

## LABORATORY VALIDATION AND FIELD VERIFICATION OF SILICA GEL SAMPLING TUBE FOR MEASURING ETHYLENE OXIDE IN AIR

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**ABSTRACT** A sampling and analytical method for the measurement of ethylene oxide (EtO) level in air was developed. The procedure was based on the entrapment of ethylene oxide in an activated silica gel adsorbent inside a sampling tube. The ethylene oxide was desorbed by distilled water and the eluent was analyzed by gas chromatography with flame ionization detector. Parameters such as breakthrough volume, method detection limit, desorption efficiency and storage tests for 14 days in ambient temperature and refrigerator were determined. Field samplings were performed at two factories in Kulim and Pulau Pinang respectively, which use ethylene oxide gas as sterilization substance. The desorption efficiency of ethylene oxide from silica gel tube was determined to be 87.8% over concentration range of 0.2 – 4.0 ppm in 3 liters of air sample. The method detection limit was determined to be 1.0 ppm whereas the precision of procedure was 10.7%. All the parameters were shown to be meeting OSHA requirements.

**ABSTRAK** Satu kaedah persampelan dan analisis untuk mengukur tahap etilena oksida dalam udara telah dibangunkan. Prosedur ini berasaskan pemerangkapan etilena oksida dalam penjerap gel silika yang telah diaktifkan dalam suatu tiub persampelan. Etilena oksida telah didejerap dengan menggunakan air suling dan eluen tersebut dianalisis dengan gas kromatografi yang dilengkapi dengan pengesan pengionan nyala. Parameter seperti keberkesanan pendejerapan etilena oksida daripada tiub silika, isipadu penembusan, had pengesanan kaedah, keberkesanan pendejerapan dan ujian penyimpanan selama 14 hari pada suhu ambien (27°C) dan suhu peti sejuk (4°C) telah ditentukan. Persampelan lapangan telah dilakukan di dua buah kilang masing-masing di kulim dan Penang yang menggunakan etilena oksida sebagai bahan pensterilan. Keberkesanan pendejerapan etilena dioksida daripada tiub gel silika telah ditentukan sebagai 87.8 % pada julat kepekatan 0.2-4.0 ppm untuk tiga liter sampel udara. Had pengesanan kaedah telah ditentukan sebagai 1.0 ppm manakala kejituan prosedur adalah 10.7 %. Semua parameter yang dikaji menunjukkan memenuhinya syarat OSHA.

**(Keywords:** Indoor air pollution monitoring, ethylene oxide (EtO), desorption efficiency, breakthrough volume)

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### INTRODUCTION

Ethylene oxide is widely used as a sterilant in the medicinal field [1] especially for heat-sensitive surgical tools and equipment [2]. It is also used as a chemical intermediate in the production of mono-, di-, and triethylene glycols; mono-, di-, and triethylene glycol ethers, ethanolamines; surface active agents and other chemicals [3]. In 1979, preliminary results of a long term carcinogenicity assay in mice were reported. In addition, an investigation of leukemia among workers potentially exposed to ethylene oxide in the Swedish factory was also reported [4]. Ethylene oxide was found to cause an increase in

incidences of mononuclear cell leukemia and peritoneal mesothelioma in the rats [5]. Ethylene oxide can chemically bond with deoxyribonucleic acid(DNA) [4] to form N-7-(2-hydroxiethyl) guanine [6], which is unstable and subsequently induces depurination in the nucleotic series [7]. Ethylene oxide exposure causes mutations, increases the rate of chromosomal aberration, sister chromatid exchange and other undesirable changes in the DNA of the mammalian cells [8]. The OSHA recommended exposure limit (REL) for ethylene oxide is 0.1 ppm as an 8-hour time-weighted average (TWA) with 10-minute ceiling limit of 5 ppm whereas the OSHA permissible exposure

limit (PEL) is 1 ppm as an 8-hour TWA with a 15-minute excursion limit of 5 ppm [9]. Many methods have been developed to monitor ethylene oxide in air such as charcoal tube [10], HBr coated charcoal tube [11], Tedlar gas bag [12], impinger [3], badge [13], 3M charcoal disc [14] passive dosimeter [15] and direct reading with IR, photoionization detector and chromatographic gas [3], gas sensor [16] and also bio-monitoring [6,17].

