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A SIMPLE SPECTROPHOTOMETRIC METHOD FOR THE QUANTITATIVE ANALYSIS OF PHOSPHATE IN THE WATER SAMPLES Nur Habibah\*1, I Gusti Ayu Sri Dhyana Putri2, I Wayan Karta3, Cokorda Dewi Widhya Hana Sundari.4, Mochammad Choirul Hadi5 Politeknik Kesehatan Kemenkes Denpasar, Jurusan Analis Kesehatan, Jalan Sanitasi No.1 Sidakarya, Denpasar Selatan 80224, Bali, Telp. (0361)710447 \*email: nur.habibah.poltekkesdps@gmail.com ABSTRACT A simple spectrophotometric method was used to determine the phosphate content in the water samples.

The method is based on the formation of molybdenum blue complex from the reaction of orthophosphate and ammonium molybdate followed by reduction with ascorbic acid in the aqueous sulfuric acid medium. The color intensity of the molybdenum blue complex is proportionally to the phosphate content in the solution. The system obeys Lambert-Beer's Law at the 890 nm in the concentration range of 0.1-1 mg P/L and the linear calibration graph was obtained with the slope, 0.6334, intercept, 0.0074 and correlation coefficient of 0.9988.

This official method was also used for the quantitative analysis of phosphate in the water samples and the satisfactory result was obtained. The range of phosphate concentration in the water samples are 0.033 – 2.943 mg P/L. Keywords: Phosphate, Phosphate analysis, spectrophotometric method, molybdenum blue complex

INTRODUCTION Phosphorous (P) is one of the most abundance element and found as crustal earth component.

Phosphorous is most commonly found as orthophosphate (Chaube and Gupta, 1983; Shyla, Mahadevaiah and Nagendrappa, 2011; Ganesh, et al., 2012; Mahadevaiah, et al., 2007). Naturally, various form of phosphorous such as ortho-, pyro-, poly-, meta-, organic, colloidal, and also suspended phosphorous are present in the water. Each of this phosphorous form can be measured as orthophosphate (Mihajlovic, et al., 2007).

Phosphates are essential element for the growth of plant and animal and also stimulate the growth of plankton and aquatic plants which provide food for fish. This may cause an increase the fish population and improved the water quality (Shyla, Mahadevaiah and Nagendrappa, 2011). Although phosphate is not toxic element, but the excessive concentration of phosphate in the natural water may caused detrimental effect to the aquatic life.

An excess concentration of phosphate in the natural water may caused excessively grow of algae and aquatic plants and using up the large amount of oxygen, so the dissolved oxygen level in the water was decreased. This condition is known as eutrophication. Eutrophication brings the detrimental effect for the aquatic life. To control the eutrophication process, EPA was recommended the maximum concentration of phosphate is 50 and 100 µg/L in a stream point phosphates entering the natural water and in a stream point that phosphates did not directly entering the natural water, respectively.

One of the greatest phosphate contributors to the natural water is detergent. A syntetic detergent consists of a surfactant, builder and other miscellaneous ingredient. Phosphates are great builders and often used as sodium tripolyphosphates or sodium/potassium phosphates. These phosphates are capable to bind several metal ions in the natural water such as calcium, magnesium, iron dan manganes, so the washing performance was increased (Pradhan and Pokhrel, 2013).

The largely used of phosphate in the various industries

such as fertilizer, detergent, beverage, food, meat, sugar and canned drinks also leads the increase release of phosphate to the natural water (Shyla, Mahadevaiah and Nagendrappa, 2011). However, the routine analysis of phosphate is importance in the areas of environmental sciences, agriculture, medicinal etc.

