Effect of Heating on the Color Formation Reaction in the Murphy and Riley Method for the Determination of Phosphate in Natural Waters

Su-Cheng Pai1*, Tzu-Yuan Wang1, Tien-Hsi Fang2 and Kuo-Tung Jiann2
1Institute of Oceanography, National Taiwan University, Taipei, Taiwan
2Department of Marine Environmental Informatics, National Taiwan Ocean University, Keelung, Taiwan

Abstract

Heating is frequently applied to enhance the reaction rate for the determination of phosphate but it may cause unexpected errors. Experiments show that the spectrum of the blue color produced by the Murphy and Riley method is subject to change at different temperatures. The variation of the molar extinction coefficient at 880 nm was found to be -48 M cm⁻¹°C⁻¹, or a relative trend of -0.22 \% °C⁻¹ w.r.t. to the absorbance at 30°C. At temperatures >60°C, the results were erratic and the change becomes irreversible. These findings suggest that the measurement should be made at room temperature and any heating process above 35°C should be avoided. A thermostated cuvette holder set at 30°C is highly recommended for both manual and flow type operation.

Keywords: Phosphate; Spectrum; Heating; Color formation reaction

Introduction

The molybdate-ascorbic acid method developed by Murphy and Riley [1] for the determination of phosphate in natural waters has been widely adopted in many scientific disciplines [2-4]. When the manual procedure is converted to an automated operation, heating is frequently applied to enhance the rate of color formation reaction for a fast throughput. For example, APHA/AWWA/WEF Standard Methods have suggested an on-line 37°C water bath [3]; Johnson and Petty [6] have employed a 50°C bath; and in an extreme case, a 95°C reactor has been used by Yuchi et al. [7]. Although heating can effectively improve the reaction rate [8,9], some scientists have encountered other problems. Benson et al. [10] have reported a phenomenon where the peak signals decreased significantly when the bath temperature was raised up to 60°C; Drummond and Maher [11] and Zhang et al. [12] have both observed noticeable silicate interferences at elevated temperatures. They have suggested that the determination should be preferably performed at room temperature.

However, we have encountered a dilemma on whether or not one should apply an on-line heating device. On our past expedition cruises, seawater samples were collected from different depths (e.g. from surface to >3000 m deep with original temperatures from 30°C down to 2°C) and all nutrients including phosphate were measured on-board by a flow injection system within 1 hour after retrieving the sampler to the deck. The use of an on-line heating bath could largely minimize the large temperature differences between samples and standards, but the uncertainty induced has yet been well evaluated. In order to clarify this problem, a series of heating experiment was carried out at higher temperatures (>30°C) and all nutrients including phosphate were measured on-board a temperature-controlled cuvette holder (Shimadzu T-83400-52) connected to a thermostat water circulation bath (Firstek B206) and controlled by a 750 W heater. A dome-type flow cuvette (Hellma, 1 cm length, 450 µL capacity) was used. The sample solution was placed in a reaction bottle immersed in a second thermostat water bath (Firstek B206) and controlled by a 750 W heater. The sample could be quickly taken up by a peristaltic pump to fill up the cuvette in 10 s.

Material and Methods

Reagents

Mixed reagent (HMoSb): A 200 mL molybdate solution (containing 5.1 g of ammonium molybdate heptahydrate) was poured gradually while stirring into a beaker filled with 180 mL of conc. sulfuric acid, followed by mixing a 100 mL of the antimony solution (containing 0.5 g of potassium antimony tartrate). The final volume was made up to 500 mL.

Ascorbic acid reagent (ASC): An amount of 13.2 g of L-ascorbic acid was dissolved in 250 mL of double distilled water.

Phosphate standard: A 10 mM P stock solution was prepared by dissolving 0.1361 g of KH₂PO₄ in 1 L of double distilled water. Diluted working standards were prepared from this stock solution.

Spiked seawater: A filtered surface seawater (salinity=33.4 psu) was prepared previously. The original concentration was <0.05 µM P. An aliquot of 3 mL of the stock standard solution was added to this seawater to make a final volume of 1 L to give a spiking concentration of 30 µM.