However, all these methods have their advantages and disadvantages such as sampling and analysis costs, sampling duration and interference. In this study, recoveries of ethylene oxide from silica gel and charcoal tubes were determined and type of adsorbent which gives higher percentage of recovery was selected to continue the validation tests. Objectives of this study were to develop a new method which is cheaper, shorter in analysis and sampling time as well as able to fulfill all the OSHA accuracy and precision requirements.

## EXPERIMENTAL

### Materials and Reagents

Silica gel powder (60 – 120 mesh) and coconut shell charcoal (35 – 50 mesh) were purchased from BHD Chemicals Ltd. (England) and E. Merck (Germany) respectively. Both adsorbents were activated at 110 °C for 24 hr before used. Ethylene oxide (EtO) with a purity of more than 99.8% was purchased from Fluka Chemika-Biochemika Company (Switzerland). The sampling tube was prepared by adding  $1.000 \pm 0.005$  g of activated adsorbent into 120 x 6 mm i.d. glass tube. Glass wool was plugged at both open ends of the tube to hold the adsorbent in place. Rubber septa were used to cap both open ends of the tube to prevent deactivation of the adsorbent due to the atmospheric humidity. All prepared sampling tubes were kept in a desiccator before used. Ethylene oxide stock standard solution was prepared by bubbling ethylene oxide gas into approximately 50 mL of water in a 100 mL volumetric flask. The lower concentration of ethylene oxide standard solution was prepared by diluting from the stock standard solution to the desired concentration with distilled water.

### Apparatus and Conditions

Air sample was collected by connecting the sampling tube to a BUCK S.S personal air

sampling pump (A.P. Buck Inc) [18]. The flow rate of the pump was calibrated daily before use. The content of the ethylene oxide in the eluent samples were analyzed by Shimadzu 9A gas chromatograph (Japan) equipped with flame ionization detector and Hitachi D-2500 Chromato-Integrator (Japan). A 2 m x 0.3 mm i.d. glass column packed with 3% Carbowax(polyethylene glycol) succinate on Chromosorb W-HP (80/100 mesh) was used for the separation of ethylene oxide from other air components. The oven temperature was programmed at 50 °C for the first 2 minutes and then ramped at 100 °C/min to 120 °C for 2 minutes. Inlet and detector temperatures were 230 °C. Nitrogen was used as carrier gas and the flow rate was 50 mL/min. Test atmosphere, which was used to determine breakthrough volume of the sampling tube was prepared by bubbling 2 ml of ethylene oxide solution in an enclosed impinger tube connected to a 1 liter round flask using purified nitrogen gas. The concentration of the ethylene oxide in the 2 mL solution was equivalent to 150 – 170 ppm for 3 liters of air sample. Relative humidity of the test atmosphere inside the flask was determined by the volume of water that has been evaporated from the 2 mL ethylene oxide solution during the bubbling process.

### Breakthrough Volume

The breakthrough volume of the sampling tube was determined by drawing air with flow rate at 150 mL/min from the test atmosphere prepared in a 1 liter round flask through two sampling tubes which were connected in series. The second sampling tube, which acts as backup tube was changed after every 1 liter of air has been drawn through both sampling tubes. Adsorbed ethylene oxide in the backup tube was eluted with distilled water and the eluent was analyzed by gas chromatograph with flame ionization detector. This test was repeated twice to ensure reproducibility.

### Method Detection Limit

Method detection limit was determined from a set of recovery data obtained from a series of liquid spiked sampling tubes. The spiked concentration was equivalent to 0.1 – 2.0 ppm in 3 liters air samples. Method detection limit was calculated by the following equation, which was obtained from OSHA: Method Evaluation Guidelines [19].

$$MDL = 3(SEE/A)$$

Where SEE is the standard error of estimate for the regression curve which obtained from a plot of chromatographic response versus amount of recovery. A is the analytical sensitivity (slope).

