CHAPTER 2

EXPERIMENTAL

2.1 Molybdenum blue method

2.1.1 Chemicals

All chemicals are analytical reagent grade and used without further purification, otherwise stated, as follows:

1) L-ascorbic acid ($C_6H_8O_6$, AR grade) (Fisher Scientific, UK)
2) Sulfuric acid (H_2SO_4, 98%, AR grade) (RCI Labscan, Thailand)
3) Hydrated potassium antimony(III) oxide tartrate (K(SbO) $C_4H_4O_6 \cdot 1/2H_2O$, AR grade) (Sigma-Aldrich, Steinheim, Germany)
4) Phosphate standard solution (1000 mg/LPO_4^{3-}, AAS grade) (Merck, Germany)
5) Ammonium molybdate tetrahydrate ($(NH_4)_6Mo_7O_{24} \bullet 4H_2O$, AR grade) (Carlo Erba, Italy)
6) Di-sodium hydrogen arsenate heptahydrate ($Na_2HAsO_4 \cdot 7H_2O$, AR grade) (Merck, Germany)

2.1.2 Reagent and solution preparations

All reagents and standard solutions were prepared using milli-Q water (Milli-Q, Millipore, Sweden).

2.1.2.1 Preparation of standard solutions

The stock phosphate standard solution was prepared by pipetting 0.5 mL phosphate standard solution (1000 ppmPO_4^{3-}**)** in 10 mL volumetric flask. Then**,** the

solution was diluted to 10 mL with milli-Q water. Phosphate standard solutions for the calibration graph were prepared daily by diluting a 50 µg/mL stock phosphate standard solution with milli-Q water to the concentration in the range of 1 to 15 µg/mL

2.1.2.2 Preparation of reagents

2.1.2.2.1 Ammonium molybdate tetrahydrate solution

Ammonium molybdate tetrahydrate (1 g) was dissolved with hot water and stood at room temperature. The volume was made up to 25.00 mL in a volumetric flask.

2.1.2.2.2 L-ascorbic acid solution

L-ascorbic acid (0.01 mol/L) was daily prepared by weight 1.76 g L-Ascorbic acid dissolved with water and made up to 25 mL in a volumetric flask.

2.1.2.2.3 Sulfuric acid solution

The 2.5 M of sulfuric solution was prepared by diluting 35 mL of 98% H_2SO_4 with water and adjusted volume to 250 mL.

2.1.2.2.4 Potassium antimony(III) oxide tartrate solution

Potassium antimony(III) oxide tartrate (0.0686 g) was dissolved in ultrapure water and was made up to 25 mL in a volumetric flask.

2.1.2.2.5 Mixed reagent

The above reagents were mixed: 50 mL H_2SO_4, 5 mL potassium antimony (III) oxide tartrate solution, 15 mL ammonium molybdate solution, 30 mL L-ascorbic acid solution and water to made up 150 mL. The reagent should be stable for 4 hours.

2.1.2.3 Single standard method for phosphate determination

The single standard solution (50 µg/mLPO_4^{3-}) was taken in different volumes into a series (0.02, 0.1, 0.2 and 0.3 mL) into 25 mL volumetric flasks before adding the reagent mixture (0.8mL) and adjusting to the mark with water. The amount of phosphate in each flask, µgPO_4^{3-}, can be calculated from the concentration (50

µg/mLPO_4^{3-}) and volume of the standard. After standing for 10 min, absorbance (880 nm) of each flask was measured. A linear calibration, which is a plot of absorbance (880 nm) vs µgPO_4^{3-} could be obtained for 1 to 15 µgPO_4^{3-}.

2.2 Determination Phosphate by using Phosphate test with mobile phone

2.2.1 Equipment/Apparatus

1) UV/VIS spectrometer model UV-1800 (Shimadzu, Japan)
2) Phosphate-test (Merckoquant®, Germany)
3) Micropipette (Eppendorf, Germany)
4) Smart phohe (iphone 4s)
5) Hand Blender non electric (BOSCH MSM6A70)
6) Hand Centrifuge
7) Kitchen Scissors
8) Food tong
9) Light control box
10) Digital balance (Professional Digital Table Top Scale 500g x 0.01g)
11) 15 ml Centrifuge tubes
12) Plastic droppers
13) 10 ml Volumetric flask
14) Battery 3K (Thai Storage Battery Public Company Limited, Thailand)
15) 1200 watt DC to AC power invertor (Chaina)
16) 20oz. Plastic Cup
17) Spatula

2.2.2 Method

2.2.2.1 Sample preparation

1) Weigh the frozen samples without packaging (1 pack)

2) Blend the frozen samples until homogenous (it was cut into small pieces for easy blending).

3) Weight 10.xx g of samples into a plastic cup.

4) Add 100 mL of milli-Q into the plastic cup then use a whisk to beat the sample solution for 2 minutes.

5) Transfer 10 mL of the sample solution into 15 ml of centrifuge tube then centrifuge for 10 min.

6) Pipet 5 mL of the supernatant solutions into the small plastic cup prepare for determination of phosphate.