Reagent strengths: The reagent strengths used in this study were slightly different from the original recipe [1] with a lower acidity to minimize Si interference and higher Sb³⁺ and ASC concentrations to increase the reaction rate [11,12]. When 2 mL of HMoSb reagent and 1 mL of ASC reagent are added to a 25 mL aliquot of sample, the final reagent strengths are: [Mo]=4.13 mM; [H⁺]=309 mM; [Sb³⁺]=0.213 mM; [ASC]=10.7 mM, with a [H⁺]/[Mo] ratio of 74.8.

Apparatus

A Shimadzu UV1800 spectrophotometer was equipped with a temperature-controlled cuvette holder (Shimadzu T-83400-52) connected to a thermostat water circulation bath (Firstek B401) with a 1 kW heater and a 700 W cooler (Figure 1). A dome-type flow cuvette (Hellma, 1 cm length, 450 µL capacity) was used. The sample solution was placed in a reaction bottle immersed in a second thermostat water bath (Firstek B206) and controlled by a 750 W heater. The sample could be quickly taken up by a peristaltic pump to fill up the cuvette in 10 s.

Experiment designs

Exp-1 Absorption spectra: A fully-developed blue color solution was transferred to the cuvette for scanning from 950 to 650 nm at different temperatures. The absorbance at 880 nm was recorded along a time scale while the thermostat was heated up step-wisely from 15 to 90°C, then cooled back down to 15°C.

*Corresponding author: Su-Cheng Pai, Institute of Oceanography, National Taiwan University, Taipei, Taiwan, Tel: 886922227358; E-mail: scpai@ntu.edu.tw

Received April 30, 2015; Accepted May 12, 2015; Published May 20, 2015

Citation: Pai S, Wang T, Fang T, Jiann K (2015) Effect of Heating on the Color Formation Reaction in the Murphy and Riley Method for the Determination of Phosphate in Natural Waters. J Environ Anal Chem 2: 139. doi:10.4172/2380-2391.1000139

Copyright: © 2015 Pai S et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.
Exp-2 Color formation at fixed temperatures: Both thermostats were adjusted to the same temperature. The reagent added sample was immediately transferred to the flow cuvette then trapped for observation of the formation curve.

Exp-3 Color formation at elevating temperature: The starting temperature was set at 25°C but the cuvette holder was heated up to 30-90°C at 15°C intervals. The sample added with reagents was transferred immediately to the heated flow cuvette. The color formation curve obtained this way was under a temperature-ramping condition.

Results and Discussion

Spectra at different temperatures

In Exp-1 two color-fully-developed phosphomolybdenum blue solutions (50 µM in freshwater and 30 µM in seawater) were scanned at different temperatures and the overlapped absorption spectra are shown in Figure 2. The change of spectra due to temperature was significant, and a knot at a wavelength of 935 nm was found for both media. On the left-hand side of this knot the absorption patterns varied in a step-wise manner at 5°C intervals. As temperature increased, the absorbance decreased like a mirror image. The molar extinction coefficient at 880 nm was found to be 23400, 22850, 22600, 21700 M⁻¹cm⁻¹ at 15, 25, 30, 50°C, respectively. The slope of the absorbance decreased like a mirror image. The molar extinction coefficient at 880 nm is shown in Figure 3. The blue colored complex at various temperatures for (A) 50 µM P in freshwater and (B) 30 µM P in seawater. The temperature settings were 25, 35-75 °C at 10°C intervals.

Color formation reaction at fixed temperatures

In Exp-2 the color formation reaction took place at fixed temperatures (20, 25, 30, 35 and 45°C) and the results are presented in Figure 5. At 20°C the curve was an “S” shape with a slow beginning, followed by an accelerating stage and then a slow ending. The time for reaching 90% of maximum absorbance (t₉₀%) was 62 s at 20°C, reduced to 38 and 24 s at 25 and 30°C, with -1% and -2% lower absorbances. At 45°C, the reaction was very fast (t₉₀%<15s), but the ending absorbance appeared to have a rising trend. This rising phenomenon can be more clearly seen in the enlarged diagrams in Figure 6 and was not found to be due to the change of the reagent blank (all reagent blanks were <0.001 at 10 min).

