

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

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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 $-48 \text{ M}^{-1}\text{cm}^{-1}\text{C}^{-1}$, or a relative trend of $-0.22 \text{ \% } ^\circ\text{C}^{-1}$ w.r.t. to the absorbance at 30°C . When the color formation reaction took place at $>35^\circ\text{C}$, the absorbance started to climb. At temperatures $>60^\circ\text{C}$, 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 [5]; 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 $>3000 \text{ m}$ 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 concentrations ($30\text{-}50 \text{ }\mu\text{M}$) in order to magnify the variation. The results will provide field analysts a quantified measure of the heating effect so they are able to choose a proper way to avoid such an uncertainty.

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 KH_2PO_4 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 $<0.05 \text{ }\mu\text{M}$ 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 $30 \text{ }\mu\text{M}$.

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 Sb^{3+} 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: $[\text{Mo}]=4.13 \text{ mM}$; $[\text{H}^+]=309 \text{ mM}$; $[\text{Sb}^{3+}]=0.213 \text{ mM}$; $[\text{ASC}]=10.7 \text{ mM}$, with a $[\text{H}^+]/[\text{Mo}]$ 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 B401H) 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 .

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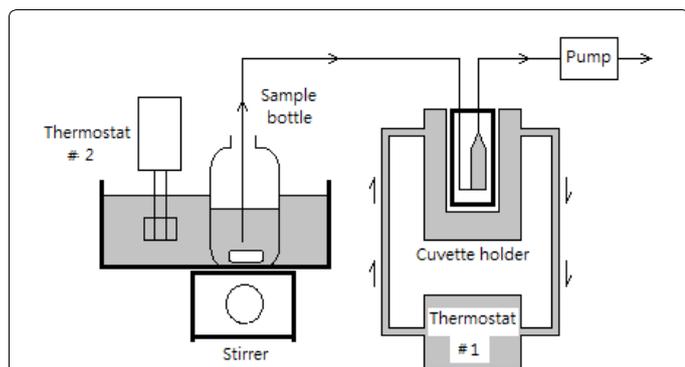


Figure 1: Apparatus used to monitor the color formation reaction in this study. The system has two thermostat baths (#1 and #2). The first one is connected to a water-circulating cuvette holder installed in a Shimadzu UV1800 spectrophotometer. A dome-type Hellma flow cuvette (1 cm, 450 μ L) is inserted 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} .

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 μ M in freshwater and 30 μ M 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 became lower as temperature increased, and vice versa.

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 23400, 22850, 22600, 21700 ... 19800 $\text{M}^{-1}\text{cm}^{-1}$ at 15, 25, 30, 50 ... 90°C, respectively. The slope of variation was found to be $-48 \text{ M}^{-1}\text{cm}^{-1}\text{C}^{-1}$ (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 15°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.

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 ($t_{90\%}$) 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 ($t_{90\%} < 15\text{s}$), 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 μ M P) in freshwater and the last one was 30 μ M P in seawater. At 45°C the absorbance for a 10 μ M P sample was 0.198 at 30 s and rose to 0.204 at 400 s, with a difference as large

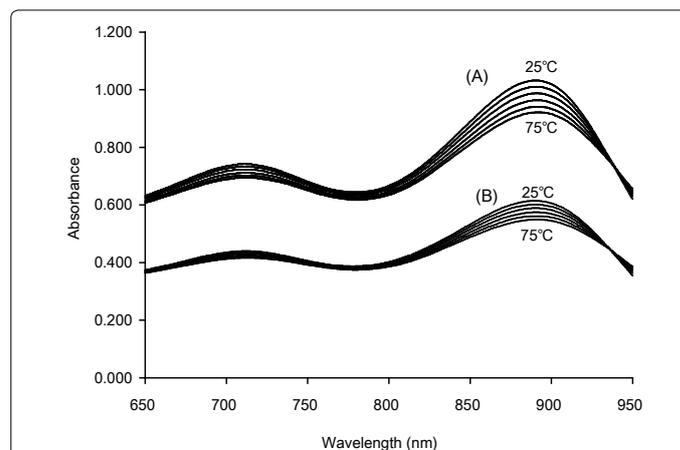


Figure 2: Overlapped spectra of the phosphomolybdenum blue complex at various temperatures for (A) 50 μ M P in freshwater and (B) 30 μ M P in seawater. The temperature settings were 25, 35- 75 °C at 10°C intervals.

