All samples were subjected to 15min of ozone gas and contained $1*10^{-3}$M NaOH.

Effect of sodium hydroxide and sodium bicarbonate on phosphate analytical curve

The effect of sodium hydroxide and sodium bicarbonate on the absorption values of phosphate was studied in the range of $1*10^{-8}$ to $1*10^{-4}$M NaH_2PO_4 (Figure 8). In the concentration range of $1*10^{-8}$ to $1*10^{-4}$M $NaHPO_4$ the equipment followed Beer-Lamer Law for both calibration curves. The slope of the calibration curve, correlation coefficient, and molar absorptivity were calculated and summarized in Table 1. Phosphate solutions containing sodium carbonate formed bubbles during spectrophotometric analysis as a result of bicarbonate reactivity with water. Variable absorption values observed for phosphate samples containing sodium

bicarbonate contributed to a large standard error observed in Figure 8. Phosphate solutions in the presence of sodium hydroxide produced a preferred light absorbing blue color complex.

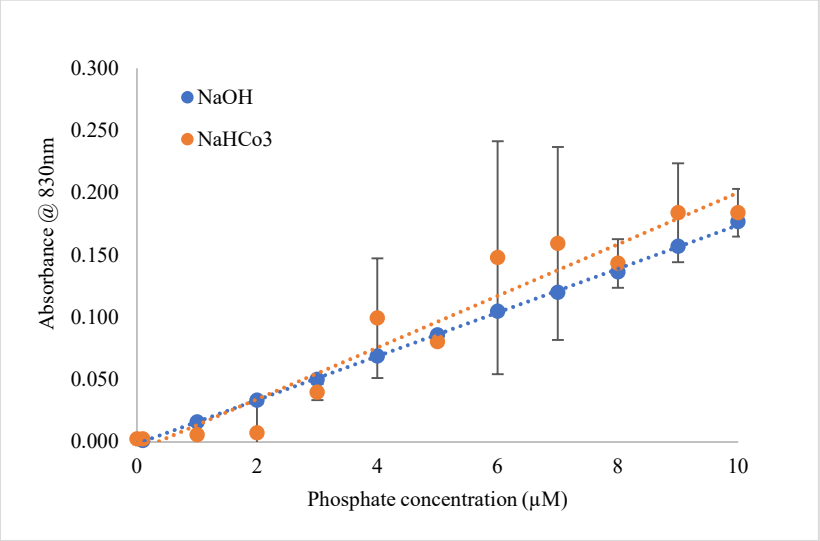

Figure 8: Calibration curve of phosphate in the concentration range from 0-10 µM NaH_2PO_4 with either sodium hydroxide ($1*10^{-3}$M) or sodium bicarbonate ($1*10^{-1}$M).

Base	Slope	Correlation coefficient R^2	Molar absorptivity ($M^{-1}cm^{-1}$)
NaOH	0.0176	0.9995	1.755E-4
$NaHCO_3$	0.0207	0.9357	2.075E-4

Table 1: The slope, correlation coefficient, and molar absorptivity of heteropoly blue complex with phosphate in the concentration range from 0-10 µM NaH_2PO_4 with either sodium hydroxide ($1*10^{-3}$M) or sodium bicarbonate ($1*10^{-1}$M).

Detection limit of glyphosate using ozonolysis and spectrophotometric analysis of phosphate product with molybdenum blue reagent

Table 2 demonstrates the percent difference between initial known glyphosate concentration and calculate phosphate concentration across samples containing $1*10^{-8}$ to $1*10^{-4}$M glyphosate. The reliable detection limit of glyphosate for the proposed method was found to be $1*10^{-5}$M and similar to previous studies utilizing spectrophotometric methods quantifying glyphosate (Glass 1981, Bhaskara and Nagaraja 2006, Nnamonu et al. 2012, Tzaskos et al. 2012). Comparison across previous studies quantifying glyphosate is difficult due to the varied concentration units reported. A glyphosate conversion table has been provided in Appendix 2.

