CHAPTER 2

EXPERIMENTAL

2.1 Apparatus

1. Polyethylene tubes, DTU, Denmark
2. Polystyrene tubes, OV chemical Co., Ltd., Thailand
3. Polypropylene tubes, OV chemical Co., Ltd., Thailand
4. Polypropylene boxes, Raaco, Denmark
5. Syringe filter, 0.45 μm cellulose acetate, Chrom Tech, Inc., England
6. Ultrasonic bath, Transsonic Digital S, Elma, USA
7. Analytical balance, sartorius Basic BA 210s, Germany
8. Universal indicator, Merck, Germany
9. Cuvette, polystyrene, 0.5-2.0 ml, OV chemical Co., Ltd., Thailand
10. Parafin oil film, Para film, USA
11. Aluminum foil, Diamond, USA
12. UV-VIS spectrophotometer, Lambda 25, Perkin Elmer, Germany

2.2 Chemicals

1. Sodium hydroxide (NaOH, MW 40.00), 99.9%, Lab scan, Ireland
2. Phosphoric acid (H_3PO_4, MW 98.00), 85%, Merck, Germany
3. Potassium choride (KCl, MW 74.55), 99.5%, Carlo Erba, Italy
4. Mercuric chloride ($HgCl_2$, MW 271.49), 99.5%, Carlo Erba, Italy
5. Sodium sulfite (Na_2SO_3, MW 126.02), 98.0%, Carlo Erba, Italy

6. Sulfamic acid (HSO_3NH_2, MW 97.08), 99.0%, Carlo Erba, Italy
7. Formaldehyde (HCHO, MW 30.02), BDH Chemicals Ltd., England
8. Pararosaniline hydrochloride ($C_{19}H_{17}N_3HCl$, MW 323.83), Fluka, USA
9. Hydrochloric acid (HCl, MW 36.46), 37%, BDH Chemicals Ltd., England
10. 1,2-cyclohexylenedinitrilotetraacetic acid monohydrate (CDTA, MW 364.35), Fluka, Japan
11. Potassium hydrogenphthalate ($COOHC_6H_4COOK$, MW 204.23), Fluka, Japan
12. 1,2-di-(4-pyridyl)ethylene, Merck, Germany
13. Sodium nitrite ($NaNO_2$, MW 69.00), 98%, Merck, Germany
14. Ammonium chloride (NH_4Cl, MW 53.41), Lab scan, Ireland
15. Ethylenediamine tetraacetic acid (EDTA, MW 292.25), 99%, Fluka, Japan

2.3 Preparation of solutions

2.3.1 Absorbing solution (potassium tetrachloromercurate; TCM), 0.04 M

A 10.86 g mercuric chloride, 0.066 g EDTA and 6.0 g potassium chloride were diluted to 1,000 ml with distilled water in volumetric flask. (Caution: mercuric chloride is highly poisonous. If spilled on skin, flush with water immediately). The pH of this reagent should be between 3.0 and 5.0. Check the pH of the absorbing solution by using pH indicating paper or a pH meter. The absorbing reagent is normally stable for 6 months.

2.3.2 Hydrochloric acid (HCl) , 1 M

A 8.3 ml of concentrated hydrochloric acid was added to 50 ml of distilled water. The solution was standed until cool and diluted to 100 ml with distilled water in volumetric flask.

2.3.3 Stock pararosaniline (PRA) solution, 0.2% w/v

A 200 mg of pararosaniline hydrochloride was dissolved in 100 ml of 1M HCl. This stock reagent is a yellowish red.

2.3.4 Working solution of pararosaniline reagent

A) Pararosaniline reagent, 0.016 % v/v

A 10 ml of 3 M phosphoric acid was added to 8 ml of PRA stock solution and diluted to 100 ml with distilled water in volumetric flask. The reagent is stable for about a month, but it has to be stored away from heat and light.

B) Pararosaniline reagent, 0.012 % v/v

A 10 ml of 3 M phosphoric acid was added to 6 ml of PRA stock solution and diluted to 100 ml with distilled water in volumetric flask. The reagent is stable for about a month, but it has to be stored away from heat and light.

C) Pararosaniline reagent, 0.008 % v/v

A 10 ml of 3 M phosphoric acid was added to 4 ml of PRA stock solution and diluted to 100 ml with distilled water in volumetric flask. The reagent is stable for about a month, but it has to be stored away from heat and light.

