CHAPTER 2

EXPERIMENTAL

2.1 Apparatus and Instrument

1. Polyethylene tubes, DTU, Denmark
2. Syringe tube 5 ml and 10 ml, Nipro, Thailand
3. GF/A (110 mm i.d) and cellulose filter paper (No 1,125 mm), (No 6, 110 nm) and (No 40, 70 nm), Whatman, USA
4. Polypropylene boxes, Raaco, Denmark
5. Membrane filter (0.45 μm, 13 mm i.d) cellulose acetate, Chrom Tech, Inc., England
6. Ultrasonic bath, Transsonic Digital S, Elma, USA
7. Cuvette polystyrene 0.5-2.0 ml, OV chemical Co., Ltd., Thailand
8. Parafin oil film, Para film, USA
9. Analytical balance, Sartorius Basic BA 210s, Germany
10. Universal indicator, Merck, Germany
11. UV-VIS spectrophotometer, Jassco V 530, Japan

2.2 Chemical

1. Triethanolamine ($C_6H_{15}NO_3$, 99%) (Fluka, Switzerland)
2. N-(1-Napthyl) ethylenediamine dihydrochloride ($C_{12}H_{14}N_2.2HCl$) (Fluka, Switzerland)
3. Sodium nitrite ($NaNO_2$) (Fluka, Switzerland)
4. Sulfanilamide ($C_6H_2N_2O_2S$) (Fluka, Switzerland)
5. Phosphoric acid (H_3PO_4) (S.D. Fine-Chem)

2.3 Preparation of solutions

2.3.1 Absorbing solution (20% v/v of Triethanolamine, TEA)

20 ml of TEA was accurately pipetted in to a 100 ml volumetric flask and adjusted to volume with deionized water.

2.3.2 Sulfanilamide solution

10.75 g of sulfanilamide was weighed and dissolved in 28 ml of phosphoric acid and adjusted volume to 500 ml with deionized water in a volumetric flask.

2.3.3 N-(1Napthyl) ethylenediamine dihydrochloride (NEDA) solution

Dissolving 0.1520 g of N-(1-Napthyl) ethylenediamine dihydrochloride dissolved in deionized water and adjusted to 100 ml in a volumetric flask.

2.3.4 Saltzmann reagent

10 ml of sulfanilamide solution and 1 ml of NEDA solution were mixed. It must be protected from light and refrigerated.

2.3.5 Nitrite standard stock solution (1,000 mg/L)

0.150 g of sodium nitrite ($NaNO_2$) was dissolved in deionize water and adjusted to 100 ml in a volumetric flask.

2.4 Optimization method for nitrogen dioxide determination

In order to construct a NO_2 test kit, a standard NIOSH (sulfanilamide-NEDA method, 1998) was chosen for determination of NO_2 in ambient air. The objective of this part of the work is to investigate the effect of variables that impose such as reaction time of color development and extraction time of sample.

2.4.1 Reaction time of color development

Reaction time for color developing has been investigated. 1 ml of 0.2-1.0 mg/L NO_2^- standard was mixed with 2 ml of Saltzmann reagent. A mix solution was left in a range of 0-25 minutes to see the stability of color solution. The absorbance of this solution was measured at 540 nm by a spectrophotometer with a 1 cm optical path length cell. Before taking the measurement, the spectrophotometer was set to zero against the reagent blank (Saltzmann reagent) solution to avoid interferences from impurities in the reagent.

2.4.2 Extraction time

Extraction is an important step that should be optimized because it leads to under or over estimation of pollutants. Extraction time was tested by spiking 20 µl of 100 mg/L of NO_2^- standard solution onto the sorbent (Whatman GF/A) in 3 diffusion tubes. After that, 2 ml of deionized water was added. After extraction in a range of 5-25 minutes, color was developed as in procedure as described in the section 2.4.1. Percent recoveries were calculated in order to see the efficiency of this condition. The Extraction efficiency was calculated in term of percent recovery as shown in equation 2.1.

$$\% \text{ Recovery} = \left[\frac{Measured \quad concentration}{Initial \quad concentration}\right] * 100 \qquad (2.1)$$

2.5 Analytical characteristics

2.5.1 Linear dynamic range

The linear dynamic range was investigated by varying concentrations of nitrite (NO_2^-) in a range of 0.02 - 10 mg/L. It was determined by plotting absorbance versus concentrations of nitrite standard solution.