Initial Stock GP Concentration (M)	Absorbance @ 830nm	Quantified glyphosate (M)	% Difference
1.00E-04	1.744	1.39E-04	32.80
1.00E-05	0.163	1.31E-05	27.11
1.00E-06	0.038	3.15E-06	103.57
1.00E-07	0.027	2.30E-06	183.37
1.00E-08	0.032	2.66E-06	198.50

Table 2: Percent difference between initial known glyphosate stock concentration and calculated phosphate concentration for samples containing glyphosate concentrations ranging from $1*10^{-8}$ to $1*10^{-4}$M.

Effect of residual phosphate on determination of glyphosate using ozonolysis and the molybdenum blue reagent

The mean percent difference between initial known glyphosate concentration and calculated phosphate concentrations for solutions containing $1*10^{-6}$ to $9.9*10^{-6}$M glyphosate and $1*10^{-6}$ to $1*10^{-5}$M phosphate are given in Table 3. Residual phosphate concentrations ranging from $1*10^{-6}$ to $1*10^{-5}$M did not influence the determination of glyphosate using ozonolysis followed by spectrophotometric analysis of phosphate product using the molybdenum blue reagent solution.

PO_4 concentration (M)	Glyphosate concentration (M)	Absorbance @ 830nm	Calculated glyphosate (M)	Percent difference (%)
1.00E-05	9.00E-06	0.161	1.30E-05	36.20
1.00E-05	1.00E-06	0.016	1.39E-06	32.48
1.00E-06	9.90E-06	0.173	1.39E-05	33.49
1.00E-06	1.00E-06	0.016	1.41E-06	34.28

Table 3: Percent difference between initial known glyphosate stock concentration and calculated phosphate concentration for samples containing glyphosate concentrations ranging from $1*10^{-6}$ to $9.9*10^{-6}$M and phosphate concentrations ranging from $1*10^{-6}$ to $1*10^{-5}$M NaH_2PO_4.

Ozone selectivity

The absorbance ratios across phosphate concentrations ranging from $1*10^{-8}$ to $1*10^{-5}$M are demonstrated in Table 4. Ozone was found not to react with phosphate samples ranging from $1*10^{-8}$ to $1*10^{-5}$M NaH_2PO_4.

Phosphate Concentration (M)	Ozonolysis A_{830}	Non-ozonolysis A_{830}	Ratio
1E-05	0.182	0.174	1.05
1E-06	0.019	0.016	1.19
1E-07	0.004	0.003	1.33
1E-08	0.001	0.001	0.79

Table 4: Absorbance values and ratio across phosphate samples containing $1*10^{-8}$ to $1*10^{-5}$M NaH_2PO_4.

Application of method

The proposed method has been applied successfully for the determination of glyphosate in water using ozonolysis followed by spectrophotometric analysis of the phosphate product with a molybdenum blue reagent solution. The reliable limit of detection of glyphosate using the proposed method is comparable, or orders of magnitude lower than concentrations of concern reported for species inhabiting aquatic environments. Glyphosate concentrations of concern for aquatic species has been reported to range from $5.91*10^{-8}$M to $3.83*10^{-3}$M glyphosate (Tsui & Chui 2003, Zhang et al. 2017). The proposed method is able to quantify concentrations of concern reported for bacterium (Tsui & Chui 2003), crustacean (Tsui & Chui 2003), cyanobacteria (Zhang et al. 2016), fish (Zhang et al. 2017), macroalgae (de Campos Olivieria et al. 2016), microalgae (Tsui et al. 2003), mollusk (Boutet et al. 2004, Matozoo et al. 2019), protozoa (Tsui & Chui 2003). When collecting field samples residual or background phosphate concentrations are likely to be present. Previously documented phosphate concentrations recorded for freshwater streams, estuaries, nearshore and offshore environments are below the lowest phosphate concentration tested (Binkley et al. 2004, Tesoriero et al. 2009, Knee et al. 2019, Wiegner et al. 2012). The proposed method is applicable in field samples from streams, estuaries, nearshore or offshore environments containing residual phosphate concentrations.

Conclusion:

The proposed method is simple, cost-effective and safe, based on the formation of colorimetrically quantifiable heteropoly blue complex from the oxidation of glyphosate by ozonolysis followed by spectrophotometric analysis of the phosphate product using a molybdenum blue reagent solution. Other methods quantifying glyphosate (i.e., HPLC) are time-demanding and expensive. The proposed method is applicable in aquatic environments such as streams and estuaries and easy to use for non-professionals, field-workers and high-school students.

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