D) Pararosaniline reagent, 0.004 % v/v

A 10 ml of 3 M phosphoric acid was added to 2 ml of PRA stock solution and diluted to 100 ml with distilled water in volumetric flask. The reagent is stable for about a month, but it has to be stored away from heat and light.

2.3.5 Sulfamic acid, 0.6% w/v

A 0.6 g sulfarmic acid was dissolved in 100 ml with distilled water in volumetric flask. The solution has to be daily prepared.

2.3.6 Formaldehyde, 0.2% v/v

A 500 µl of formaldehyde (36 to 38%w/w) was diluted to 100 ml with distilled water in a volumetric flask. The solution has to be daily prepared.

2.3.7 Buffered formaldehyde

A 530 µl formaldehyde (36 to 38% w/w) solution and 0.204 g of potassium hydrogenphthalate and 2 ml of 0.05 M disodium(trans-1,2

cyclohexylene dinitrilo) tetraacetate (Na_2CDTA) were diluted to 100 ml with distilled water in a volumetric flask (Lícia *et al*., 2005).

2.3.8 Stock Na_2CDTA solution, 0.05 M

A 1.82 g 1,2-cyclohexylenedinitrilotetraacetic acid monohydrate (CDTA) and 0.4 g NaOH were diluted to 100 ml with distilled water in a volumetric flask (Lícia *et al*., 2005).

2.3.9 Sulfite standard solution

A 100 mg/l stock standard solution of sulfite were prepared by dissolving 0.0160 g of Na_2SO_3 and 0.0100 g of ethylenediamine tetraacetic acid (EDTA) were diluted to 100 ml with distilled water in a volumetric flask. Working standard solution of SO_2 were prepared by diluting stock standard solution of Na_2SO_3 with potassium tetrachloromercurate 0.04 M. The Na_2SO_3 standard was freshly prepared for every day measurement.

2.3.10 Ammonium solution, 100 mg/l

A 0.0298 g of ammonium chloride was dissolved in 100 ml with distilled water in a volumetric flask.

2.3.11 Nitrite solution, 1000 mg/l

A 0.1530 g of sodium nitrite was dissolved in 100 ml with distilled water in a volumetric flask.

2.3.12 Ozone solution, 100 mg/l

A 0.0069 g of 1,2-di-(4-pyridyl)ethylene was diluted to 25 ml with distilled water in a volumetric flask.

2.3.13 Hydrochloric acid solution, 100 mg/l

A 2.74 ml of 0.1 M hydrochloric acid was diluted to 100 ml with distilled water in a volumetric flask.

2.4 Optimization of method for construction of a SO_2 test kit

In order to construct a SO_2 test kit, a standard US EPA method (Pararosaniline Method, 1982.) was chosen for determination of SO_2 in ambient air. The objective of this part of the work is to investigate the effect of the variables that impose on the complexation process involving in sulfite determination such as concentration of color development reagent (pararosaniline), volume and reaction time of sulfamic acid**,** type and volume of formaldehyde reagent, reaction time of color development and interferences (NO_2, HCl, NH_3 and O_3). Therefore, the influences of these variables are verified by the experiment. Diagram of standard US EPA method for SO_2 determination is shown in Figure 2.1.

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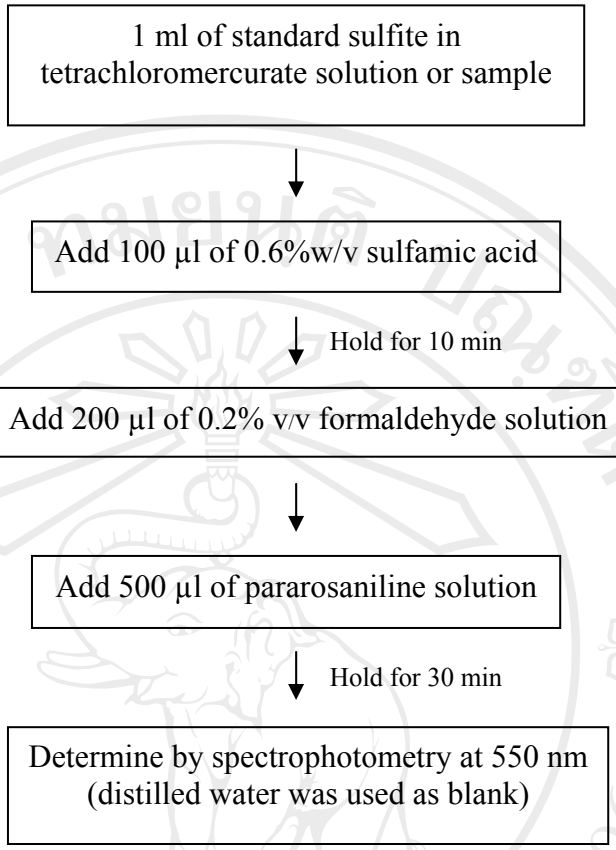


