CHAPTER 2

EXPERIMENTAL

2.1 Chemicals

- 2.1.1 Formaldehyde, 35% purity, AR grade, (Aldrich, USA)
- 2.1.2 4, 5-dihydroxy-2, 7-naphthalenedisulfonic acid disodium salt

dihydrate (Fluka, Sigma-Aldrich, USA)

- 2.1.3 Sulfuric acid reagent grade (Merck, Germany)
- 2.1.4. Deionized Water
- 2.1.5 Sodium bisulfite granular, AR grade, (Mallinckrodit, USA)
- 2.2 Instruments and apparatus
- 2.2.1 Passive sampler
 - 1. Formaldehyde passive samplers (SKC inc., USA)
 - 2. GF and cellulose filter paper (Whatman, USA)
 - 3. Syringe tube 5 ml (Nipro, Thailand)
 - 4. PTFE Thread seal tape (id 15 mm) (Daiichi, STV Association Co., Ltd.,

Japan)

5. Parafin oil film (Para film, USA)

2.2.2 Exposure chamber

1. Anemometer (testo, Japan) ang Mai University

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- 2. Hygrometer (Digital Hygro-thermometer, Japan)
- 3. Soap bubble Flow meter
- 4. Air pump (GAST, USA)
- 5. Hot plate
- 2.2.3 UV-VIS spectrophotometer (Lambda 25 Perkin Elmer, Germany)

2.3 Preparation of Sampling Tubes

2.3.1 Adsorbent conditioning

Celluloses and glass fiber filters were used as adsorbent as detail provided in

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Table 2.1 Detail of adsorbent

Yable 2.1. Table 2.1 Detail of adsorbent			
	Pore size	Applications	
Cellulose filter Whatman No.1	11 µm	Routine lab application student analysis	
(Qualitative filter types)		2121	
Cellulose filter Whaman No.6	2.5 µm	High retention, ideal for chemical	
(Qualitative filter types)		analysis	
Cellulose filter Whatman No.40	8 µm	Gravimetric analysis, collection of trace	
(Quantitative filter types)	IT A	element	
GF filters Whatman GF	1.5 μm	Use for water and air pollution official	
12	66630	test methods	
GF filters Whatman GF/A	1.6 µm	Gravimetric determination of airborne	
	UNI	particulates	
GF filters Whatman GF/C	1.2 µm	Standard in filtering waste water or for	
<u>สิทธิมหาวิ</u>	ทยา	cell harvesting	

Cole-Parmer international, USA

Chiang Mai Univers Before impregnation, the filter specified is pre-washed in de-ionized water for approximately 12 hours and dried at the 60 °C in oven. The filter paper was cut into a circle with a same size of internal diameter (13 mm) of diffusion tube.

2.3.2 Sorbent of formaldehyde

Determination of formaldehyde in ambient air by using PP-tube (13 x 50 mm) containing a cellulose filter treated with 1% NaHSO₃ solution. The formaldehyde vapor passes through a diffusion barrier and is adsorbed on bisulfite-impregnated paper contained within the monitor. The formaldehyde collected is analyzed by desorbing the formaldehyde-bisulfite adducts from the exposed filter in the sampler using deionized water (NIOSH, 1994). The reaction is performed as shown in figure.2.1.Then, analyzed by the chromotropic acid (CTA) method (Balmat and Meadows, 1985)



Figure 2.1 Reaction of bisulfite adduct

Formaldehyde vapor present in react with bisulfite then form stable complex, formaldehyde bisulfite adduct which desorbed with deionized water and reacted with chromotropic acid in the presence of sulfuric acid to form a purple mono-cationic chromogen. The absorbance of this colored solution is read in a spectrophotometer at 580 nm. Although the chemistry of the color formation is not well-established, the following reaction mechanism is proposed in acidic solution (Figure 2.2).



