CHAPTER II

THEORY AND LITERATURE REVIEW

Before 19th century, people in the world died from infectious waterborne diseases such as dysentery, typhoid and cholera. After early that century waterborne diseases have decreased dramatically due to the uses of chemical disinfectants in water treatment [4]. Despite human triumph over waterborne pathogens, in 1974 Rook [58] reported the relationship between chlorination in drinking water and formation of disinfection by-products such as chloroform, which was probable human carcinogen. Disinfectants have been proven to protect water from microorganisms (waterborne disease). However, an unintended health hazard due to their by-products can occur. For this reason, researchers have focused on using disinfectants and determination of disinfection by-products.

In this chapter, drinking water treatment process, disinfectants, formation of disinfection by-products (DBPs) and analytical methods of DBPs in drinking water are reviewed.

2.1 Drinking water treatment processes [59]

The purpose of drinking water treatment is to remove pathogens, toxic chemical and aesthetic contaminants from raw water, especially surface water such as rivers, lakes and reservoir. The processes consist of coagulation, flocculation, sedimentation, filtration and disinfection as illustrated in Figure 2.1.

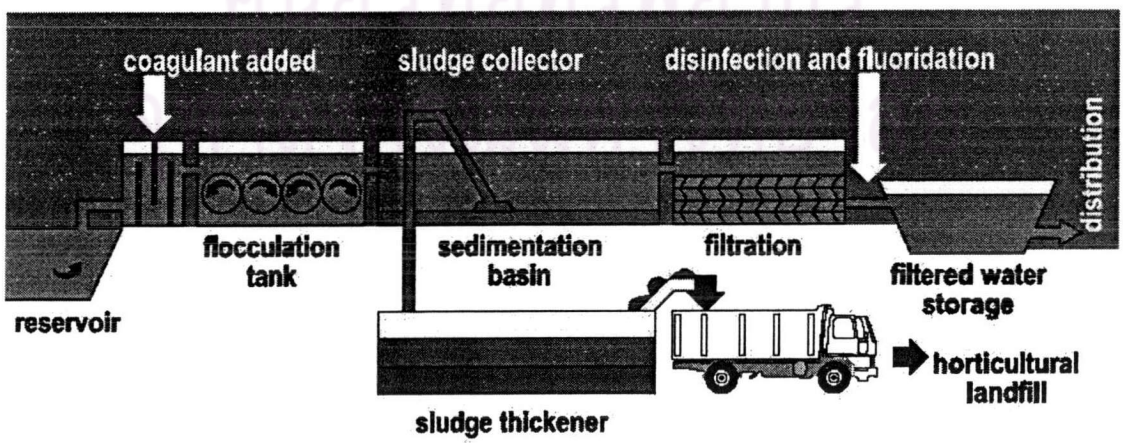


Figure 2.1 Conventional water treatment processes [60].

Coagulation is a process to neutralize the charges on the surface particles. Flocculation is a process to agglomerate neutralized particles by chemically joining or bridging them together. Sedimentation is a process to remove flocculated flocs from processed water by gravity. Filtration is a process to separate non-settable and destabilized particles from process water using a porous medium such as sand. The final process before distributing water into the distribution system is disinfection. This process serves two main purposes. The primary purpose of disinfection is to kill or inactivate pathogens. The secondary purpose is to provide a disinfectant residual in finished water and prevent microbial regrowth in water distribution system. Although the majority of pathogens are removed by coagulation, flocculation, sedimentation and filtration, disinfection is a critical process to protect the public from contracting water borne diseases.

2.2 Disinfectants

Disinfectants are chemical agents used to eradicate and inactivate the pathogens in the water treatment processes. Chlorine, chloramine, ozone and chlorine dioxide are common disinfectants for water treatment. Each of those disinfectants has advantages and disadvantages in the terms of efficacy, cost, stability, ease of application and formation of DBPs [61,62]. In most water treatments including in Thailand, chlorine is the most widely used disinfectant in the water treatment process. This might be due to that chlorine is effective disinfectant against a wide range of bacteria, virus and other microorganisms. Moreover, chlorine provides adequate residual throughout the distribution system in order to preserve the water from microbial recontamination [63]. The chemistry of chlorine disinfectant and the formation of their DBPs are described in the next section.