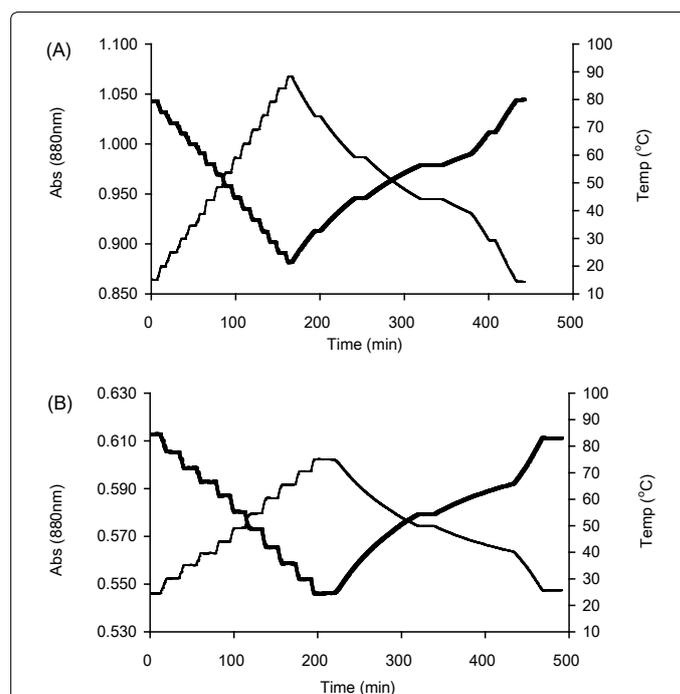
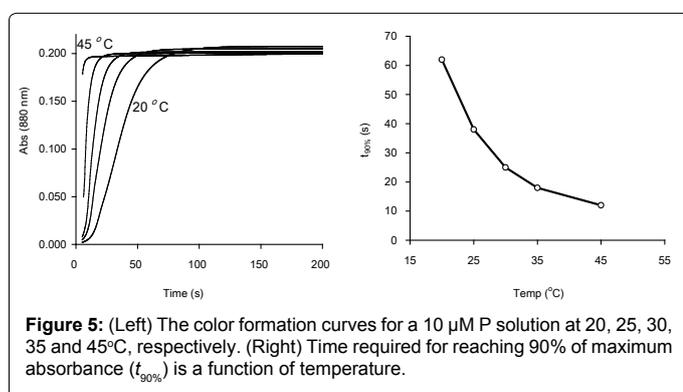
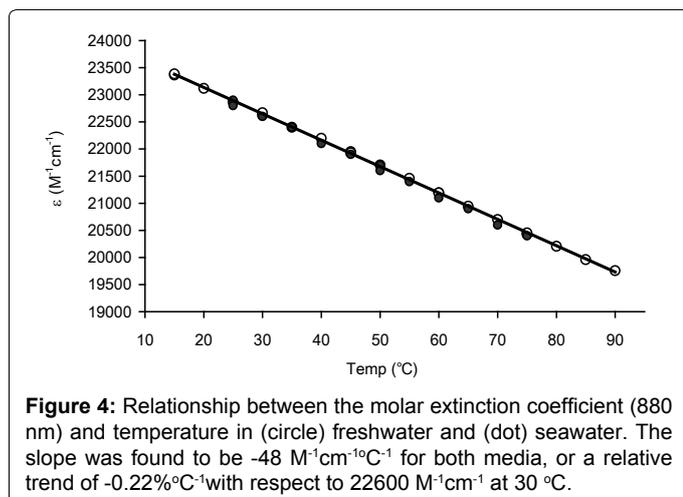


Figure 3: Observation of the variation of absorbance at 880 nm by changing the temperature of the cuvette holder. (A) 50 μ M P in freshwater (B) 30 μ M P in seawater.



as $+3\%$ or a slope of $+0.5\% \text{min}^{-1}$. This climbing trend continued even after 400 s, and the slope was more obvious at the relatively low concentration comparing to $+0.25\% \text{min}^{-1}$ at $30 \mu\text{M}$ and $+0.16\% \text{min}^{-1}$ at $50 \mu\text{M}$, respectively. For the seawater medium, the phenomenon was almost the same as for freshwater. It reveals that, the molecular 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.