Figure 2.2 Purple mono-cationic chromogen (Fritz, 1966)

2.3.3 Passive Sampling Tube

Diffusion polypropylene (PP) tube was adapted from 5 ml PP syringe tube. The passive sampler (Figure 2.3) consists of a polypropylene tube (50 mm long and 15 mm i.d.), the impregnated filter (Whatman 6) and Teflon membranes (PTFE thread seal tape) (Figure 2.4). Before impregnation, the filter is prewashed in de-ionized water for about 2 hours and dried at 60°C in the oven for 12 hours. The impregnation step is done by put the filter paper in the diffusion tube, add 100 μ l of 1 % bisulfife solution to the filter and close the open end with a Teflon membrane to reduce meteorological effect. Storage is needed before and after exposure by keeping at 4 °C in the refrigerator. The filter is extracted by DI water following with (CTA) method and then measured by spectrophotometer at 580 nm.



Figure 2.3 (a) dimension of passive sampler (b) Self-constructed passive sampler



2.4Analytical methodology

2.4.1 Preparation of calibration curve of formaldehyde

To a series of test tubes or vials 1.0, 5.0, 10.0, 15.0, 20.0, 25.0 and 30.0 μ l of standard formaldehyde solution were added. The volume was adjusted to 2 ml with 1% sodium bisulfite solution. Color was developed and its absorbance was measured as described in topic 2.3.2. A reagent blank was used to subtract its absorbance from all the standards as well as the samples. A calibration curve was prepared by plotting the corrected absorbance (y axis) vs. micrograms of formaldehyde (x axis). The slope and intercept were determined form the calibration curve.

2.4.2 Desorption (extraction) of filter paper

After exposure of passive samplers, formaldea bithyde vapor were trapped as sulfite adduct on the filter. After that it was extracted by 3 ml of deionized water in diffusion tube of each diffusion tube. The diffusion was immediately stopped and allowed eluting for 30 minutes. After that 3 ml aliquot of the eluate was transferred to a test tube for color development. The aliquot was added with 2 ml 1% sodium bisulfite solution.

2.4.3 Color Development

1.0 ml of chromotropic acid solution was added to each sample and mixed well 5 ml of concentrated sulfuric acid and well mixed. Then, slowly and carefully added. The samples were allowed to cool to room temperature and measured the absorbance at 580 nm by 1 cm burettes. Distilled water was used in the reference cell. A reagent blank was carried through all the steps of the sample analysis. Subtract was blank. The absorbance of the reagent from that of the standards and samples were compared to the calibration curve to obtain the amount of formaldehyde present.

2.4.4 Linear dynamic range (LDR)

Formaldehyde was prepared in a concentration range of $0.5 - 14 \mu g/ml$ in test tube. After that the CTA method was applied and the solutions were measured by spectrophotometry. Linear dynamic range was determined by plotting absorbent value *versus* concentration. The linearity of the response was determined by considering the correlation efficiency.

2.4.5 Detection limit

In analytical chemistry, the detection limit, or LOD (limit of detection), is the lowest quantity of a substance that can be distinguished from the absence of that substance (a *blank value*) within a stated confidence limit (generally 1%). There are a number of different "detection limits" that are commonly used. These include the instrument detection limit (IDL), the lower level of detection (LLD), the method detection limit (MDL) and the level of quantitation (LOQ). The IDL is often used to estimate the rest of the detection limits and a relationship can be established between these detection limits as IDL: LLD: MDL: LOQ = 1:2:4:10 (EPA 1996).

a) Method Detection Limit (MDL)

Many times there is more to the analytical method than just doing a reaction or submitting it to direct analysis. For example it might be necessary to heat a sample that is to be analyzed for a particular metal with the addition of acid first (digestion). Additional steps in an analysis add additional opportunities for error. Since detection limits are defined in terms of error, this will naturally increase the measured detection limit. This detection limit (with all steps of the analysis included) is called the MDL. The practical method for determining the MDL is to analyze 7 samples through the entire analytical process. The standard deviation is then determined. The one-sided t distribution is determined and multiplied versus the determined standard deviation. For seven samples (with six degrees of freedom) the t value is 3.14. In this study MDL was used in Limit of Detection (LOD) (EPA Gibbons, 1996).