2.3 Chlorine disinfectant

Chlorine disinfectant is available in granular or powdered form as calcium hypochlorite ($Ca(OCl)_2$) or in liquid form as sodium hypochlorite (NaOCl; bleach). In water treatment plants, chlorine is used in the form of gaseous chlorine or hypochlorite (OCl^-). When added to the water, chlorine is hydrolyzed, producing hypochlorous acid (HOCl, chlorine oxidation state: +I) and hydrochloric acids follow:

$$Cl_2 + H_2O \rightleftharpoons HOCl + Cl^- + H^+ \qquad (2.1)$$

Hydrochloric acid is a strong acid and is completely dissociated into hydrogen and chlorine ions. Hypochlorous acid is a weak acid with a pK_a of approximately 7.5 at 25 °C, and it dissociates into hydrogen ions and hypochlorite ions:

$$HOCl \rightleftharpoons H^+ + OCl^- \quad (2.2)$$

In chlorination, it is believed that chlorine(0) and chlorine(+I) compounds work as primarily disinfecting agents by denaturing enzymes or proteins, inactivating microorganisms, and in some cases, physical disrupting cell membranes [64].

2.4 Disinfection by-products (DBPs)

Disinfection by-products are term used to describe a group of organic and inorganic compounds formed during water disinfection. Disinfection by-products are formed by the reaction between disinfectants and natural organic matters (NOM) and/or inorganic substances present in water. The formation of DBPs can be expressed as follow:

$$NOM + HOCl \longrightarrow \text{Disinfection by-products} \quad (2.3)$$

In addition, when water contains bromide, chlorination can also produce a variety of brominated by-products. Bromide is oxidized by chlorine(I) to give bromine(I). This reaction is irreversible:

$$ClO^- + Br^- + H_2O \longrightarrow Cl^- + HOBr + OH^- \quad (2.4)$$

Thus, bromination reactions abound during chlorination. In this fashion, a mixture of brominated, chlorinated and bromochlorinated by-products are formed during disinfection:

$$NOM + HOCl + HOBr \longrightarrow \text{Disinfection by-products} \quad (2.5)$$

The speciation and concentration of DBPs in water are affected by several parameters and treatment conditions including chlorine dosage, concentration of chlorine and nature of NOM (mainly fulvic and humic substances), chlorine contact time, pH, temperature of water and concentration of bromide ion [65]. Nowadays approximately 500 DBPs have been reported [66]. The distribution of halogenated by-products proportional to the total organic halogen (TOX) in chlorinated drinking water was shown in Figure 2.2.

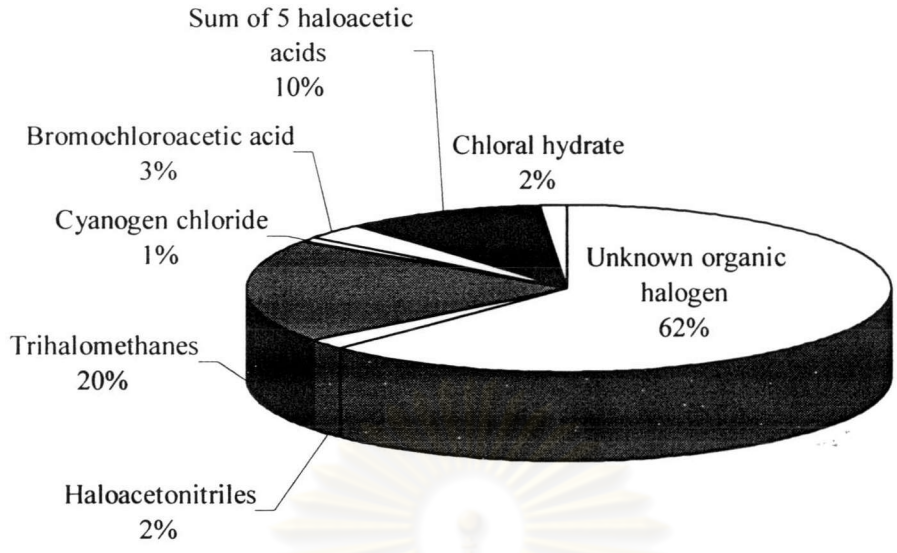


Figure 2.2 Relative amount of halogenated DBPs proportional to the total organic halogen in chlorinated drinking water [7].