2.5.2 Calibration curve

A calibration curve was constructed by plotting concentrations of nitrite standard solution versus absorbance obtained from spectrophotometry. Nitrite solutions were prepared from 1,000 mg/L stock standard solution and diluted in a range of 0.02-1.0 mg/l. After that the mixtures of Saltzmann reagent and standard solutions were measured by spectrophotometry using reagent as blank.

2.5.3 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 for quantitative assays for low levels of compounds in the sample matrices and used particularly for determination products or low levels of active constituent in a product (Gibbons, 1996). The limits of detection and quantification were calculated as follows:

$$LOD = 3*SD \quad (2.2)$$

$$LOQ = 10*SD \quad (2.3)$$

Where: SD = mean of standard deviation from measurement of nitrite standard solution.

2.5.4 Repeatability and reproducibility

Repeatability was the results of standard deviation of measurements repeated by the same analyte on the same instrument within a short time period (IUPAC Compendium of Chemical Terminology, 1997). The repeatability in this work was checked by 10 times continuously measurement of 0.2 mg/l nitrite standard solution by spectrophotometer at 540 nm. Reproducibility (IUPAC Compendium of Chemical Terminology, 1997) was the closeness of agreement between independent results obtained with the same method on identical test material but under different conditions (different operators, different apparatus, different laboratories and after different intervals of time). Reproducibility of the system was pursued by preparing 10 solutions of 0.2 mg/l nitrite followed by analysis in the same manner. The results were estimated by standard deviation (SD) and the relative standard deviation (%RSD)

2.6 Passive sampler for NO_2 determination

A passive sampler used in this work consists of a diffusion tube containing filter paper impregnated with triethanolamine. The diffusion tube is fixed vertically, the opened end upright, in the protective shelter to protect effects of meteorology as shown in Figure 2.1