There are four samples shown in Figure 6 with three tested concentrations (10, 30 and 50 µM P) in freshwater and the last one was 30 µM P in seawater. At 45°C the absorbance for a 10 µM P sample was 0.198 at 30 s and rose to 0.204 at 400 s, with a difference as large as 0.550. In Exp-3 the absorbance difference was 0.540 at 30 s and rose to 0.550 at 400 s, with a difference as large as 0.550.

The observation on the effect of changing temperature to the molar extinction coefficient at 880 nm is shown in Figure 3. The blue colored solution was again transferred to fill the cuvette. The cuvette holder was first cooled down to 15°C, and then the thermostat was heated up in a step-wise manner at 5°C intervals. As temperature increased, the absorbance decreased like a mirror image. The molar extinction coefficient at 880 nm was found to be 30400, 29400, 28200, 26200, 24000 M⁻¹cm⁻¹ at 15, 20, 25, 30 and 35°C, respectively. The slope of variation was found to be -48 M⁻¹cm⁻¹°C⁻¹ (Figure 4) or a relative trend of -0.22 %°C⁻¹ w.r.t. the absorbance value at 30°C. After reaching the highest 90°C, the cooler of the thermostat was switched on and the temperature was dropped also in a step-wise manner. At the ending temperature of 90°C, the absorbance went back to almost the identical value as that of the starting 15°C. In the seawater medium the trend and slope were almost the same as that in freshwater. The change due to temperature variation was apparently reversible in this experiment.

Figure 1: Apparatus used to monitor the color formation reaction in this study. The system has two thermostat baths (1 and 2). The first one is connected to a water-circulating cuvette holder installed in a Shimadzu UV1800 spectrophotometer. A dome-type Hellma flow cuvette (1 cm, 450 µL) is inserted into the holder. The sample is placed in a bottle immersed in a second thermostat. Upon adding reagents, the mixture is quickly taken up to fill the cuvette by a peristaltic pump at a rate of 20 mL min⁻¹.

Figure 2: Overlapped spectra of the phosphomolybdenum blue complex at various temperatures for (A) 50 µM P in freshwater and (B) 30 µM P in seawater. The temperature settings were 25, 35-75 °C at 10°C intervals.

Figure 3: Observation of the variation of absorbance at 880 nm by changing the temperature of the cuvette holder. (A) 50 µM P in freshwater (B) 30 µM P in seawater.
values as described in Exp-1. The effect of heating in Exp-3 was no longer reversible.

**Conclusion**

The negative aspect of heating on the Murphy and Riley method for the determination of phosphate has long been overlooked by scientists but evidences found in this study reveals that the uncertainty induced by the temperature difference should not be ignored. In general, the molar extinction coefficient of the final blue color at 880

---

**Reaction at ramped temperatures**

In Exp-3 the sample (50 µM P) was maintained at a fixed starting temperature of 25°C and the cuvette holder was heated up by setting the thermostat at 30, 45, 60, 75 and 90°C, respectively. The results were somewhat erratic. All curves shown in Figure 7 tangle with each other in the first hour and the readings appear to stabilize only after 180 min. Enlarged diagram (Figure 7) gives a clearer pattern. Only at 30°C is the structure of the blue complex might be different if the reaction takes place at a temperature higher than 35°C. Exp-2 did not continue to add higher temperatures as water vapor started to condense on the wall of the sample bottle.

---

**Figure 4:** Relationship between the molar extinction coefficient (880 nm) and temperature in (circle) freshwater and (dot) seawater. The slope was found to be -48 M⁻¹cm⁻¹°C⁻¹ for both media, or a relative trend of -0.22%°C⁻¹ with respect to 22600 M⁻¹cm⁻¹ at 30°C.

**Figure 5:** (Left) The color formation curves for a 10 µM P solution at 20, 25, 30, 35 and 45°C, respectively. (Right) Time required for reaching 90% of maximum absorbance (t₉₀%) is a function of temperature.