Figure 2.1 Determination of SO_2 by the standard US EPA method

2.4.1 Concentration of color forming reagent

The 100 µl of 0.6 %w/v sulfamic acid was added into 1 ml of sulfite standard in tetrachloromercurate solution with concentration in a range from 0 - 0.8 mg/l were prepared and allowed to react for 10 min. Then 200 µl of 0.2 %v/v formaldehyde and 500 µl of pararosaniline solutions were added. The various concentrations of pararosaniline solution in a section 2.3.4 were tested and left for 30 min. After that, the maximum absorbance of this solution and reagent blank were measured at 550 nm by using a spectrophotometer with a 1 cm optical path length cell by using distilled water as blank.

2.4.2 Volume of sulfamic acid

The volume of 0.6 %w/v sulfamic acid was studied in a range from 25- 400 µl that were added into 1 ml of 0.1 mg/l sulfite in tetrachloromercurate solution. It was left for 10 min before adding 200 µl of 0.2 %v/v formaldehyde. After that the 500 µl of appropriate concentration of pararosaniline solution tested in a section 2.4.1 was added and left for 30 min. The maximum absorbance of this solution and a reagent blank (a mixed solutions without sulfite) were measured at 550 nm by a spectrophotometer with a 1 cm optical path length cell by using reagent blank as blank.

2.4.3 Reaction time of sulfamic acid

Once the appropriate volume of 0.6 %w/v sulfamic acid in a section 2.4.2 was added into 1 ml of 0.1 mg/l sulfite in a tetrachloromercurate solution. After that the reaction time of sulfamic acid varied from 0-20 min was studied. Then the experiment was carried out according to the procedure as described in a section 2.4.2.

2.4.4 Volume of formaldehyde

After the appropriate concentration of pararosaniline solution, volume and reaction time of 0.6 %w/v sulfamic acid (sections 2.4.1, 2.4.2 and 2.4.3) were obtained. Then volumes of formaldehyde (100-500 µl) used for a formation of color solution was tested. The procedure was described in the section 2.4.2.

2.4.5 Type of formaldehyde reagent

Two kinds of formaldehyde solution; 0.2 %v/v formaldehyde reagent and buffered formaldehyde solution, were used for comparing their effects on color intensity of sulfite solution. Again, the procedure for determining standard sulfite solution concentration in a range from 0.05–1.0 mg/l were prepared.

2.4.6 Reaction time of color development

The reaction time of color development was studied after all conditions were achieved. A mix solution was left in a range from 0-30 min to see the stability of color solution and the procedure was mentioned in a topic 2.4.2.

2.5 Effect of the interferences on sulfite solution

The procedure for determining sulfite solution was tested on its stability to the interferences such as HCl, NH_3, NO_2 and O_3, which are common gases in the atmosphere. Different concentrations of each interferences 0.01, 0.10 and 1.00 mg/l were added into the 0.10 mg/l sulfite solution. After that the modified US EPA method was applied and the solutions were measured by spectrophotometry.

2.6 Analytical characteristics

2.6.1 Linear dynamic range (LDR)

Sulfite standard solution was prepared in different concentrations in a range of 0.002-1.50 mg/l in test tubes. After that the modified US EPA method

was applied and the solutions were measured by spectrophotometry. Linear dynamic range was determined by plotting absorbance values versus concentrations of sulfite solution.

2.6.2 Calibration Curve

A calibration curve was constructed by plotting the different concentrations of standard sulfite solution versus absorbances obtained from spectrophotometry. Sulfite solutions were prepared from 100 mg/l standard stock solution that was diluted with 0.04 M TCM in a range of 0.006-0.5 mg/l. After that the modified US EPA method was applied and the solutions were measured by spectrophotometry by using reagent blank as blank. The linearity of the response was determined by considering the correlation coefficiency.