In the recent years, several methods have been developed to monitoring the phosphate levels in the natural water. Several methods such as complexogravimetry (Mamadal and Kundu, 2005), colorimetry (Khrisnamurthy and Suryanarayana, 1982); Williams, et al., 1982), spectrophotometry (Chaube and Gupta, 1983; Shyla, Mahadevaiah and Nagendrappa, 2011; Ganesh, et al., 2012; Mahadevaiah, et al., 2007; Mihajlovic, et al.,

2007, Hayashi, Dazuka and Ueno, 1960; Borissova and Mitroplitska, 1979; Motomizu, Wakimoto and Kyoji, 1984; Gutschik, 1985 and Smeller, 1995), atomic absorption spectrometry (Christian and Feldman, 1968), flow-injection spectrophotometry (Motomizu and Mitsuko, 1984), ion chromatography (Ruiz and galceran, 2005) and also HPLC (Botker, et al., 1994).

Some of these methods offer disadvantages such as requiring complicated and expensive equipments and the few of these are also involving the extraction procedurs, so obviously limiting the practice method in the common laboratory (Shyla, Mahadevaiah and Nagendrappa, 2011). Among these methods, spectrophotometric method involve ammonium molybdate are the most commonly practiced method.

This common method offer some advantages such as simple, easy, cheap, hence this method is much suitable for routine analysis of phosphate in the water sample. The determination of phosphate in this method is based on the reaction between orthophosphate and ammonium molybdate in the aqueous acidic condition, followed by its reduction by various reducing agent, for example ascorbic acid.

The result of this reaction is molybdenum blue complex, with the maximum absorbance at the wavelength range of 800-900 nm. The color intensity of this blue complex is proportionally with the phosphate content in the sample (Pradhan and Pokhrel, 2013). In order to increase the accuracy of this method, several modification have been conducted.

Some reducing agents such as thiorea, sodium sulfide and also hidrazyne sulphate were used for the substitution of ascorbic acid, due to the lake stability of this reducing agent (Shyla, Mahadevaiah and Nagendrappa, 2011; Mahadevaiah, et al., 2007; Pradhan and Pokhrel, 2013). Although some earlier paper stated that the official spectrophotometric method involve ascorbic acid has the limitation due to the stability

of ascorbic acid, but this present work proved that ascorbic acid still powerful to provide the good accuracy for the phosphate routine analysis.

**MATERIAL AND METHOD Instrumentation** A UV-Vis spectrophotometer Biochrom Libra S-12 was used for absorbance measurements at the wavelength range of 800-900 nm. Radwag balancing digital was also used to weigh all the reagents. **Reagents Preparation** All the reagents were prepared by dissolving chemical analytical grade reagents in the distilled water.

**Standard phosphate solution A** stock solution of phosphate (500 mg P/L) was prepared by dissolving of 2.195 g potassium dihydrogen phosphate anhydrate into a glass beaker, then transferred into a 1000 mL volumetric flask and diluted with the distilled water. Subsequently, the series of phosphate working solution was prepared by further dilution of stock solution.

**Sulfuric acid** Sulfuric acid 5 N was prepared by dilution of 70 mL concentrated sulfuric acid (~ 36N) with distilled water in a 500 mL volumetric flask to the mark. **Ammonium molybdate solution** Dissolved 20 g of ammonium molybdate in about 100 mL distilled water, then transferred into a 500 mL volumetric flask and diluted to the mark. **Potassium antimonyl tartrate** Dissolved 1.375 g of potassium antimonyl tartrate in about 300 mL distilled water in a 500 mL volumetric flask and diluted to the mark.

Ascorbic acid solution Dissolved 1.76 g ascorbic acid into a 100 mL volumetric flask and diluted to the mark.

Mixed solution Mixed solution was prepared by mixing 50 mL of solution a, 15 mL of solution b, 5 mL of solution c and 30 mL of solution c. The mixed solution cannot be used if the color turned into blue. This mixed solution was stable for 4 hours.

Determination the maximum wavelength of the reduced phosphomolybdate complex solution The absorption spectrum (Fig.1) of the reduced phosphomolybdate complex solution was determined by using 50 mL of 0.8 mg P/L phosphate working solution and 8 mL of mixed solution.

Subsequently this solution was stored in a room temperature for about 10 min. The absorbance of this solution was measured at 800-900 nm against blank solution.