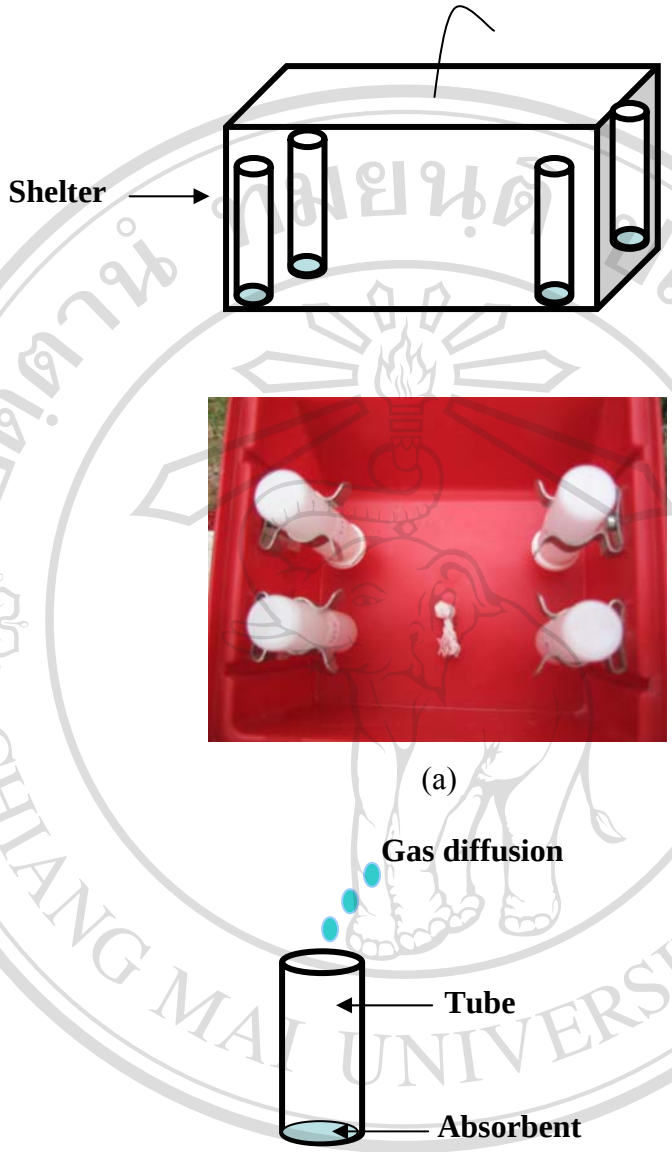

Figure 2.1 The configuration of passive samplers (a) and the gas diffusion pathway (b)

2.7 Methodology of ambient NO_2 determination

2.7.1 Preparation of passive samplers

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

2.7.2 Sampling procedure

The passive sampler consists of a diffusion tube containing a filter impregnated with absorbing chemicals. The sampling tube is fixed in the protective shelter to protect them from light and exposure above the ground at least 1.5 meter. Sampling is started by removal of a cap from the tube. The sampler is exposed at the sampling site allowing unrestricted movement of air around them. In each experiment, samplers are simultaneously exposed with unopened samplers being used as blanks. An exact exposure time is recorded for further calculation. In case that the analysis can not be processed immediately after the exposure, the samplers and blanks were stored in a refrigerator prior to analysis to minimize background contamination.

2.7.3 Transportation

When the sampling is complete, the exposed passive diffusion tubes are collected, immediately closed with caps and were kept in the airtight container along transportation.

2.7.4 Sample preparation

After extraction process, the solution was filtered through 0.45 µm cellulose acetate helping of glass syringe in order to get rid of contaminated particles, which could disturb absorbance measurement by spectrophotometry.

2.8 Sampling site

The sampling of NO_2 in ambient air has been carried out at the Pollution Control Department air quality station, Yupparaj Wittayalai School (18° 47' 28.43"N, 98° 59' 24.26"E) as shown in Figure 2.2.