### Desorption Efficiency

The desorption efficiency of ethylene oxide from the sampling tube was determined by spiking with an equivalent weight of ethylene oxide in distilled water (2 µL) into the sampling tube. The concentrations of ethylene oxide which were spiked into the sampling tubes were equivalent to 0.2, 1.0, 2.0 and 4.0 ppm in 3 liters air sample. Each concentration was repeated for six times. Spiked sampling tubes were stored in the dark at room temperature overnight. Spiked ethylene oxide was eluted with 10 mL of distilled water and the recovered amount of ethylene oxide in the eluent was determined using GC-FID. Similar procedure was used to determine the desorption efficiency of ethylene oxide from coconut shell charcoal sampling tube.

### Stability Test

Forty-five sampling tubes which contained amount of ethylene oxide equivalent to 2 ppm ethylene oxide for 3 liters air samples were prepared by spiking 50 µL ethylene oxide solution into each tubes. Three tubes were eluted on the same day after spiking. The other twenty one tubes were kept at 5 °C in a refrigerator and the other twenty one tubes were kept in dark condition inside a drawer at room temperature. On every other day within 14 days, three tubes from each group were eluted by 10 mL of distilled water and the amount of recovered ethylene oxide in each tube was determined using GC-FID. An ethylene oxide solution was

also tested for stability within 14 days in a refrigerator. Precision of overall procedure at 95% confidence level was equivalent to ± 1.96SEE by assuming a normal distribution of the values about the storage tests regression curve and uniformity of the entire range of the curve. SEE is the total standard error of the overall procedure for each storage test including the personal air sampling pump variability (SP). Precision of overall procedure was calculated by the following mathematical equation [19].

$$SEE = [ (SEE_R)^2 + (SP)^2 ]^{1/2}$$

Where SEE<sub>R</sub> is the standard error of estimate for the regression curve of each storage test.

### Field Sampling

Simple random field samplings were carried out in two factories which used ethylene oxide as a sterilization substance for their products in Kulim Industrial Area, Kedah and Bayan Lepas Free Industrial Zone, Pulau Pinang. Air samples adjacent to the sterilization chamber were collected by drawing 3 liters of air through the sampling tubes using 150 mL/min flow rate. The open ends of the sampling tubes after sampling were sealed with aluminium foil and capped with rubber septum before transported in a thermostat box containing blue ice to the laboratory for analysis. All the sampling tubes were kept in a refrigerator before analysis. The obtained field sampling results were used to calculate time weighted average by using the following mathematical equation [20, 21].

$$TWA = \sum x_i t_i / \sum t_i$$

Where x<sub>i</sub> is the concentration of the ith air sample and t<sub>i</sub> is the averaging time of the ith air sample. The obtained TWA results were further converted to 8-hr TWA. Locations of the sampling site in MM and BB factories are shown in the **table 1**.

Table 1: Locations of the sampling site in MM and BB factories.

MM factory	Sampling sites	BB factory	Sampling sites
Awaiting Sterilization Area	a & b	Awaiting Sterilization Area 1 & 2	A & B
Degassing Area	c & d	Control Room 1 & 2	C & D
Quarantine Area	e & f	Degassing Area	E & F
Packaging Area	g	Operator Room	G
Incineration Room	h	Loading Area	H & I
EtO Gas Tank Room	I	Outside Factory	J & K
Loading Area	j	Quarantine Area 1 & 2	L & M

Note: MM represents the factory situated in Kulim industrial area, Kedah and BB represents the one situated in Bayan Lepas Free Industrial Zone, Pulau Pinang.

**RESULTS AND DISCUSSION**

As shown in **Table 2**, the recoveries of ethylene oxide from silica gel tubes (average  $83.9 \pm 2.8\%$ ) were higher than the recoveries obtained from charcoal tubes (average  $17.1 \pm 4.2\%$ ). The low recovery shown by charcoal tubes may be due to

their high surface activity and strong adsorptive behavior which may induced chemical reactions during storage and desorption of the samples [22]. Based on these results, silica gel sampling tube was selected to continue the following validation tests due to the obtained high recovery.