2.6.3 Limit of detection (LOD) and limit of quantification (LOQ)

In analytical chemistry, the LOD is the lowest concentration of the analyte that can be detected with a given degree of confidence. LOQ is a parameter of quantitative assays for low levels of compounds in sample matrices and is used particularly for the determination of impurities and degradation products or low levels of active constituent in a product (Gibbons, 1996). The limits of detection and quantification were calculated as follows:

$$LOD = 3 \times SD \quad (2.1)$$

$$LOQ = 10 \times SD \quad (2.2)$$

2.6.4 Repeatability and Reproducibility

Precision is a measure of random errors, and may be expressed as repeatability and reproducibility. Repeatability is the closeness of agreement between mutally independent test results obtained with the same method on identical test material in the same laboratory by the same operator using the same equipment within short intervals of time. Reproducibility is the closeness of agreement between test results obtained with the same method on identical test material in different laboratories with different operators using different equipment (Gibbons, 1996).

The repeatability of the system was determined by repeating measurements of a 0.10 mg/l sulfite solution by spectrophotometry for 10 times under the same conditions. The reproducibility of the system was pursued by preparing 0.10 mg/l sulfite solution for 10 times and each solution was measured by spectrophotometry for only 1 time. Absorbance values obtained from each test were calculated for their average, SD and %RSD.

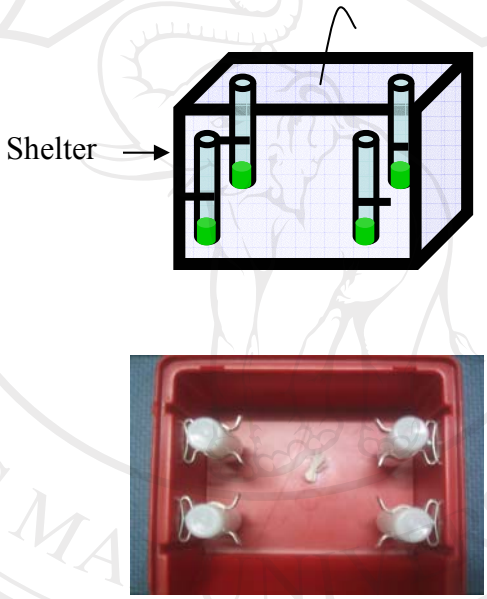
2.6.5 Stability of pararosaniline methyl sulfonic acid complex

A 0.1 mg/l sulfite solution was prepared from the stock standard solution (100 mg/l) in 0.04 M TCM. After that, an experiment was processed as the method mentioned in the topic 2.4. An aliquot of solution was assayed every 2 min in a period of 40 min at room temperature for determination of SO_2 by spectrophotometry.

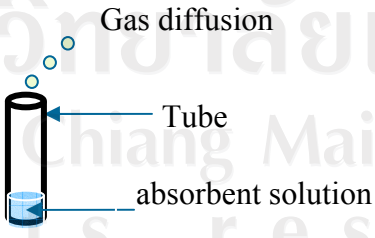
2.7 Passive sampler for SO_2 determination

2.7.1 Configuration of passive samplers

A passive sampler used in this work consists of a diffusion tube containing absorbent solution. The diffusion tube is mounted vertically, the opened end upright, in the protective shielding to protect the effects of meteorology as shown in Figure 2.2.



(a)



(b)

Figure 2.2 The configuration of passive samplers (a) and the gas diffusion pathway (b)

2.7.2 Methodology of ambient SO_2 determination

A) Preparation of passive samplers

A diffusion tube has one opened end, which can be closed with a cap. To minimize background contamination, all the components of a diffusion tube were cleaned up by 30 minutes sonicating and rinsed twice with distilled water before drying at 60^{o}C overnight. The exposed tube can be reused after cleaning.

B) Sampling procedure

The passive sampler consists of a diffusion tube and absorbing chemical. Sampling is started by remove the cap. The samplers are exposed at the sampling site allowing unrestricted movement of air around them. The sampling tube was wrapped with an aluminum foil in order to protect them from sun light. After that the tube was fixed in a protective shelter to minimize the wind turbulence effects and other meteorological conditions and hanged above the ground at least 1.5 meter. In each experiment, the samplers are simultaneously exposed together with unopened samplers being used as blanks. Each set of passive sampler consists of 3 blank tubes and 5 collecting tubes. The exact exposure duration (start and end times) was recorded for calculation of SO_2 concentrations. The experiment was carried out at the air quality monitoring station of Pollution Control Department (PCD) in Yupparaj Wittayalai School. Their concentrations were

compared to values obtained from the PCD monitoring station which SO_2 is detected by fluorescence technique.