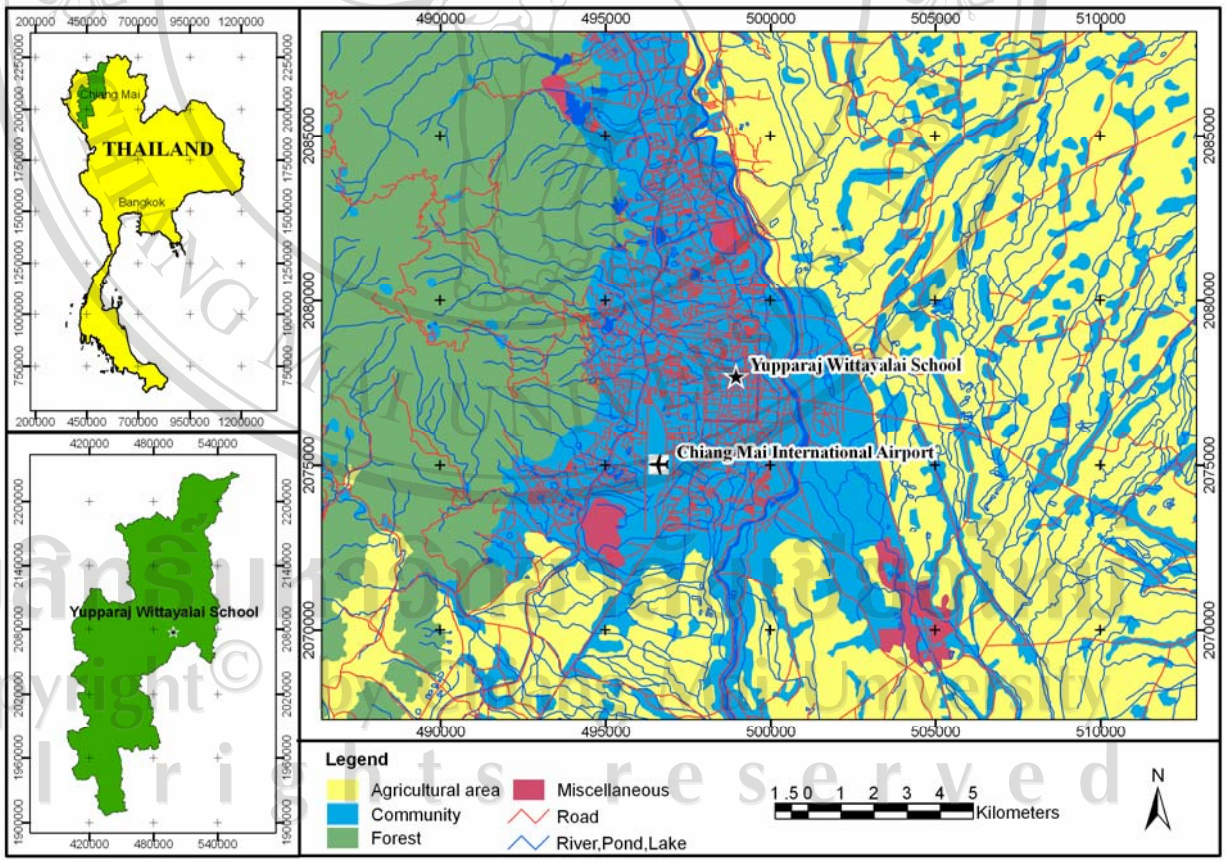

Figure 2.2 Sampling site

2.9 Development of passive samplers

2.9.1 Selection of sorbent support

Different of physical and chemical properties of filter papers represent efficiency for absorption of NO_2 in ambient air. This work aims to test capacity of each filter type on absorptivity of NO_2 in air. Three types of cellulose filter and a glass fiber filter (GF/A) were tested to be used as the sorbent support for NO_2 as detail provided in the Table 2.1.

Table 2.1 Sorbent for NO_2 in air

Sobent type	Pore size	Applications
1. Cellulose filter (Whatman No.1)	11 µm	Routine lab application student analysis
2. Cellulose filter (Whaman No.6)	2.5 µm	High retention, ideal for chemical analysis
3. Cellulose filter (Whatman No.40)	8 µm	Gravimetric analysis, collection of trace element
4. GF filter (Whatman GF/A)	1.6 µm	Gravimetric determination of airborne particulates

Source: Cole-Parmer international USA, 1998

Four types of adsorbent have been investigated to compare their absorptivities for nitrogen dioxide. Each filter type was cut into the circle with the same size of an internal diameter of the polypropylene diffusion tube (1.3 cm) and put into a diffusion tube. A 50 µl of 20 % triethanolamine (TEA) (Kirby *et al,* 2000) was added to the filter using micropipette. A set of sampling consisted of 5 sampling tubes and 3 blank tubes (un-opened tube). Then all of diffusion tubes were attached in the protective

shelter by fixing the opened end upright and exposed for 24 hours outdoor. After sampling, nitrogen dioxide concentrations obtained from each sorbent type were determined by spectrophotometry and compared.

2.9.2 Cleaning of the sorbent filter

Method of filter cleaning was investigated to seek for the lowest contaminant in the absorbent filter.

A) A filter paper was soaked in deionized water about 30 minutes and dried in an oven at 60 ^{0}C for 24 hours and left in a desiccator.

B) A filter paper was soaked in deionized water about 30 minutes and rinsed twice, following with 15 minutes sonication in deionized water and twice rinsing. After that it was dried in the oven at 60 ^{0}C for 24 hours and left in a desiccator prior use.

Cleaned filter papers were put in a diffusion tubes. After that 50 µl of 20 % TEA was added. The diffusion tubes were exposed for 24 hours outdoor.