As indicated in Figure 2.2, trihalomethanes and haloacetic acids are two major groups of organic disinfection by-products identified in chlorinated water. Several toxicological and epidemiological studies have demonstrated their negative health effect. The US EPA has summarized the health information on the carcinogenic potential of THMs and HAAs in Table 2.1.

Table 2.1 Status of health information for THMs and HAAs [63].

Compound	Cancer group[a]	Detrimental effects
Chloroform	B2	Cancer, liver, kidney and reproductive effect
Bromodichloromethane	B2	Cancer, liver, kidney and reproductive effect
Chlorodibromomethane	C	Nervous system, liver kidney and reproductive effect
Bromoform	B2	Cancer, nervous system, liver and kidney effect
Dichloroacetic acid	B2	Cancer, reproductive and developmental effects
Trichloroacetic acid	C	Liver, kidney, spleen and developmental effects

[a] B2: Probable human carcinogen (sufficient evidence from animal studies), C: Possible human carcinogen (limited evidence from animal studies and inadequate or no data in human).

2.4.1 Trihalomethanes (THMs)

Trihalomethanes (THMs) are organic compounds based on methane (CH_4) that three hydrogen atoms are substituted by halogen atoms. Substituted atoms may be fluorine, chlorine, bromine, iodine or combination thereof. These compounds are formed in drinking water primarily as a result of chlorination of organic matter present naturally in raw water supplies. The THMs that are most commonly present in drinking water are chloroform ($CHCl_3$), bromodichloromethane ($CHCl_2Br$), chlorodibromomethane ($CHClBr_2$) and bromoform ($CHBr_3$) [67]. Their structures, names, chemical formula and common acronyms were shown in Table 2.2 and their properties were summarized in Table 2.3.

Table 2.2 Structure, names, chemical formula and common acronyms of four THMs.

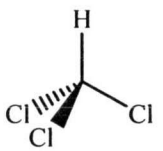
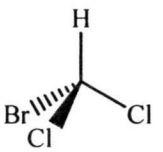
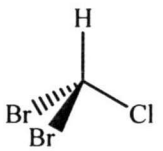
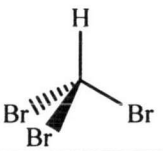
Structure	Name	Formula	Acronyms
	Trichloromethane or Chloroform	$CHCl_3$	TCM
	Bromodichloromethane	$CHCl_2Br$	BDCM
	Chlorodibromomethane	$CHClBr_2$	CDBM
	Tribromomethane or Bromoform	$CHBr_3$	TBM

Table 2.3 Trihalomethanes and some property [68].

Property	$CHCl_3$	$CHCl_2Br$	$CHClBr_2$	$CHBr_3$
Molecular weigh (g/mol)	119.38	163.8	208.3	252.77
Melting point (°C)	-64	-55	<-20	6-7
Boiling point (°C)	62	90	116-122	149
Density (g/cm^3)	1.49 at 20°C	1.97 at 25/25°C	2.38	2.9 at 20/4°C
Vapor pressure (mm)	160 (20°C) 245 (30°C)	-	-	5.6 (25°C)
Solubility (mg/L)	8000 (20°C) 9300 (25°C)	-	-	3190 (30°C)
Henry's law constant at 25 °C (k_H/kPa m^3mol^{-1}) [69]	0.43	-	-	0.047
Log P^* [70]	1.97	2.10	2.24	2.38
Respond of ^{63}Ni ECD Counts ($g \times 10^{-16}$) [47]	2.1	16.3	12.9	4.33

* P is *n*-octanol/water partition coefficient.

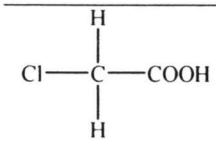
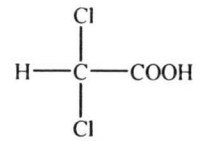
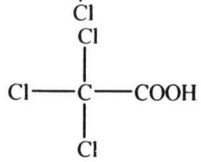
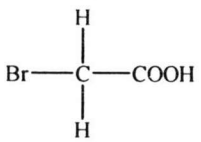
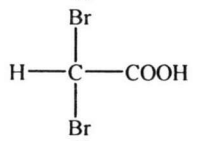
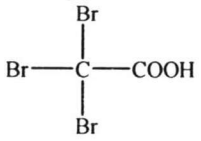
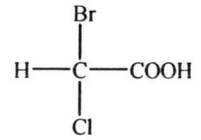
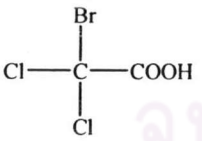
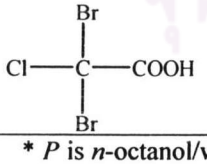
THMs are classified as volatile compounds because the Henry's law constant at 25 °C lies between 10^{-5} - 10^{-3} atm·m^3/mol (101.325 kPa = 1 atm) [71]. Therefore, gas chromatography is the suitable instrumentation for separation and analysis of THMs.