Determination the color stability of the reduced phosphomolybdate complex solution The color stability of the reduced phosphomolybdate complex solution was studied by using using 50 mL of 0.8 mg P/L phosphate working solution and 8 mL of mixed solution.

The absorbance of this solution was measured immediately after the addition of mixed solution for about 45 min. The results are shown in Fig 2. Recommended procedure A series of 250 beaker glass was prepared. Pipetted 50 mL of various phosphate working solution to the each glass. One drop of phenolphthalein was added to the glass.

If the solution is turned into pink-red, a number of 5 N sulfuric acid was added to the the solution until the pink color was disappeared. Subsequently, 8 mL of mixed solution was added to the each glass. The solutions were stored at room temperature for about 30 min. The absorbances of the solutions were measured at 890 nm against blank solution.

Application procedure for the water samples Waste water samples were collected from the various household laundry industry. The waste water samples was filtered through a Whatmann filtered paper and stored in a bottle sample at 4 °C. Aliquots of the sample were used for the determination of the phosphate content by using the recommended procedure.

Fig. 1.

The absorption spectrum of the reduced phosphomolybdate complex solution from the solution of 50 mL of 0.8 mg P/L phosphate working solution and 8 mL of mixed solution

RESULT AND DISCUSSION The simple spectrophotometric method for the phosphate measurement in this work was adapted from the official phosphomolybdate method with ascorbic acid as a reducing agent (BSN, 2005).

This method is much simple, compared with the other developed method. This method provides some advantages such as, simple, easy, cheap and also carried out without any complex preparation sample procedure. Hence, this simple method is suitable for the routine determination of phosphate in common laboratories.

The quantitative determination of phosphate in this work is based on the formation complex between orthophosphate with ammonium molybdate and potassium antimonyl tartrate in the aqueous sulfuric acid medium, followed by its reduction with ascorbic acid (BSN, 2005). In the acidic medium, orthophosphate and molybdate ion condense into molybdophosphoric acid, which upon selective reduction produces blue color, due to the formation of molybdenum blue complex.

Under the optimized condition, the color intensity of the molybdenum complex was proportional to the amount of phosphate content in solution (Shyla, Mahadevaiah and Nagendrappa, 2011; Ganesh, et al., 2012; Mahadevaiah, et al., 2007). Since the silicate was interfere to this phosphomolybdate method, some modification method was performed.

In this work, complexing, reducing and masking agent was mixed before the addition to the phosphate working solution and water sample (BSN, 2005). As the previous report, the addition of masking agent such as tartaric acid before the formation of molybdophosphate species is the one of the effective method for the decreasing of silicate interference (Yaqoob, Nabi and Worsfold, 2004).

The other paper stated that the reduction molybdophosphoric acid with ascorbic acid or tin (II)-chloride in the presence of potassium antimonyl tartrate was effectively decrease the silicate interference and increasing the sensitivity of the phosphomolibdenum blue method. These authors stated also that the antimony ions play as catalyst (Murpy and Riley, 1962).

\_ In order to determine the maximum wavelength of this blue complex, the absorbance of phosphate working solution at the concentration of 0.8 mg P/L was measured in the wavelength range of 800- 900 nm. From Fig. 1, conclude that the maximum wavelength was obtained at 890 nm with the absorbance 0.466. Hence, the absorbance of phosphate working solution and water sample should be measured at this wavelength.

The color stability of the complex was one of the important parameters that affecting the phosphate result of the method. Therefore, the color stability of the molybdenum complex was studied carefully. Some previous works were developed a new method with substituting ascorbic acid with the others reducing agent, such as hydrazine sulphate, sodium sulphide, tin(II)- chloride and also thiourea due the stability of ascorbic acid Shyla, Mahadevaiah and Nagendrappa, 2011; Ganesh, et al., 2012; Mahadevaiah, et al.,

2007; ; Mihajlovic, et al., 2007). Based on the result which has shown in Fig. 2, we can conclude that the molybdenum blue complex resulted from the reduction process by the ascorbic acid has good stability. The color of molybdenum blue was stable with no significant different in absorbance from the initial mixing process up to 45 min. after mixation process.