C) Sample transportation

When the sampling is done, the exposed passive samplers were collected and immediately closed with caps. The end time of exposure was also recorded. The samplers were kept in the airtight container along transportation back to the lab.

D) Sample preparation

After the exposure, the volume of absorbing solution decreased due to evaporation. Therefore, absorbing solution in each tube was adjusted back to 2 ml with 0.04 M TCM. After that the modified US EPA method was applied and the absorbances of the solutions were measured by spectrophotometry.

E) Calculation of SO_2 concentration in ambient air

SO_2 concentration in ambient air was calculated using an equation 1.4. The unit of microgram per cubic meter ($\mu g/m^3$) was converted to part per billion by volume (ppbv) as shown in an appendix A.

2.7.3 Development of passive samplers

TCM was used for trapping SO_2 in ambient air. A 2 ml of 0.04 M TCM was used as absorbing solution by directly filled into the diffusion tube. Then it was exposed at the sampling site for 72 hrs (3 days).

A) Diffusion tube types

Types of diffusion tube (Figure 2.3) with different lengths and internal diameters (i.d.) were tested to find out the best conditions for SO_2 determination**.** Three types of diffusion tube including polyethylene (PE ; 54 mm length and 14.8 mm i.d.), polystyrene (PS ; 98 mm length and 13.8 mm i.d.) and polypropylene (PP ; 56 and 93 mm length and 14.8 mm i.d.) were used as diffusion tubes for setting up of passive samplers. Their efficiencies in term of SO_2 determination have been compared. Each tube type (5 replicates and 3 blanks) was exposed outdoors for 3 days at Yupparaj Wittayalai School, where the PCD air quality monitoring station is located. Then SO_2 concentrations were determined by spectrophotometry under the optimum conditions. The concentration of SO_2 in each test was calculated by using the median value of 5 replications subtracted with median blank value.

Figure 2.3 diffusion tubes (a) PE; 54 mm length and 14.8 mm i.d., (b) PP; 56 mm length and 14.8 mm i.d., (c) PP; 93 mm length and 14.8 mm i.d. and (d) PS; 98 mm length and 13.8 mm i.d.

B) filtration process of SO_2

Once appropriates type of diffusion tube in a section A was obtained, a step of sample filtration prior to measurement by spectrophotometry is tested. The solution was divided into 2 parts, where the first part was directly measured by spectrophotometry while the other part was filtered by 0.45 µm cellulose filter with helping of glass syringe. The absorbances obtained from both procedures were recorded and compared both in terms of technique (using cellulose filter and no filtration process) and time consuming.

C) Sampling period for SO_2

After appropriate conditions in the section A and B, were obtained, sampling duration was tested by exposure of 7 sets of the samplers at the same station mentioned before. A set of the samplers (5 sampling tubes and 3 blanks) was taken every 24 hours until day 7 for determination of SO_2 concentrations and comparison to the concentrations of the PCD station from the same exposure period.

2.7.4 Validation of passive sampler

An accuracy of the results obtained from the passive sampler and spectrometric measurement was evaluated by comparing with a fluorescence technique obtained from standard active monitoring located at site (Yupparaj Wittayalai School). The active monitoring data was obtained from the pollution control department (PCD) of Thailand.

Precision is expressed as the standard deviation (SD) and percentage relative standard deviation (% RSD), which is calculated by the equations 2.1 and 2.2 (Carl Peter and Jo, 1993).

$$SD = [(Xi-X)^2/ (n-1)]^{½} \quad 2.3$$

$$\%RSD = [SD/ X] * 100 \quad 2.4$$

Where

Xi = individual value in data
X = mean of data
n = number of measurements

Precision of the passive sampling technique was checked by determining the replication of diffusion tubes both for samplers and field blanks.

2.8 SO_2 test kit

Test kits are self-contained analytical kits that use a chemical reaction that produces color to identify contaminants, both qualitatively and quantitatively (http://www.clu-in.org/char/technologies/color.cfm). There are numerous advantages to using test kits in the environmental field, including speed, portability, ease of use and low cost per sample. These kits tend to be screening analytical methods. The change in color indicates the presence of the target compound, while the compounds are quantified if the intensity of the color produced can be compared with the color of standards of known concentrations. The level of certainty will vary depending on whether the intensity of the color is compared visually with a standard color chart.