Table 2: Percentage recovery of ethylene oxide from silica gel and coconut shell charcoal sampling tubes

No. silica gel tubes	% recovery	No. charcoal tubes	% recovery
1	$85.9 \pm 2.6$	1	$19.6 \pm 0.9$
2	$87.3 \pm 4.3$	2	$21.1 \pm 0.6$
3	$83.6 \pm 1.1$	3	$21.4 \pm 0.2$
4	$80.0 \pm 0.8$	4	$11.6 \pm 2.3$
5	$81.4 \pm 3.3$	5	$13.5 \pm 2.6$
6	$85.1 \pm 1.2$	6	$15.6 \pm 1.4$
Mean	$83.9 \pm 2.8$	Mean	$17.1 \pm 4.2$

The method detection limit which was determined by a set of recovery data from a series of ethylene oxide solution spiked standards is  $0.583 \mu\text{g}$  in  $3 \mu\text{L}$ , which is equivalent to  $0.11 \text{ ppm}$  in 3 liters air sample at ambient condition. The mean desorption efficiency for ethylene oxide over the concentration range of  $0.2 - 4.0 \text{ ppm}$  is  $87.8 \pm$

$3.6\%$  (**Table 3**). The obtained mean desorption efficiency fulfils the OSHA standard requirements, which is more than  $75\%$  [19]. The bias of the desorption efficiency result is found to meet EPA QA/QC protocols bias for spike recovery which is  $\pm 25\%$  [22]. The obtained desorption efficiency result is used as correction factor to field sampling test results.

Table 3: Recovery of ethylene oxide from silica gel sampling tubes at various spiked concentrations

concentration ( for 3 liters air sample)	0.2	1.0	2.0	4.0
% recovery(desorption efficiency)	$80.8 \pm 4.1$	$80.7 \pm 2.4$	$89.8 \pm 2.5$	$91.0 \pm 2.0$
	$81.9 \pm 8.7$	$90.6 \pm 4.3$	$94.1 \pm 3.3$	$90.9 \pm 1.3$
	$81.3 \pm 9.4$	$86.1 \pm 9.2$	$95.7 \pm 2.8$	$87.8 \pm 1.9$
	$84.1 \pm 9.4$	$87.6 \pm 5.0$	$99.5 \pm 9.4$	$83.9 \pm 2.0$
	$84.1 \pm 7.2$	$87.2 \pm 3.9$	$98.1 \pm 3.3$	$83.2 \pm 1.5$
	$90.7 \pm 2.0$	$75.0 \pm 4.1$	$100.5 \pm 7.1$	$81.9 \pm 3.0$
Mean	83.8	84.5	96.3	86.5
Standard Deviation	3.6510	5.7141	4.0021	4.0159
Relative Standard Deviation	4.3559	6.7596	4.1566	4.6453
Combine Standard Deviation	3.6084			
Average % recovery	87.8			
Accuracy(%)	-16.2	-15.5	-3.7	-13.6

According to **Figure 1**, the breakthrough volumes for the first and second run are approximately 17 and 18 liters. The breakthrough is considered has occurred when the amount of ethylene oxide detected in the second sampling tube exceeds 0.10% of the total ethylene oxide prepared in the 1 liter round flask.