2.4.2 Haloacetic acids (HAAs)

Haloacetic acids (HAAs) are acetic acids that one or more hydrogen atoms are replaced by halogen atoms at the alpha position (the position next to the carboxylic group). Halogen atoms may be fluorine, chlorine, bromine, iodine or combination of them. HAAs are colourless and fairly stable. There are nine species of HAAs that are most commonly present in drinking water [67]. Five HAAs (HAA5) i.e., mono-, di-, trichloroacetic acid and mono-, and dibromoacetic acids are regulated under the disinfectants/disinfection by-products by the US EPA. The maximum contamination level (MCL) of HAA5 in drinking water has been set at 60 µg/L [12]. Six HAAs (HAA6), including HAA5 and bromochloroacetic acid, are also regulated under information collection rule (ICR) by the US EPA that applied to water distribution

systems serving more than 100,000 people [72]. Their structures, names, chemical formula, common acronyms, log P and pK_a of nine HAAs were shown in Table 2.4.

Table 2.4 Structure, names, chemical formula and common acronyms of nine HAAs.

Structure	Name	Formula	Acronyms	Log P^* [73]	pK_a [73]
	Monochloroacetic	$ClCH_2CO_2H$	MCAA	0.22	2.87
	Dichloroacetic	Cl_2CHCO_2H	DCAA	0.92	1.26
	Trichloroacetic	Cl_3CCO_2H	TCAA	1.33	0.51
	Monobromoacetic	$BrCH_2CO_2H$	MBAA	0.41	2.89
	Dibromoacetic	Br_2CHCO_2H	DBAA	1.693	1.47
	Tribromoacetic	Br_3CCO_2H	TBAA	3.459	2.13
	Bromochloroacetic	$BrClCHCO_2H$	BCAA	1.14	1.39
	Bromodichloroacetic	$BrCl_2CCO_2H$	BDCAA	2.31	1.09
	Dibromochloroacetic	Br_2ClCCO_2H	DBCAA	2.907	1.09

* P is n-octanol/water partition coefficient.

The pK_a values of the HAAs range from 0.5 to 2.9. Therefore, HAAs are present in neutral water as dissociated forms which are soluble and non-volatile. As a result, analysis by GC is more difficult. HAAs must be derivatized to their volatile

ester derivatives (esterification). Alternative techniques such as high-performance liquid chromatography (HPLC) [73], ion chromatography (IC) [74] and capillary electrophoresis (CE) [75] may avoid the requirement for derivatization but are not applicable for drinking water analysis because of their high detection limits compared to the GC method [29,33].

2.5 Standard methods for analysis of trihalomethanes and haloacetic acids in water sample

The US EPA method and the Standard Method were used for determination of THMs and HAAs in water sample. Their methods for THMs and HAAs were listed in Table 2.5 and Table 2.6, respectively.

Table 2.5 Standard methods for analysis THMs in drinking water [76,77].

Method	Analytical method	Detection
US EPA 502.2	Purge and trap	GC-photoionization-electrolytic conductivity detection
US EPA 524.2 Standard Method 6232C	Purge and trap	GC-MSD
US EPA 551.1 Standard Method 6232B	LLE (MTBE or pentane)	GC-ECD

Table 2.6 Standard methods for analysis HAAs in drinking water [76,77].

