



CHAPTER III

MATERIALS AND METHODS

Apparatus

- A Hewlett Packard HP 5890 gas chromatograph equipped with Electron Capture Detector (ECD) and Hewlett Packard HP 3394 A integrator
- A Griffen No.7 constant temperature water bath (Griffen&George Ltd., GT. Britain)
- 1 mL Gas tight syringe (Halmilton Company, Switzerland)
- 50.00 μ L Micro syringes (Halmilton Company, Switzerland)
- Pipettes 1.00, 3.00 and 5.00 mL
- Volumetric flasks 10.00, 25.00, 100.00, 1000.00 mL
- Cylindrical 50 mL
- 60 mL serum vials (see appendix A)
- gray septa, aluminum foils, aluminum caps; (see appendix A)
- Manual hand operated crimper, Hewlett Packard (see appendix A)
- Water sampler
- Lux meter
- Chlorine and pH test kit
- Thermometer
- A Hewlett Packard HP 5890 gas chromatograph equipped with mass selective detector (MSD) HP 5972 B

Chemicals

1. Chlorinated Hydrocarbon Standards

All of the standard are analytical reagent grade. Methylene chloride and carbon tetrachloride were purchased from E.Merck, Darmstadt, Germany. Chloroform was purchased from J.T. Baker Chemical Company, Deventer, Holland.

1,1,1-Trichloroethane and Trichloroethylene were purchased from Carlo Erba, Italy. The density of the standard chemicals are shown in Table 3.1.

Table 3.1 Density of chlorinated hydrocarbons standards used in the headspace study.

Name of compound	Density at 20° C (g/mL)
Methylene Chloride (CH ₂ Cl ₂)	1.316
Chloroform (CHCl ₃)	1.473
Carbon tetrachloride (CCl ₄)	1.590
1,1,1,-Trichloroethane (CH ₃ CCl ₃)	1.326
Trichloroethylene (CHCl ₃)	1.462

2. Organic Solvents

Absolute methanol, analytical reagent grade, was purchased from E. Merck, Darmstadt, Germany.

3. Salt

Anhydrous sodium sulfate (AR Grade) was obtained from E.Merck, Darmstadt, Germany. They were heated in an oven at 150 °C for 2 hours and were kept in desiccator before being used.

4. Double Distilled Water

Double distilled water was distilled by the Yamato distiller model WA-52 R (Yamato Scientific Co, Ltd.Tokyo, Japan) and boiled at 100°C for 1 hour in 1000 mL beaker.

Primary Data Collection

1. Questionnaire was designed based on the Bangkok Metropolitan Administration Regulation. One hundred and four swimming pools were recruited for this study. They were randomly selected from 291 pools from every district in Bangkok area (number of registered swimming pools is 291 pools in 1994).

Each swimming pool staff was interviewed to complete the questionnaire. The detail of questionnaire was displayed in Appendix B.

Swimming pool water collection

The sampling sites of this study were classified into four groups as follows :

1. Hotel swimming pool	10	samples
2. Academic swimming pool	10	samples
3. Public swimming pool	8	samples
4. Club swimming pool	8	samples

They were randomly selected from 104 swimming pools that had been interviewed by questionnaire before.

1. Sampling Method

Sample collection was divided into 2 groups

Group 1: Thirty-six samples were collected at deep area and shallow area in each swimming pools. Samples were collected at 20 cm. depth from surface water.

Shallow area refers to the area of swimming pool of which the depth from surface of water to the bottom of swimming pool shall not exceed 1.50 metre.

Deep area refers to the area of swimming pool of which the depth from surface of water to the bottom of swimming pool exceed 1.50 metre.(Bangkok Metropolitan Administration, 1987)

group 2: Four sampling sites were selected from all types of swimming pools for water sample collection. At each sampling site, the water was collected every

three hours, from 6 a.m. to 6 p.m. for two days. The samples were collected at 20 cm and 60 cm depth from the water surface.

Two 250 mL glass bottles were used for each swimming pool. The bottle was held underwater at about 20 cm. Water was allowed to fill the entire bottle. The bottle was closed below the surface of the water to prevent the introduction of air to the sample. For samples collected at 60 cm. depth, a water sampler was used to collect the water.

Thirty mL of the water sample was placed in a 60 mL vial which contained 13 g of anhydrous sodium sulfate. The vial was closed with aluminium foil, gray rubber septa and aluminium cap sequentially, and then the vial was tightly crimped with manual hand operated crimper.

The water samples were transported to the laboratory where they were kept in a refrigerator. Then, they were analysed within 5 days.

Four parameters were measured at the sampling sites. Method for the measurement of each parameter was depicted in Table 3.2

Table 3.2 Method for the measurement of each parameter

Parameter	method
Chlorine residual	Pool Test Kit
Temperature	Thermometer
pH	Pool Test Kit
Light intensity	Lux Meter
Halogenated hydrocarbons	Gas Chromatography

2. Water Sample Analysis

2.1 Glassware Preparation

All glassware, including serum vials were cleaned with detergent, diluted HNO₃ (1:1), water and rinsed with double distilled water respectively and dried in an oven at 150°C at least 3 hours before being used.

2.2 Preparation of the Standard Solution

2.2.1 The Standard Solution for the Headspace study using GC with ECD as a Detector.

The single component standard stock solutions of each chlorinated hydrocarbon i.e., methylene chloride, chloroform, carbon tetrachloride, 1,1,1-trichloroethane and trichloroethylene in methanol were prepared quantitatively with 50 μ g microsyringe and diluted to the mark in 25.00 mL volumetric flasks with methanol. The volume of each chlorinated hydrocarbon was calculated from the density as shown in Table 3.1 and their concentration were shown in Table 3.3.