The steps taken by EPA to arrive at the MDL are outlined as follows:

(1) Measurements are taken on at least seven samples of the prepared solution.

Results are tabulated and the standard deviation of the data set is taken:

(2) Using the degrees of freedom from the data set and the appropriate confidence level (usually 1%), the critical t-value is looked up using reference tables:

 $s^{2} = \frac{1}{n-1} \left[\sum_{i=1}^{n} (x_{i} - \overline{x})^{2} \right]$

 $t_{\nu,\alpha} = (look - up value)$

2.16

2.15

(3) The MDL is computed as the product of the standard deviation and the critical t-value:

$MDL = t_{\nu,\alpha} \times s$ 2.17 Constant of Quantitation (LOQ) $MDL = t_{\nu,\alpha} \times s$ 2.17 Chiang Mai University

The limit of quantitation is the lowest amount of the analyte in the sample that can be quantitatively determined with defined precision under the stated experimental conditions. The limit of quantitation is a parameter of quantitative assays for low levels of compounds in sample matrices and is used particularly for the determination of impurities and/or degradation products or low levels of active constituent in a product.

The LOQ may be determined by preparing standard solutions at estimated LOQ concentration (based on preliminary studies). The solution should be injected and analysed n times (normally 6-10). The average response and the standard deviation (SD) of the n results should be calculated and the SD should be less than 20%. If the SD exceeds 20%, a new standard solution of higher concentration should be prepared and the above procedure repeated. The LOQ is X + (10 x SD) <u>http://www.apvma.gov.au</u>.

The MDL and LOQ were done in this study by using ten of reagents blank and analyzed by CTA method following with measurement by spectrophotometry at 580 nm. The data of concentration value were evaluated to standard deviation value with the equation 2.15. After that the MDL was calculated with the equation 2.17.

2.5 Formaldehyde Exposure Chamber System

The system used for the laboratory experiments is a dynamic chamber in which the variables: concentration of contaminant, temperature, humidity and wind velocity can be controlled (see Figures 2.5 and 2.6). The formaldehyde exposure chamber was constructed for testing an efficiency of developed passive samplers. The passive samplers were placed inside the chamber in order to test their efficiency of formaldehyde absorbing by determination of % recovery.

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Figure 2.6 Formaldehyde Exposure Chamber System

Air Pump 2) valve 3) AC column 4) Silica gel column 5) Humidifier unit 6) Cooling box 7) HCHO generator unit 8) Mixing chamber 9) Exposure chamber 10) Sampling holder 11) Anemometer 12) Hygrometer 13) Flow meter 14) Exhaust pump 15) Gas collector

The Formaldehyde Exposure Chamber System has been constructed to test the developed passive samplers under controlled conditions of formaldehyde concentration, temperature, humidity, and wind velocity. The system consists of five distinct units: 1) humidifier unit 2) formaldehyde vapor unit 3) mixing chamber unit 4) exposure chamber 5) gas collector.

2.5.1 Humidifier unit

The humidifier unit consists of water vapor generator and cooling box (Figure 2.7). In order to achieve different levels of humidity inside the exposure chamber, the air was purified by directly flowed to the activated charcoal and silica gel column and then to the glass vial contained water that was heated by the water bath at the control temperature. Levels of relative humidity were measured by a hygrometer coupled to an exposure chamber. Required humidity level can be set by mixing of dried air flow (through drying columns and humidifier unit) with humid air (water steam), then the water vapor flows to a cooling box (contained cooling water) to adjust the humidity inside the chamber.