The relative humidity of the test atmosphere inside the 1 liter round flask was approximately  $88.2\%$  at  $28 \text{ }^\circ\text{C}$ , which fulfils OSHA's test atmosphere conditions that are approximately  $80\%$  at  $22.2 \text{ }^\circ\text{C}$  [23]. This relative humidity is also quite similar to the atmospheric relative humidity in Malaysia that is  $94\%$  on a sunny day

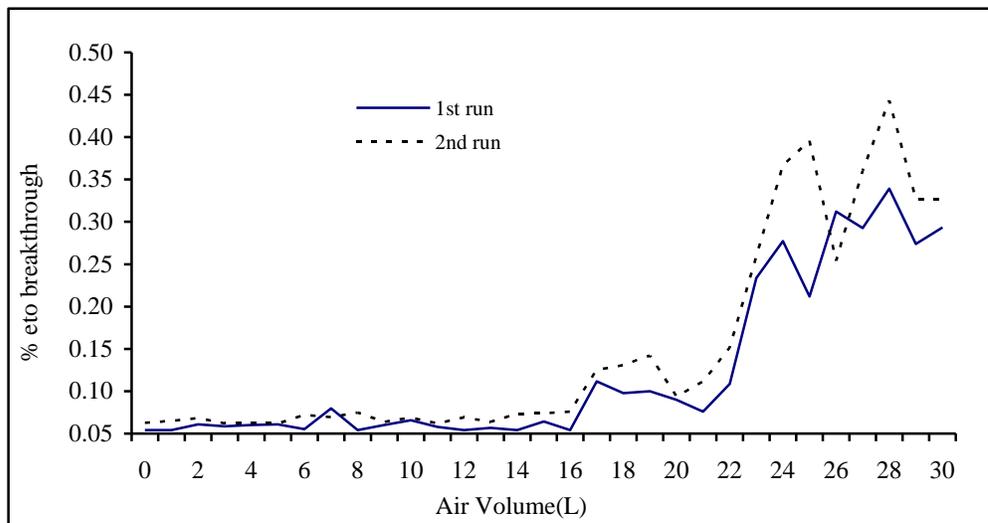


Figure 1: Determination of breakthrough volume.

[24]. After 30 liters air sample has been drawn through the sampling tubes, amount of ethylene oxide that has adsorbed in the first sampling tube is 74.8% in the first run and 63.5% in the second run based on the total ethylene oxide prepared in the 1 liter round flask. This shows that silica gel sampling tube is able to adsorb ethylene oxide up to these values before breakthrough occurs.

Silica gel sampling tubes which were spiked with ethylene oxide equivalent to 2 ppm in 3 liters air sample can be stored up to 14 days in the refrigerator and 2 days at ambient temperature (Figure 2). Recovery of ethylene oxide from the sampling tubes after 14 days storage in a refrigerator and ambient temperature were 83.9% and 21.8%, respectively (Figure 2).

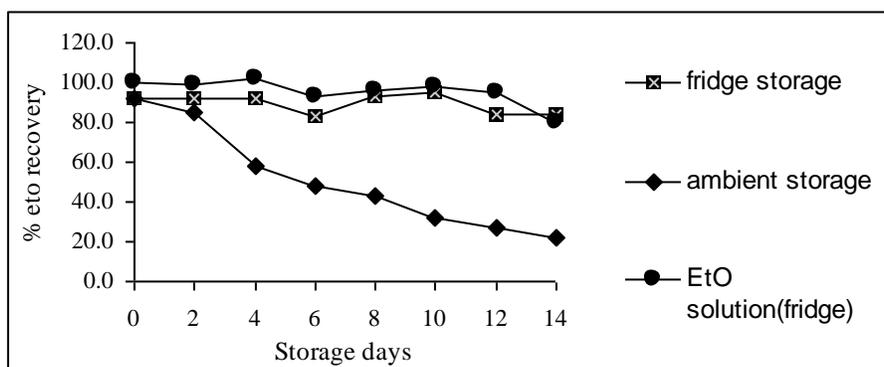


Figure 2: Stability test of ethylene oxide (EtO) in the silica gel sampling tube at room and fridge temperatures.