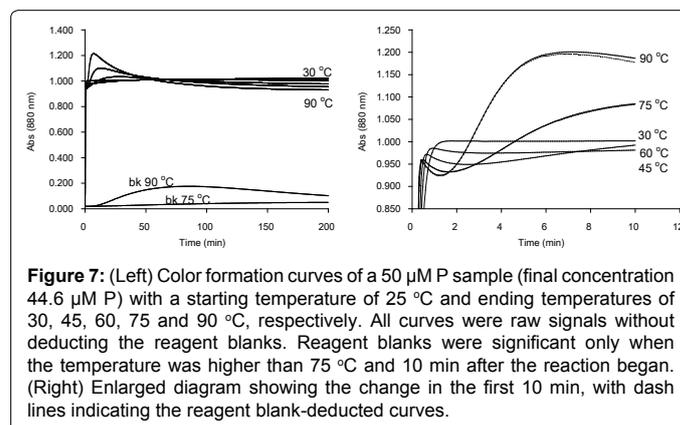
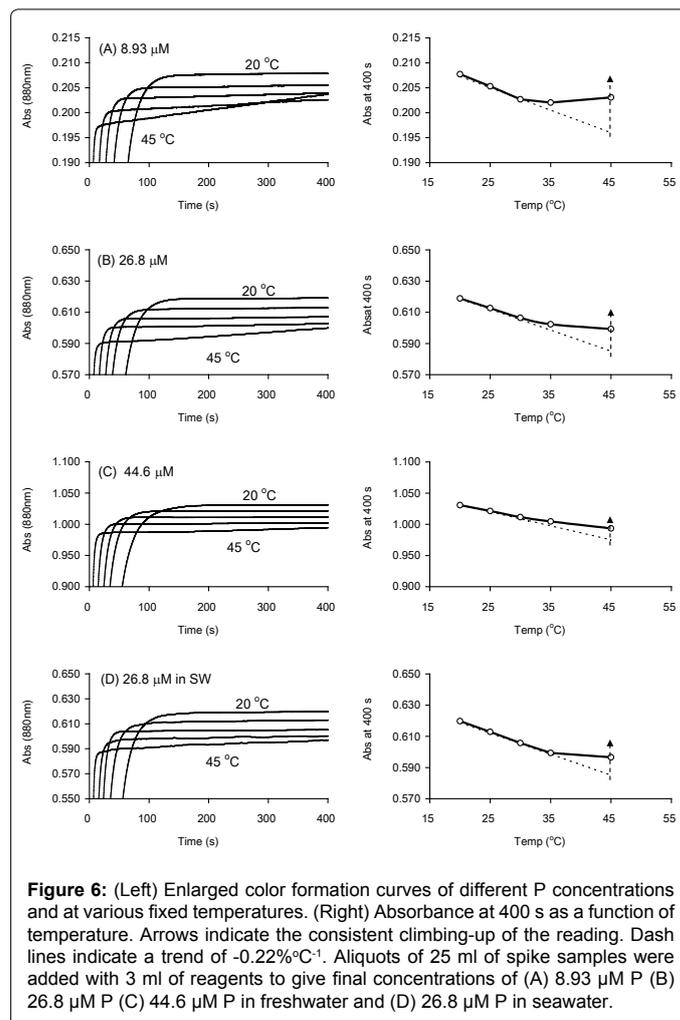
Reaction at ramped temperatures

In Exp-3 the sample ($50 \mu\text{M P}$) 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 curve in a normal shape. At 45 °C the blue color formed quickly in 30 s, but dropped a little afterward, and then rose again gradually after 3 min. At 60 °C the curve dropped after 30 s, rose after 2 min. At 75 °C the amplitude of the drop-rise became magnified, and after 15 min the absorbance dropped again. At 90 °C the curve ran high quickly to an absorbance of 1.2 at 7 min which was $+20\%$ in excess of the normal reading. After 7 min the absorbance dropped down gradually. The cause of this hump is unclear, and it is not parallel with the reagent blanks which became obvious only at $> 75 \text{ °C}$ after 10 min. An extended observation was made by allowing the blue color to form at high temperatures and then cool down to 25 °C . The final absorbance readings did go high but did not go back to the expected

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



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.

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