Figure 2.7 Humidifier units

2.5.2 Formaldehyde generator unit

The formaldehyde generator unit is shown in Figure 2.8. It consists of two glass vials containing formaldehyde solution. They were put in the box for temperature controlling. As the air flow through formaldehyde generator unit then formaldehyde solution was conversed to vapor phase above the solution and purged to the mixing chamber. The mass of vapor delivery and flow were controlled by valve controller. The mass of formaldehyde vapor delivery can be monitored by collecting under the water and determined with Chromotropic acid (CTA) method. The flow rate was measured by the soap bubble flow meter.



Figure 2.8 Formaldehyde vapor delivery unit

2.5.3 Mixing chamber

The mixing chamber is where the water and formaldehyde vapor were mixed and diluted by air before going to the exposure chamber, so that thoroughly mixed air stream reaches the exposure chamber. The mixing chamber made of glass vial and coated with Teflon spray in order to make its surface hydrophobic.

2.5.4 Exposure chamber

The mixed dilution air system directly flowed to the exposure chamber where the passive samplers were placed. A diagram of the exposure chamber is presented in

Figure 2.9. The chamber consists of a glass box with a dimension of $0.3 \times 0.5 \times 0.375$ m which contains approximately 5.6 L. It was coated with the Teflon spray for hydrophobic surface. Anemometer for wind speed measurement and a hygrometer for humidity and temperature measurement were set on the top of the chamber. A fan inside the chamber was also set for control the wind speed. Ten passive samplers were positioned at the sampling holder at the top of the chamber during the exposure. The exposure chamber unit was insulated by the foam.



2.5.5 Gas collector unit

The gas collector unit was used for monitoring amount of formaldehyde inside the exposure chamber. It consists of 4 glass vials contained distilled water. They were connected in line as shown in Figure 2.10. The inlet air was trapped under the water. After that, a 2 ml aliquot of the solution in each vial was transferred to a test tube for color development and measured with spectrophotometry.



The passive samplers were placed inside the chamber in order to test their efficiency of formaldehyde absorbing by determination % recovery of each parameter. The formaldehyde concentration inside the chamber can be determined by calculation of mass of gas delivery rate (μ g/min) (using gas collector) per flow rate of formaldehyde gas (L/min).

2.7 Optimization of sampler

2.7.1 Detection limit of sampler

The limit of detection (LOD) is the lowest concentration of the analyte that can be detected with a given degree of confidence. The limit of quantification (LOQ) is the smallest concentration that can be confidently quantified. The limits of detection and quantification were calculated as follows:

 $LOD = 3 \times SD$

2.2

Each of 10 unexposed field blank tubes was kept at 4 °C for 8 and 24 hours and analyzed by the CTA method. Detection limit of formaldehyde was calculated from 3 times of standard deviation of those 10 concentration values.

2.7.2 Determination of total air resistance

During sampling the flux of gas through all sections of the passive gas sampler is equal, i.e., gas is not adsorbed on the walls. The total air resistance can be calculated by summation of all resistances that influence diffusive transport of the gas to the sampling filter. The Teflon filter over the inlet prevents turbulent diffusion inside the sampler. Equation 2.3 is used to calculate total air resistance calculates total air resistance (Gillettr, *et al.* 2000).

Total air resistance (m⁻¹) =
$$\frac{LR}{AR} + \frac{LF}{AF} + \frac{LN}{AN} + \frac{LBL}{AR}$$
 2.3

AR area of ring $1.32 \times 10^{-4} (m^2)$

AF total pore area of Teflon filter

AN Total pore area of steel mesh

LF thickness of Teflon filter

LR length of ring 5×10^{-2} (m)

LN thickness of steel mesh) (m)

LBL laminar boundary layer depth 5×10^{-3} (m)

A is the total area of all pores in the filter. If only membrane is used the member L_N/A_N from esq. (1.9) must be dropped out. For the boundary layer theory is that the boundary layer thickness is 5 x 10⁻³ m indoors (Ayer *et al* 2000). However, LF/AF value can not be calculated through the dimension of filter membrane. Then, comparison between enclosed membrane and without membrane exposed can be determining LF/AF value from the experiment. The membrane constant values was determined using two sets of 5 samplers enclosed membrane and without membrane and without membrane exposed and calculate for K_F constant. Five of passive samplers (without membrane) and another 5 of (with membrane) were exposed simultaneously in the chamber for 4 hours and determined by CTA method.