Method	Analytical method	Derivatization method	Detection
US EPA 552, Standard Method 6251B	LLE (MTBE)	Diazomethane	GC-ECD
US EPA 552.1	Sorption to anion exchange resin	Acidic methanol	GC-ECD
US EPA 552.2	LLE (MTBE)	Acidic methanol	GC-ECD
US EPA 552.3	LLE (MTBE or TAME)	Acidic methanol	GC-ECD

THMs can be isolated from the aquatic matrix by P&T and LLE methods followed by direct analysis with GC-ECD or GC-MSD. For the determination of HAAs with GC, derivatization prior to analysis is necessary. Methylation of HAAs is

to convert the carboxylic group of HAAs to their methyl ester derivatives that are more volatile, more thermally stable, more selective and more analytical response [78]. Diazomethane and acidic methanol are two of the chemical choices in standard methods for HAAs derivatization. In several literatures, diazomethane has been reported for its toxicity, mutagenicity, carcinogenicity and irritancy [23,78]. For this reason, derivatization with acidic methanol was frequently used. The mechanism of acidic methanol derivatization involves nucleophilic acyl substitution reaction of a carboxylic group with an alcohol to form ester. The reaction is carried out under acidic condition, as described in Figure 2.3.

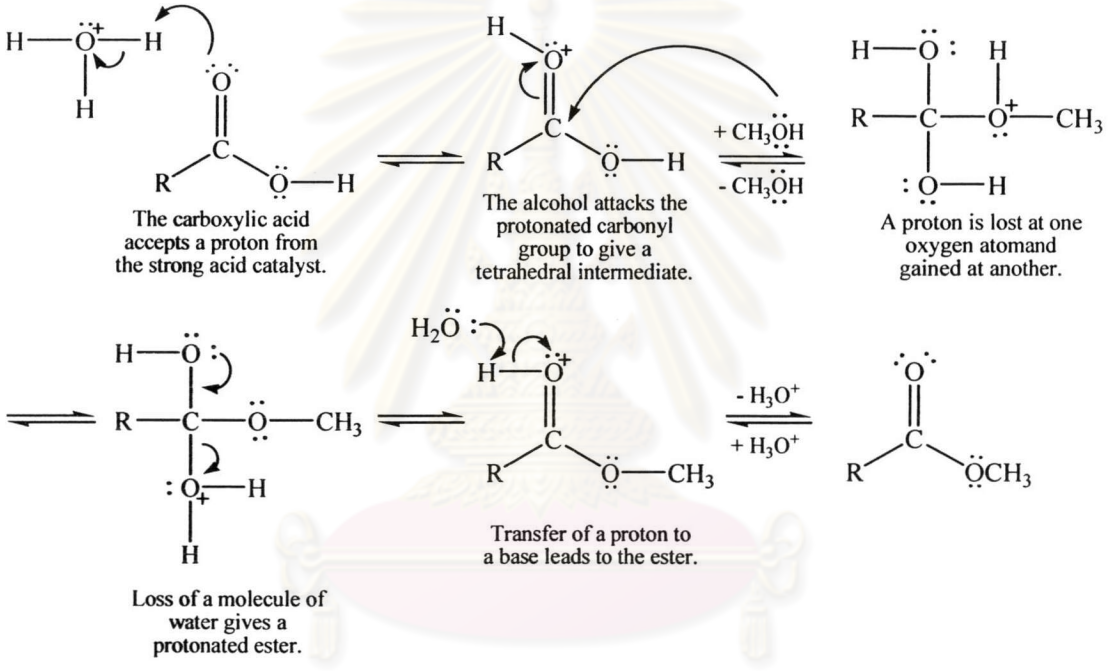


Figure 2.3 Mechanism for the reaction of acid-catalyzed esterification [79].

Methyl esters of haloacetic acids are more volatile than free acids, giving a possibility of gas chromatographic analysis. Moreover, methyl esters provide more symmetrical GC peak and low adsorption on difference parts of the equipment.

2.6 Gas chromatography-electron capture detector (GC-ECD) [80]

Gas chromatography (GC) is a separation method according to the partition of sample components between a stationary phase and a gas phase. The sample components are vaporized in the injection port and carried by the carrier gas through the separation column contained in the thermostat-controlled oven. The separation is

accomplished based on their vapor pressures and solubility in the stationary phase under programmed temperature. At the end of the column, the carrier gas and the separated components are transferred through a detector, where the signals are generated and the quantities of the components are determined. The schematic of gas chromatographic system is illustrated in Figure 2.4.