2.9.3 Comparison of diffusion tubes

Types of diffusion tubes (Figure 2.3) with different lengths and internal diameter (i.d.) were test. Two types of diffusion tube including polyethylene (PE; 5.4 cm long and 1.4 cm i.d.), polypropylene (PP) applied from 5 ml (5.3 cm long and 1.3 i.d.) and 10 ml (7.7 cm long and 1.6 cm i.d) syringe tubes were used as diffusion tubes for setting up of passive samplers. The glass fiber filter (GF/A) was used as the sorbent, which was allowed to reach bottom of the tube and impregnated with 50 µl of

20% TEA. Their efficiencies for NO_2 absorbtion have been compared by 24 hour exposure outdoor of 5 replicates and 3 blanks.

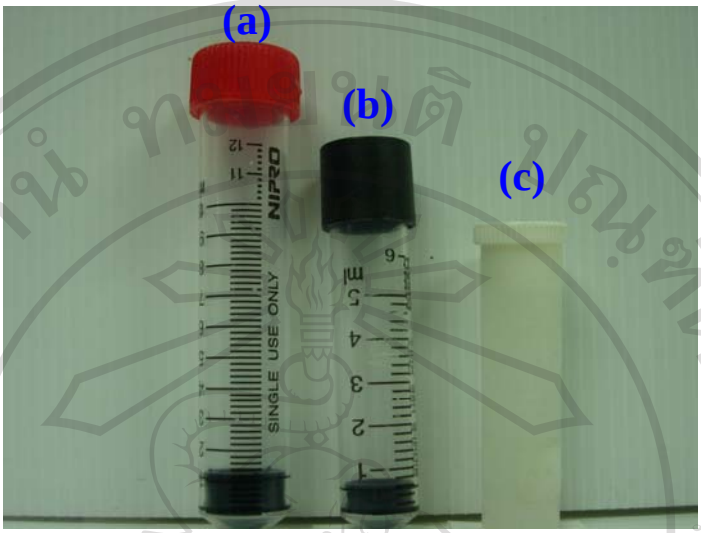

Figure 2.3 Diffusion tubes (a) PP; 7.7 cm long and 1.6 cm i.d (b) PP; 5.3 cm long and 1.3 cm i.d. (c) PE; 5.4 cm long and 1.4 cm i.d.

2.9.4 Comparison of diffusion tubes with membrane and without membrane

A Teflon membrane is placed at the open end of the diffusion tube in order to obtain a sampler free wind speed effect (Gerboles, 2005). It was expected that membrane would limit the losses of water of the absorbing solution. In this study two set of samplers including use of the protective shelter and use of the membrane together with protective shelter were tested and compared.

The PP diffusion tubes (5.3 cm long and 1.3 cm i.d), containing impregnated filter (GF/A) was used as a sampler in this test. One set of sampler was prepared ordinary, while another set was extra-prepared by using Teflon membrane (Polytetrafluoroethylene: PTFE) closed at the open end of the tube. They were exposed at the sampling site for 24 hours. After that NO_2 concentrations were determined and compared.

2.9.5 Sampling period

After appropriate condition in topics 2.9.1 – 2.9.4 were obtained, sampling duration was test using 4 sets (5 sampling tubes and 3 blanks) of samplers at the reference site. A set of the samplers was collected after 24 hours, 3, 5 and 7 days exposure. Nitrogen dioxide concentrations obtained from each sampling was compared with values obtained from chemiluminescence measurement of the PCD air quality monitor station at the same sampling duration.

2.10 Validation of passive sampler

An accuracy of the results obtained from the passive sampler and spectrometric measurement was evaluated by comparing with chemiluminescence technique obtained from standard active monitoring at the sampling site.

Precision is expressed as the standard deviation (SD) and percentage relative standard deviation (% RSD), which is calculated by the equation 2.4 and 2.5 (L.Bernstein, 1993).

$$SD = \left[\frac{(Xi - \overline{X})^2}{(n-1)} \right]^{1/2} \qquad (2.4)$$

$$\%RSD = \left[\frac{SD}{\overline{X}} \right] * 100 \qquad (2.5)$$

Where

Xi = individual value in dada

$\overline{X}$ = mean of data

n = number of measurements

Precision of the passive sampling technique was calculated based on number of the replication of diffusion tubes.