2.2.2.2 Determination of phosphate by using the test strips

When the test strips was dipping into the sample solution, the phosphate will be react with the reagent on the reaction zone. It was reducing to molybdenum blue when the sulfuric acid was dropping onto the reaction zone. The procedure for phosphate test is as follows:

1) Immerse the reaction zone of the test strip in the sample extract solution for 1 second.

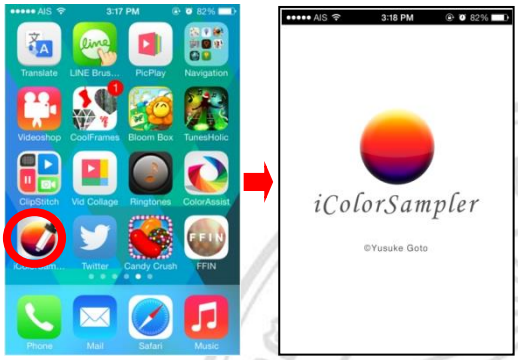
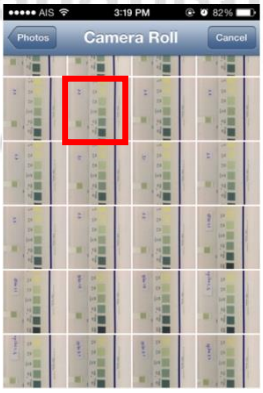
2) Drop the reagent and place on the reaction zone and allow to react for 15 second. Allow excess liquid to run off via the strip onto an absorbent paper towel.

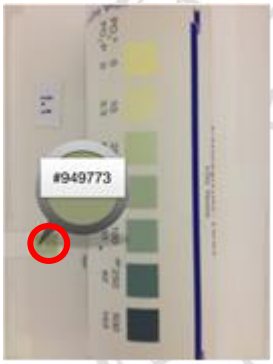
3) After 1 min determine with which color field on the label the color of the reaction zone coincides most exactly by take a photo with iphone4S

The label color of the standard chart as show the rang of phosphate concentration 0 to 500 mgPO_4^{3-}/L which can be used to approximate the amount of phosphate in the rough.

2.2.2.3 Measurement the R intensity by using application of an iphone4S

The R intensity of the photo was measuring by using the application of an iphone4S (icolorsampler for iphone version 1.0.0 @2012, YUSUKE GOTO), It's can be download from the apple store. The R intensity measurement step as follow:

	1. Open the icolorsampler (version 1.0.0 @2012, YUSUKE GOTO) application for iphone
	2. Choose the photo of the test strips for measure R intensity by select the photo menu.
	3. Choose the photo

	4. Use the finger touch the picture for get bigger of it.
	5. Use the finger touch the area of the photo for measure the R intensity.
	6. Touch the code of RGB intensity that appeared

	7. Get the RGB intensity

2.3 Linear of calibration curve from R intensity

The calibration graph was plotted between R values of color intensity against concentrations of PO_4^{3-} (25-100 ppmPO_4^{3-}). It was constructed by measurement color intensity from digital image of standard chart for phosphate test. The application icolorsampler (for iphone version 1.0.0 @2012, YUSUKE GOTO) was used for color intensity measuring in this research.

2.4 The control light box

The box was built to control the light and any effect from environment. It was made from white feature board (7 cm x 9.5 cmx 5 cm) on top of the box to drill a hole 2 x 2 cm for placing the camera (iphone4S) on this hole. Inside the box has a small box (height 5.5 cm) to put the standard chart and test strips. All sides of the box will be closed, it is shown in the Fig. 2.1. The intensity of the color on the reaction zone was detected using the icolorsampler (for iphone version 1.0.0 @2012, YUSUKE GOTO) for an iphone 4S in the flash-off mode.

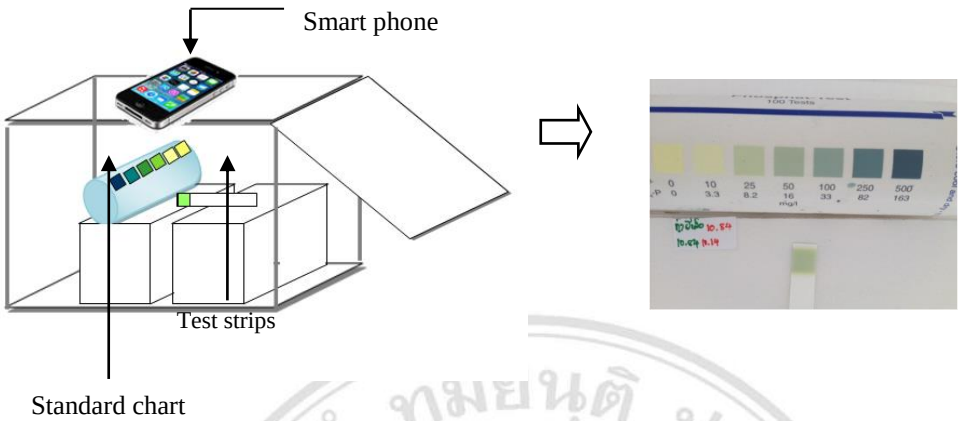


Figure 2.1 Light control unit