**Figure 6:** (Left) Enlarged color formation curves of different P concentrations and at various fixed temperatures. (Right) Absorbance at 400 s as a function of temperature. Arrows indicate the consistent climbing-up of the reading. Dash lines indicate a trend of -0.22%°C⁻¹. Aliquots of 25 ml of spike samples were added with 3 ml of reagents to give final concentrations of (A) 8.93 µM P (B) 26.8 µM P (C) 44.6 µM P in freshwater and (D) 26.8 µM P in seawater.

**Figure 7:** (Left) Color formation curves of a 50 µM P sample (final concentration 44.6 µM P) with a starting temperature of 25°C and ending temperatures of 30, 45, 60, 75 and 90°C, respectively. All curves were raw signals without deducting the reagent blanks. Reagent blanks were significant only when the temperature was higher than 75°C and 10 min after the reaction began. (Right) Enlarged diagram showing the change in the first 10 min, with dash lines indicating the reagent blank-deducted curves.
nm has an inverse relationship with temperature. If the color formation reaction takes place at temperatures much higher than 35°C, erratic results can happen and it is irreversible even if the solution cools back down to room temperature. The use of heating at more than 35°C should be avoided in any case. When a batch of samples having a wide temperature range is to be measured, or when a sample is just digested for total P measurement, they should be left to equilibrate with the surrounding temperature before adding reagents. For manual operation a temperature-controlled cuvette holder set at a fixed 30°C can be used for optimal precision and accuracy. For automated analysis the same cuvette holder can be used to reduce the possible variation due to the temperature effect.

Acknowledgments
The authors would like to thank B.W. Tang of Shih-Shin Instrument Co., Taipei, Taiwan for his suggestion on cuvette holder. This project was supported by the National Science Council, Taipei, Taiwan under a contract no. NSC-97-2611-M-002-002-MY3.

References
1. Murphy J, Riley JP (1962) A modified single solution method for the determination of phosphate in natural waters. Anal Chim Acta 27: 31-36.
2. Going JE, Eisenreich SJ (1974) Spectrophotometric studies of reduced molybdooantimonylphosphoric acid. Anal Chim Acta 70: 95-106.
3. Eisenreich SJ, Bannerman RT, Armstrong DE (1975) A simplified phosphorus analysis technique. Environ Lett 9: 43-53.
4. Jarvie HP, Withers PJ A, Neal C (2002) Review of robust measurement of phosphorus in river water: sampling, storage, fractionation and sensitivity. Hydrol Earth Syst Sci 6: 113-132.
5. Figueras MJ, Inza I, Polo FL, Fellu MT, Guarro J (1996) A fast method for the confirmation of fecal streptococci from M-enterococcus medium. Appl Environ Microbiol 62: 2177-2178.
6. Johnson KS, Petty RL (1982) Determination of phosphate in seawater by flow injection analysis with injection of reagent. Anal Chem 54: 1185-1187.
7. Yuchi A, Ogiso A, Muranaka S, Niwa T (2003) Preconcentration of phosphate and arsenate at sub-ng ml⁻¹ level with a chelating polymer-gel loaded with zirconium (IV). Anal Chim Acta 494: 81-86.
8. Pai SC, Yang CC, Riley JP (1990) Effects of acidity and molybdate concentration on the kinetics of the formation of the phosphoantimonymolybdenum blue complex. Anal Chim Acta 229: 115-120.
9. Sjosten A, Blomqvist S (1997) Influence of phosphate concentration and reaction temperature when using the molybdenum blue method for determination of phosphate in water. Wat Res 31: 1818-1823.
10. Benson RL, Truong YB, McKelvie ID, Hart BT (1996) Monitoring of dissolved reactive phosphorus in wastewaters by flow injection analysis. Wat Res 30: 1959-1964.
11. Drummond L, Maher W (1995) Determination of phosphorus in aqueous solution via formation of the phosphoantimonymolybdenum blue complex. Re-examination of optimum conditions for the analysis of phosphate. Anal Chim Acta 302: 69-74.
12. Zhang JZ, Fischer CJ, Orther PB (1999) Optimization of performance and minimization of silicate interference in continuous flow phosphate analysis. Talanta 49: 293-304.