2.11 Nitrogen dioxide test kit

Test kits are self- contained analytical kits that use a chemical reaction that produces color to identify contaminants, both qualitatively and quantitatively (Borisuttichun, 2008). There are numerous advantages to use test kits in the environmental field, including simple equipment, ease of use, quickly to analysis and low cost per sample. 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 standard of know concentrations. The level of certainty will vary depending on whether the intensity of the color is compared visually with the standard color chart (http://www.clu-in.org/char/technologies/color.cfm).

2.11.1 Construction of NO_2 test kit and user instruction

Nitrite standard solutions were prepared in 0.03, 0.06, 0.10, 0.40, 0.80, 0.15 mg/l by serial dilution of the 1,000 mg/l stock solution. Then Saltzmann reagent was used for color development and NO_2 determination. The mixtures were applied for construction of NO_2 standard color chart. Moreover, NO_2 concentrations (mg/l) were also calculated into ambient concentration (ppbv). After that color chart related to ranges of NO_2 concentration was produced.

The NO_2 test kit composes of a polypropylene diffusion tube, a plastic shelter, chemical reagents, a standard color chart, a plastic dropper and a disposable syringe. A test procedure is as follow.

1) Remove the cap of the diffusion tube out and put GF/A filter paper in a diffusion tube after that add 2 drop of 20% triethanolamine (absorbing solution).

2) Fix the sampling tube in a protective shelter and hang at the sampling site above ground at least 1.5 meter. A recommended sampling duration is 1 day. However, an accurate exposure time has to be recorded.

3) At the end of sampling time, extract by adding 2 ml of deionized water using a syringe, shake and hold for at least 10 minutes.

4) Transfer 1 ml of solution in to small glass tube. Add 2 ml Saltzmann reagent close the cap and mix well. Wait 10 minutes until color development was completed. After the specified time, color of the sampler solution was compared to the standard color of nitrogen dioxide chart to find out its concentration. When the developed color lies between 2 standard colors, read out value between the 2 standard values.

2.11.2 Stability of test kit reagent

A 0.2 mg/l nitrite solution was prepared from the stock standard solution. After that add Saltzmann reagent for color developed and the solutions were measured by spectrophotometry. Saltzmann reagent was kept in an amber bottle at room temperature in the dark condition. An aliquot of solution was assayed every week. Starting from week 1 (day 0) until absorbtion decreased.

For testing stability of absorbing solution (20% TEA), the sampler were exposed in the field and NO_2 concentrations were determined by spectrophotometry.

2.11.3 Comparison of NO_2 test kit with spectrophotometry and chemiluminescence techniques

After NO_2 test kit based on passive sampling technique was constructed, NO_2 concentration obtained from the color chart were compared with those from spectrophotometry and chemiluminescence 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.

2.11.4 Questionnaires

As a mechanism for obtaining information and opinion, questionnaires have a number of advantages and disadvantages when compared with other evaluation tools. 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 (Burgess, T.F., 2001).

The random sampling used probability the principle at 95% confidence level. In survey questionnaires, 100 students in Chemistry Department, Faculty of Science, Chiang Mai University as a representative of the population (Total = 3,000 persons) were randomly chosen. The test form for survey research is show in Table 2.2. Nitrite standard solution was prepared in different concentrations including 0.03, 0.04, 0.06,

0.10, 0.20, 0.40, 0.60, 0.80, 1.00 and 1.50 mg/l in the glass tubes by serial dilution of 1,000 mg/l stock solution.

The selected population compared colors of the solution in the glass tubes with the standard color chart and ticked the mark (✓) of the matching color in the box indicates the measured value of the sample. In case that the color lies between 2 standard colors, values in both boxes related with colors must be ticked. Complete the test (10 tubes) is needed to be done completely using the same procedure.

Table 2.2 Test form for survey research

Tube number	**Concentration of nitrite (mg/l)**						
	0.03	**0.06**	**0.10**	**0.40**	**0.80**	**1.50**	**comment**
1							
2							
3							
4							
5							
6							
7							
8							
9							
10							