Low recovery obtained in the 14 days ambient storage test may be due to the high surface activity of silica gel which induces chemical reactions during storage [22]. Results obtained in the refrigerator storage test fulfils OSHA requirements [19], that the recovery (uncorrected for desorption efficiency) must be above 75% after storage and a change in recovery of more than 10% in 15 days storage was a significant uncorrectable bias and must be avoided. Amount of ethylene oxide in aqueous solution still

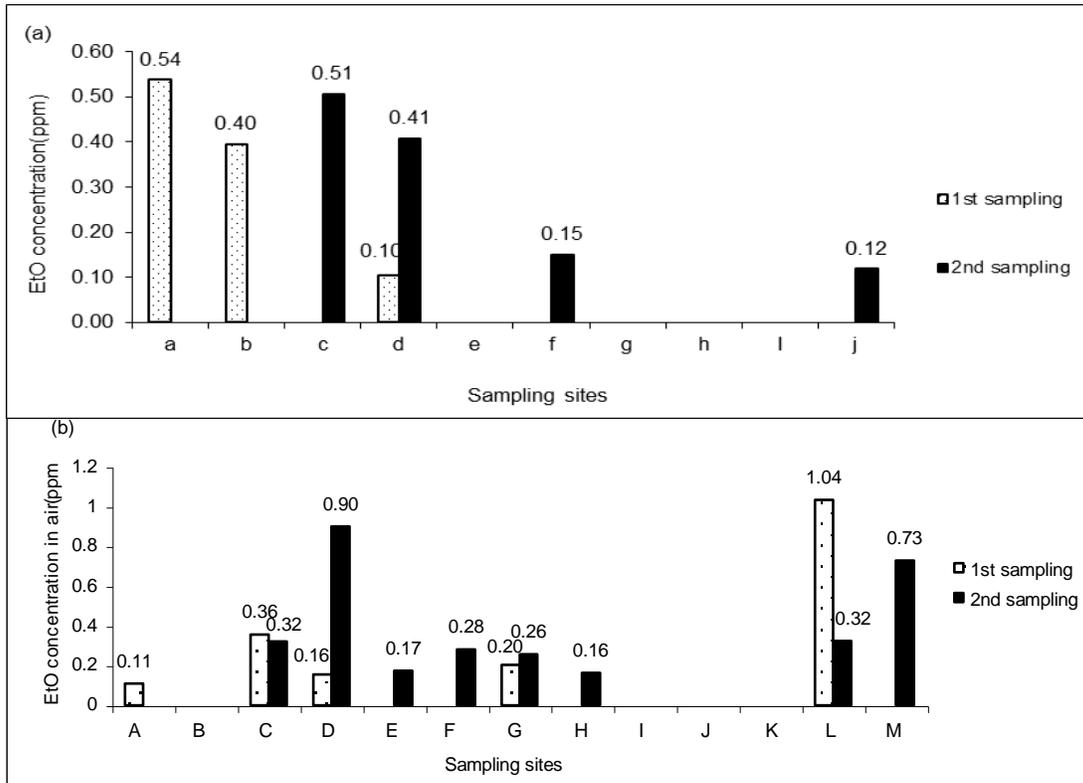
remained at 90% after 12 days storage in a refrigerator at about 5 °C (Figure 2).

The precision of overall procedure at 95% confidence level for 14 days refrigerator and ambient storage test are ±10.7% and ±15.1%, respectively. ±3% of the sampling or pumping error [18] is included in this calculation. The obtained results are less than ±25% at 95% confidence level which is stipulated by OSHA [19].

As shown in **Figure 3a**, ethylene oxide was detected in awaiting sterilization area and degassing area in MM factory in the first sampling event. The detected ethylene oxide concentrations are 0.54, 0.40 and 0.10 ppm,

respectively. Ethylene oxide was detected in degassing area, quarantine area and outside the factory in the second sampling event with the concentrations at 0.51, 0.41, 0.15 and 0.12 ppm (**Figure 3a**).

Figure 3: (a) Ethylene oxide (EtO) concentration (ppm) in the MM factory, (b) Ethylene oxide (EtO) concentration in BB factory



Note: Bars did not show in graph for sampling sites where no ethylene oxide was detected (lower than method detection limit).