2.8.1 Construction of a SO_2 test kit and user instruction

Sulfite standard solutions were prepared in a range of 0.006-1.20 mg/l by serial dilution of the 100 mg/l stock solution. Then the US EPA method was applied for color development and SO_2 determination. The mixtures were applied for construction of SO_2 standard color chart. Colors of sulfite standard solutions from different concentrations were classified into 6 levels. Moreover, SO_2 concentrations (µg/ml) were also calculated into ambient concentration (ppbv). After that color chart related to ranges of SO_2 concentration was produced.

The SO_2 test kit composes of PP diffusion tube, aluminum foil, plastic shelter, chemical reagents (4 bottles), standard color chart, disposable syringe and spoon (in handy plastic case). This kit can be used for 50 tests. A test procedure is as follows.

1) Remove the cap of the diffusion tube out and add 2 ml of Reagent-A (absorbing solution; tetrachloromercurate) to the tube and wrap with an aluminum foil.
2) Fix the sampling tube in a protective shelter and hang at the sampling site above ground at least 1.5 meter allowing unrestricted movement of air around them. A recommended sampling duration is 3 days. However, an accurate exposure time has to be recorded.
3) At the end of sampling time, if the volume of absorbing solution was decreased, it must be adjusted back to 2 ml by a syringe and mix the solution.
4) Pull 1 ml of the solution and transfer into a small glass tube. Add 1 spoon of Reagent-B (sulfamic acid do not overfill it), 0.3 ml of Reagent-C (buffer formaldehyde) and 0.5 ml of Reagent-D (pararosaniline), respectively, closes the cap and mix well.
5) Wait 10 minutes until color development was complete. After the specified time, color of the sample solution was compared to the standard color of sulfur dioxide chart to find out its concentration (stability of color fading within 25 min). When the developed color lies between 2 standard colors, read out value between the 2 standard values.

Note Keep all reagents in a cool, dry, dark place and out of the reach of children. Caution: Reagent-A (absorbing solution) is highly poisonous. Avoid contact with skin, especially with eyes. If spilled on skin, flush with water immediately. Avoid generating or breathing dust, do not ingest and wash hands after use. SO_2–kit reagent can be kept and used for 3 months under normal storage.

2.8.2 Stability of test kit reagent

A 0.1 mg/l sulfite solution was prepared from the stock standard solution (100 mg/l) in 0.04 M TCM. After that the modified US EPA method was applied and the solutions were measured by spectrophotometry. All reagents were kept in amber bottles at room temperature in a dark condition. An aliquot of solution was assayed every week. Starting from week 1 (day 0) until absorbtion decreased.

2.8.3 Analysis of sulfur dioxide in air samples by SO_2 test kit in comparison with spectrophotometry and fluorescence techniques

After SO_2 test kit based on passive sampling technique was constructed. The results obtained from modified US EPA method (color development) following with reading of SO_2 concentration by test kit color chart was compared with those from spectrophotometry and fluorescence techniques (PCD's air quality monitoring station). In detail, affecting parameters such as reagent stability and questionnaires were also tested to ensure quality and capacity of the test kit.

2.8.4 Questionnaires (Burgess, 2001)

Questionnaires are an inexpensive way to gather data from a potentially large number of respondents. Often they are the only feasible way to reach a number of reviewers large enough to allow statistically analysis of the results. A well-designed questionnaire that is used effectively can gather information on both the overall performance of the test system as well as information on specific components of the system. If the questionnaire includes demographic questions on the participants, they can be used to correlate performance and satisfaction with the test system among different groups of users.

The random sampling use probability that principle at 95% confidence level. In survey questionnaires, the researcher randomly chosed 100 students in Chemistry Department, Faculty of Science, Chiang Mai University as a representative of the population (Total = 3,000 person). The questionnaire for survey research is shown in Table 2.1. Sulfite standard solution was prepared in different concentrations including 0.006, 0.50, 0.10, 0.15, 0.20, 0.30, 0.40, 0.60, 0.80, 1.0 and 1.2 mg/l in glass tubes by serial dilution of the 100 mg/l stock solution. Then the US EPA method was applied for color development. Compare color of solution in a glass tube with the standard color chart and tick the mark (√) of matching color in a box indicates the measured value of the sample. When the color lies between 2 standard colors, tick in both boxes. Complete the test (11 tubes) using the same procedure. Questionnaire for survey research is shown in Table 2.1.

Table 2.1 Questionnaire for survey research

Tube number	Concentration of sulfite (mg/l)						
	0.006	**0.1**	**0.2**	**0.4**	**0.8**	**1.2**	**Comment**
1							
2							
3							
4							
5							
6							
7							
8							
9							
10							
11							