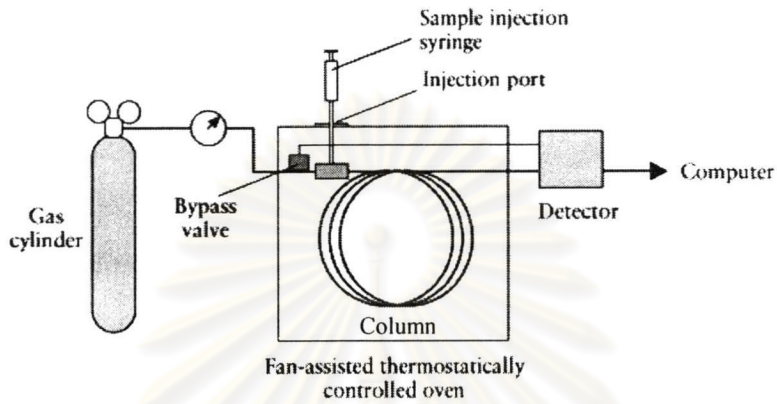


Figure 2.4 Schematic diagram of a typical gas chromatograph [81].

There are many detectors which can be used in gas chromatography. Different detectors will give different types of selectivity. The electron capture detector (ECD) is one of many detectors in GC that is selective to compounds containing atom with high electronegativity such as chlorine, fluorine and bromine. In ECD, the electron (beta radiation) is generated by the radioactive ^{63}Ni to ionize some of the carrier gas (typical nitrogen) producing a current between a biased pair of electrodes, as shown in Equation 2.6 and Equation 2.7. When organic molecules that contain atom with high electronegative pass by the detector, the emitted electrons are captured and the current measured between the electrodes is reduced, resulting in a reduction in the baseline current. The change in current provides the signal for the electron-capturing compounds:

$$^{63}Ni \longrightarrow \beta^- \qquad (2.6)$$

$$\beta^- + N_2 \longrightarrow 2e^- + N_2^+ \qquad (2.7)$$

$$A + e^- \longrightarrow A^- \qquad (2.8)$$

The mathematical relationship of this process is similar to Beer's Law. Thus, the extent of the absorption or capture is proportional to the concentration of the analyte. A schematic of a typical ECD was shown in Figure 2.5.

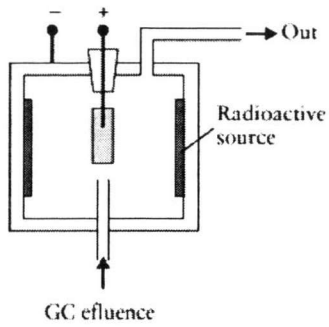


Figure 2.5 Schematic of a typical electron capture detector [81].

2.7 Development of sample preparation technique for THMs and HAAs

Since the detection of disinfection by-products in chlorinated drinking water, there are several sample preparation methods for determination of disinfection by-products especially for THMs and HAAs. Liquid-liquid extraction (LLE) is one of the oldest and most widely used technique. LLE involves distribution of sample components between two immiscible liquid phases. Typically, LLE is performed in a separatory funnel by agitating the mixture to disperse drops of one liquid into the other, then allowing the drops to coalesce so that the bulk organic liquid phase is separated from the aqueous sample phase. Despite its widespread uses, it is considered high consumption of toxic organic solvent, time consuming, tedious, multistage operation and often tiresome formation of emulsions [34-36]. Driven by the need to overcome these drawbacks, new sample preparation techniques have been developed.

Analysis of THMs by direct aqueous injection (DAI) has been developed by Grob [82]. In this technique, the sample is directly injected into the GC column. The advantage of this procedure is rapid and simple preparation of the sample without any pretreatment steps except filtration if necessary. However, it requires frequent maintenances because aqueous injection into a GC will rapidly deteriorate system performances. Moreover, contamination of the injector and the GC column with sample matrix due to non-volatile compounds and salts are also disadvantage. Furthermore, attaining a stable baseline is often difficult to attain [37,38].