Ethylene oxide was detected in awaiting sterilization area 1, control room 1 and 2, operator room and quarantine area 1 and 2 in BB factory in the first sampling event. The detected concentrations are 0.11, 0.36, 0.16, 0.20 and 1.04 ppm, respectively (**Figure 3b**); whereas in the second sampling event, ethylene oxide was detected in control room 1 and 2, degassing area, operator room, unloading area 1 and quarantine area 1 and 2 with concentrations ranging from 0.16 – 0.90 ppm (**Figure 3b**).

weighted averages of the ethylene oxide level in MM factory are 0.05 and zero ppm in the first and second sampling events respectively; whereas in BB factory are 0.08 ppm and 0.12 ppm in the first and second sampling events. All of the calculated 8 hour time weighted averages are below OSHA permissible exposure limit (PEL) and action level that are 1 ppm and 0.5 ppm [9]. However, the calculated 8 hour time weighted averages of the ethylene oxide level in BB factory in the second sampling event is above the NIOSH recommended exposure limit (REL) that is 0.1 ppm [9]. Therefore, BB factory may require further monitoring to ensure the concentration of ethylene oxide in the air surrounding the sterilization chamber is below

The detected levels of ethylene oxide in factories MM and BB are below OSHA excursion limit (5 ppm for 15 min) and NIOSH ceiling limit (5 ppm for 10 min). The calculated 8 hour time

the NIOSH's REL. However, ethylene oxide in the air surrounding the sterilization chamber in both factories is considered safe if OSHA regulations are imposed.

As shown in **Figure 4**, the concentration of ethylene oxide in BB factory increases obviously from 1300 to 1530 in the first sampling event and from 1140 to 1338 in the second sampling event.

This is due to the ethylene oxide was released from sterilization chamber when treated products

were unloaded from the chamber. Besides, ethylene oxide can also be released from the treated products after unloading from the chamber [3]. According to OSHA substance safety data sheet for ethylene oxide [25], workers who carry out the unloading task need to wear oxygen mask to protect them from exposing to ethylene oxide gas. Similar results were obtained by a team of researchers in the measurement of ethylene oxide at one of medical sterilization sites in Japan [26].

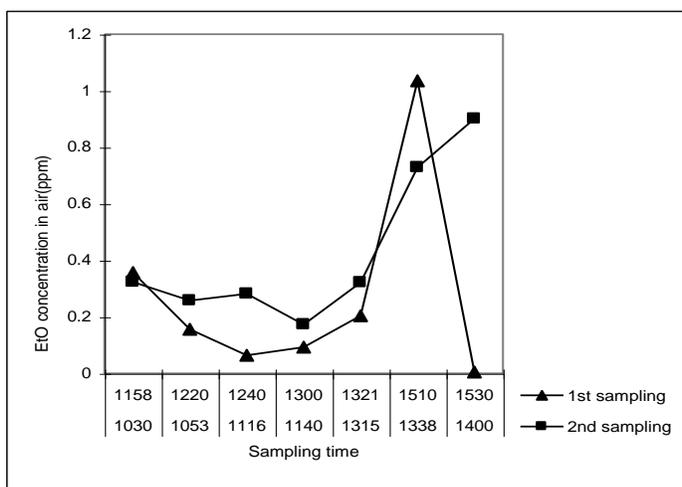


Figure 4: Ethylene oxide (EtO) concentrations in BB factory throughout the sampling time.

## CONCLUSION

In conclusion, an accurate and precise method with cheaper cost and shorter analysis and sampling time was developed in this study. In this method, water was used as a solvent to extract ethylene oxide from the sampling tube. This is because water is a cheaper solvent and less toxic compared with other organic solvents such as carbon disulfide, benzene and dimethylformamide, which were used to extract ethylene oxide and 2-bromoethanol from the charcoal tube in HBr coated charcoal methods [10, 11]. Furthermore, no solvent peak is produced during the analysis of sample with GC-FID. Therefore, time per analysis can be shortened compared to other methods which use dimethylformamide, carbon disulfide and benzene as solvent.

## ACKNOWLEDGEMENTS

We thank to the MM and BB company managers to allow us to collect the air samples in their factory. This project is financially supported by Universiti Sains Malaysia.

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