2.7.3 Optimization types of impregnated paper

Types of impregnated paper were tested to find out the suitability of adsorbent with polypropylene (PP) tube. Six different types of filter paper including Whatman No.1, No.6 and No.40, Glass Fiber, Glass Fiber/A and Glass Fiber /C were tested.

Ten passive samplers were prepared for each paper type and exposed for 4 hours in the exposure chamber which contained 14.5 mg/m^3 formaldehyde at 60 % relative humidity, 30 °C and zero wind speed.

2.7.4 Optimization types of diffusion tube

Types of diffusion tube were tested to find out the suitability of diffusion length with Whatman No.6 adsorbent. Three types of tube including polypropylene, poly- ethylene (PE) and polystyrene (PS) were selected. Ten samplers of each tube type were exposed for 4 hours in the exposure chamber which contained 6.4 mg/m³ formaldehyde at 50 % relative humidity, 31 °C and zero wind speed.

2.7.5 Desorption efficiency and number of filter layers

Number of filter layers was considered to affect on absorption capacity. Therefore the selected filter paper (Whatman No.6) was cut into a circle with a same size of internal diameter of the diffusion tube. Numbers of filter paper layer ranked from 1 to 5 were put in the tubes with 6 replications of each. $20 \ \mu g \ (20 \ \mu l \ of 1 \ \mu g/\mu l)$ of formaldehyde solution were spiked onto each filter set and then desorbed by 3 ml DI water for 60 minutes prior to analysis with spectrophotometry.

2.7.6 Stability of sample

Thirty five samplers were exposed to 28.7 mg/m³ formaldehyde at 80 % relative humidity 30 °C for 4 hours in the exposure chamber. After that, five samplers of those were analyzed by spectrophotomerty within a day. Half of the remain samplers (15 tubes) was stored at 4 °C in a refrigerator and another half was kept at room temperature (approximately 28±2°C). Each set (5 samplers) of those was analyzed every 7 day under the same condition as previous until the samplers were finished.

2.8 Validation of sampler

An accuracy of the results obtained from the passive sampler was evaluated by calculation with concentration level in the exposure chamber. 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 (Carl Peter and Jo, 1993).



Precision of the passive sampling technique was checked by determining the replication of diffusion tube of samplers.

2.8.1 Effect of wind velocity

Ambient air face velocity can affect the performance of diffusive samplers because it may influence the effective diffusion path length. Under conditions of low external wind speeds, the effective diffusion path length may increase. Under conditions of high external wind speeds, the effective diffusion path length may decrease. This is because a 'boundary layer' exists between the stagnant air within the sampler and the turbulent air outside and contributes to the effective diffusion path length.

The effect of face velocity on sampler performance, a series of experiments was performed, where sets of five samplers were exposed under conditions corresponding to wind velocities of 0.0, 1.0 and 1.5 m/s. Wind velocity varies according to the voltage applied to fans and measured with an anemometer placed inside the exposure chamber. These experiments were carried out for 4 hours at a relative humidity of $80\pm3\%$, a temperature of 30 ± 2 °C and a formaldehyde concentration about 3-18 mg/m³, which corresponds to a condition of pollution.

2.8.2 Effect of relative humidity

High humidity levels may affect the sorption capacity of adsorbing material and boundary layer on the barrier membrane. In this test, different levels of relative humidity were simulated with a hygrometer coupled to an exposure chamber by varying proportions of dried air flow (through drying Columns and humidifier unit) mixed with humid air (water steam), to vary required humidity level: 30% (low relative humidity), 60% (medium), and 80% (high). Sets of five passive samplers were simultaneously exposed to the chamber by containing 3 -16 mg/m³ formaldehyde, at facial velocity of 0.0 m/s, for 4 hours. Average formaldehyde concentration in atmosphere inside exposure chamber measured with both passive samplers and the gas collector.