Table 3.3 The concentration of the single component standard stock solution in methanol.

Compound	Volume of Standard used (μ l)	The concentration of the standard stock solution in methanol (ppm)
Methylene chloride	19	1000.16
Chloroform	17	1001.64
Carbon tetrachloride	16	1017.60
1,1,1-trichloroethane	19	1007.76
Trichloroethylene	17	994.16

2.2.2 The Standard Mixture Solution of Methylene chloride, Chloroform, Carbon tetrachloride, 1,1,1-trichloroethane and Trichloroethylene in Methanol.

The standard mixture solution containing 30.00, 30.04, 3.05, 3.02 and 2.98 ppm of methylene chloride, chloroform, carbontetrachloride, 1,1,1-trichloroethane and trichloroethylene, respectively and were prepared by pipetting 3 mL of methylene chloride and chloroform and pipetting 0.3 mL of carbon

tetrachloride, 1,1,1-trichloroethane and trichloroethylene stock solution as shown in Table 3.3 into a 100.00 mL volumetric flask and diluting to the mark with methanol.

2.3 Gas Chromatographic Conditions

Gas Chromatographic conditions used in this study are shown in Table 3.4.

2.4 Headspace Condition

The optimum headspace analysis condition used in analysis of the water samples are shown in Table 3.5.

Table 3.4 The gas chromatographic conditions used ECD as a detector for the study of mixture component solution.

GC Parameter	GC Condition
Analytical Column	25 m× 0.32 mmID., HP-5 (5% phenyl Methyl silicone) Capillary column
Temperature Program	36 °C (1 min.) to 100 °C (10 min.) at 5 C°/min.
Split Ratio	100:1
Carrier Gas	Helium
Flow Rate of Carrier Gas	0.7 mL/min.
Make up Gas	Nitrogen
Flow Rate of Make up Gas	40.00 mL/ min.
Detector	Electron Capture Detector (ECD)
Detector Temperature	300°C
Inlet Temperature	150°C

Table 3.5 The optimum headspace analysis conditions used in the analysis of the water samples

Parameter	Condition
Equilibration time	60 minutes
Temperature	60°C
Liquid to gas phase volume ratio	30 : 30 in 60 mL serum vial.
Injection volume	1.00 mL
Salt used	13.00 g of anhydrous Na ₂ SO ₄

Triplicate analyses

2.5 Standard Calibration Curves

Various concentrations of standard mixture of interested compounds in aqueous solution are prepared for constructing the standard calibration curves of each interested compound. These standard mixtures in aqueous solutions are analysed by the headspace technique under the identical condition and GC condition as the analysed samples. Then the peak area of each standard compound obtained from the chromatogram is plotted against the initial concentration of the standard. The curve should be linear for a particular system. The slope and y-intercept can be calculated by the linear least square method from a linear equation.

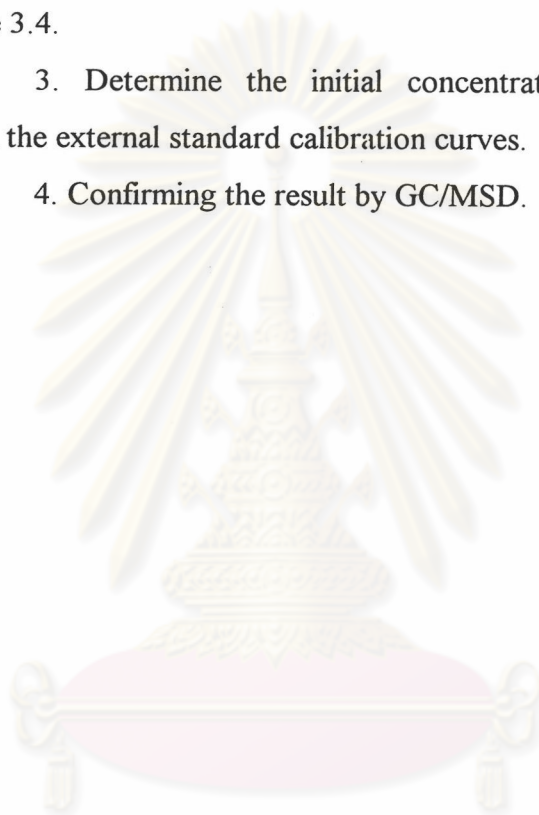
The exact volume of the vapor phase from the equilibrated unknown samples is then chromatographed. Finally, the initial concentration of the interested compound in the aqueous solution can be determined by substitution the peak area of the interested compound into the equation.

2.6 Determination of Halogenated Hydrocarbons in Water Samples

Water Samples were analysed by headspace analysis technique under the optimum headspace condition and under the GC condition as mentioned above. The concentration of the interested component in water samples were

determined by means of external standardization methods. The procedure was described as follow;

1. Place sample into a constant temperature water bath with the temperature is set at 60°C for 60 min.
2. Withdraw 1.0 mL of vapor phase from the serum vial by using 1 mL gas tight syringe and inject into gas chromatograph under GC condition as described in Table 3.4.
3. Determine the initial concentration of each halogenated hydrocarbon from the external standard calibration curves.
4. Confirming the result by GC/MSD.



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