Based on this result, the measurement of absorbance was conducted 30 min. after the addition of the mixed solution. In order to obtain the accurate results, ascorbic acid solution should be used before 7 days after its preparation because ascorbic acid solution was stable for one week at 4 °C. Under the optimum experimental condition, the calibration graph was determined in the concentration range of 0.1-1 mg P/L at a wavelength of 890 nm. Fig. 3 showed that the system obeys Lambert- Beer's Law and linier calibration graph was obtained with slope, 0.6334, intercept, 0.0074 and correlation coefficient of 0.9988.



— Fig. 2.

Effect of time on the color stability of the reduced phosphomolybdate complex solution from the solution of 50 mL of 0.8 mg P/L phosphate working solution and 8 mL of mixed solution

The correlation coefficient value showed that there was a proportional correlation between absorbance and phosphate concentration in the measured solution. Subsequently, the recommended procedure was applied to determine the phosphate content in the water samples.

The obtained results are shown in Table 1. The measurement results showed that most of the waste water samples contain of phosphate with the relative high concentration. The range of phosphate concentration in the waste water samples are 0.033 – 2.943 mg P/L. The release of waste water into the natural water influences the water quality and also the aquatic life.

The highly phosphate concentration in the waste water, which entering the natural water caused the increasing of phosphate content in the natural water. In the mild concentration, phosphates are essential element for the aquatic life.

— Fig. 3. Linier calibration graph of phosphate working solution at 890 nm

Table 1.

Determination of phosphate in water samples	Sample	Absorbance	Phosphate
Concentration (mg P/L)	W1	0.151	0.249±0.0011
	W2	0.072	1.246±0.0033*
	W3	0.070	1.222±0.0089*
	W4	0.179	2.943±0.0022*
	W5	0.098	0.166±0.0033
	W6	0.024	0.050±0.0022
	W7	0.274	0.443±0.0056
	W8	0.345	0.556±0.0011
	W9	0.014	0.033±0.0011
	W10	0.037	0.693±0.0011*
	W11	0.156	2.580±0.0011*
	W12	1.097	1.743±0.0658*
	W13	0.047	0.085±0.0033
			*Phosphate concentration after dilution factor

Phosphates are required for the growth stimulates of plankton and aquatic plants, which provide food for fish, so the fish population and water quality was improved.

However, the excess of phosphate concentration in the natural water caused detrimental effect to the aquatic life. The excessive concentration of phosphate in the natural water may caused excessively grow of algae and aquatic plants, which is using up the large amount of oxygen and decreasing the dissolved oxygen level in the water. This condition is known as eutrophication.

The natural water became eutrophic when the range of phosphate concentration reaches up to 0.035-0.1 ppm. In order to control the eutrophication process, EPA was regulated the maximum concentration of phosphate in a stream that did not directly enters into the natural water that is 100 µg/L, so the routine analysis of phosphates content both in the waste waters and natural waters are important.

Hence, the practical, accurate and simply method are required for the routine analysis. The measurement results proved that the spectrophotometric method by using ascorbic acid for the phosphate measurement provide the accurate results, practical and also simple, so this method is suitable for routine quantitative analysis of phosphate in the water sample.

CONCLUSIONS A simple spectrophotometric method for the routine determination of phosphate in the natural water was studied. This method was adapted from the official method.<sup>19</sup> The presented results proved that this method is working satisfactorily and suitable for routine analysis of phosphate.

This method is also provide some advantages, such as simple, easy, cheap, hence, this main method is still powerfull for phosphate determination in water. REFERENCES Badan Standarisasi Nasional, 2005, SNI Air dan Air Limbah-Bagian 31; Cara Uji Kadar Fosfat dengan Spektrofotometer Secara Asam Askorbat. Borissova, R. & Mitropolitska, E., 1979, Spectrophotometric determination of phosphate ions with the system cerium (III)-Arsenazo III, Talanta 26, 543–547. Botker, H. E.,

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