2.8.3 Effect of temperature

Since the diffusion coefficient is a function of temperature, the effective sampling rate will be affected by the fluctuation of temperature. Based upon theoretical predictions, the diffusion coefficient is proportional to the square root of the absolute temperature (Fuller *et al.*, 1966). Three sets of 10 passive samplers were simultaneously exposed for 4 hours in the chamber containing 2.5 -15 mg/m³ formaldehyde, at 15, 30, 40 °C and facial velocity of 0.0 m/s. Average formaldehyde concentrations in the atmosphere inside the exposure chamber were measured with both passive samplers and the gas collector.

2.8.4 Effect of sampler orientation

The ambient air face velocity and orientation of the sampler can affect the performance and reliability of diffusive samplers because they may influence the effective diffusion path length (Gair and Penkett, 1995). The diffusion path length is a

function of the length and cross-sectional area of the diffusion space within the sampler. Both low and high wind speeds may be sources of error. If wind speed falls, the effective path length may increase, causing a decrease in uptake rates. Conversely, high wind speeds may reduce the effective path length and increase the uptake rate (Gair and Penkett, 1995).

In the orientation experiment 15 samplers were placed at the holders (5 vertical (upright) and 5 vertical (upside down) and 5 horizontal directions) and exposed at the workplace of Language Institute, Chiang Mai University for 24 hours. After that, they were collected in polyethylene bag and brought back for determination of formaldehyde concentration. Significant difference was determined using Randomized Complete Block design (RCB) test with a 95% confidence level.

2.8.5 Effect of reverse diffusion

Reverse diffusion can occur if the bonding of the target compounds to the collection medium is not sufficiently strong. Therefore, tests were performed to ascertain whether there were any losses of collected, but incompletely retained, analyte during sampling. In diffusive sampling the vapor concentration of the target compound at the surface of the adsorbing medium is expected to be very low in comparison to the concentration in the surrounding atmosphere. If the concentration differential becomes negative, loss of the analytes to the air could occur, which could be misinterpreted as being due to sampler saturation, since the effect would be the same.

The aim of this test was to check if back diffusion takes place in the samplers. The possibility of reverse diffusion occurring was investigated by exposing 10 diffusive samplers loaded at known formaldehyde concentration (26 mg/m^3) 50 % relative humidity and 26° C in the exposure chamber. After 4 hours, 5 diffusive

samplers were removed from chamber and the other 5 samplers were continuously exposed for another 4 hours of clean air. Concentrations of formaldehyde collected were determined. Significant difference determined using the RCB test with a 95% confidence level.

2.8.6 Sampling time

A set of 5 samplers and 3 field blank tubes was exposed at the workplace site for referent period of times including 8 and 24 hours, 2, 3, 4, 5, 6 and 7 days. After the sampling was completed, the samplers as well as field bank were collected and determined for formaldehyde concentrations.

2.8.7 Comparison of self constructed passive sampler and commercial passive sampler

A set of 3 field blank and 5 self constructed samplers and 1 of commercial passive sampler (SKC inc.) (Figure. 2.11) were exposed at the workplace site for different periods of time including 8, 24 hours and 7 days. Significant difference was determined using RCB test with a 95% confidence level.

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2.2 cm Figure 2.11 Formaldehyde Passive Sampler (SKC inc.)

The speciation of passive sampler of SKC inc. and Self constructed passive sampler (SCS) is shown in Table 2.2.



22 mm	13 mm
97 mm	50 mm
Glass tube	PP tube
Glass fiber filter	Cellulose
1% bisulfite	1% bisulfite
0.03 ppm	0.05 ppm
2500 baht	~20 baht
CTA method	CTA method
E Contraction	SIT
	22 mm 97 mm Glass tube Glass fiber filter 1% bisulfite 0.03 ppm 2500 baht CTA method

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