Headspace extraction (HS) is the common technique for quantitative and qualitative analysis of volatile organic compounds from a variety of matrices. This technique has existed since the late 1950s [83] and is still actively used. A typical headspace analysis involves the partition of volatile analytes between the liquid or

solid sample and the headspace in a temperature controlled sealed vial. Once the concentration in the headspace of the vial reaches equilibrium with the concentration of the analyte in the sample matrix, a portion of gas phase is transferred into the GC for analysis. HS does not require complicated instrumentation. It is relatively inexpensive, solvent free method and can be automated. HS has been applied to the analysis of THMs in water samples [21,22]. Considerable efforts have been devoted to analysis of polar compounds such as HAAs [23,24]. However, it is relatively low sensitive compared to LLE [39,40]. As a result, HS may be suitable for analysis of samples with higher contents of volatiles. In order to overcome this disadvantage, purge and trap (P&T) has been developed. Like HS, P&T relies on the volatility of the analysis to achieve extraction from the matrix. An inert gas is purged through the sample and the volatiles are transferred to an absorbent trap. The trap is then heated and the volatiles are thermally desorbed and transferred to the GC for analysis. This technique enables lower detection limits than HS and is possible to determine solutes with lower vapor pressure [38]. P&T is most frequently used technique for volatile organic compound in water samples when GC is used. It has been approved by US EPA as standard method for determination of THMs [76]. However, P&T requires complex and expensive instrument. When compared to LLE, P&T requires longer analysis time. Moreover, leaking and carry over may occur in the system [41,42].

In 1990, Arthur and Pawliszyn [84] have developed the sample preparation technique termed solid-phase microextraction (SPME). This technique successfully redresses the limitations that are inherent in the traditional LLE method due to its solvent free sample preparation technique. In SPME, a thin fused silica fiber coated with a stationary phase is exposed to the sample or the headspace and the target analytes partitioning from the sample matrix in to the fiber coating. After extraction, the fiber can be directly transferred to the heated injection port of GC where the analytes are thermally desorbed for subsequent analysis. This technique has been applied to analysis THMs [27,28] and HAAs [29-32]. An important feature of SPME is that sampling, extraction, preconcentration and sample introduction are incorporated into single step [84]. Although this method offers many advantages, SPME fiber is relatively fragile, expensive and having limited lifetime; moreover, several studies have shown that carryover of analytes on the fiber between analyses is possible [43-45]. To overcome these shortcomings, novel sample preparation techniques have been quested and developed.

In the last few years, solvent microextraction involving the miniaturization of the LLE has been developed and shown to be an alternative sample preparation [49,56,57]. The general idea behind this technique is the great reduction in the volumetric ration of the sample and extracting phase resulting that high enrichment factor can be achieved. There are two main methodologies that evolved from the solvent microextraction. The first methodology is solvent microextraction based on hanging droplets or single-drop microextraction (SDME) that is a SPME related concepts. SDME involves the extraction of analytes into a microdrop of organic solvent immiscible with water, which is suspended on a teflon rod [85] or a tip of microsyringe [86]. Unlike SPME fibers, drops can be renewed for each extraction. It is an elegant method that overcomes the limitation fiber coating availability as a wide variety of solvents and trapping agents can be used. SDME is simple for analytes extraction and/or preconcentration, fast, inexpensive, effective and virtually solvent free sample pretreatment technique. However, problems with drop stability and lack of sensitivity have been reported [50,51].

In order to develop a more robust format for SDME, solvent microextraction based on the use of porous hollow fiber membrane has been developed. The membrane is served as an interface between the organic solvent (acceptor solution) and the sample (donor solution) where the analytes are extracted from the aqueous sample through the thin film of organic solvent that is impregnated in the pores into the organic solvent inside the lumen of the hollow fiber membrane. Using pores of the polymeric membrane as a support for organic solvent can overcome the instability of drop in SDME. In addition, because the pores of the hollow fiber membrane are so small, it shows some selectivity for large molecules that may be extracted with the organic solvent. So, this technique can be used to extract analytes from complex and dirty matrices such as biological fluids, mixtures of soil and water (slurry), for examples [52-57].

2.8 Liquid-phase microextraction (LPME)

The "Liquid-phase microextraction, LPME" technique is one set up of hollow fiber protected solvent microextraction which was presented by Pedersen-Bjergaard and Rasmussen in 1999 [52]. The set up of this technique was shown in Figure 2.6. Two conventional medical syringe needles are inserted through a septum and the two ends are connected with a piece of hollow fiber membrane. Before using hollow fiber

membrane, the hollow fiber is immersed for several seconds in an organic solvent (typical *n*-octanol or dihexyl ether) in order to immobilize the solvent in the pores. After impregnation, the hollow fiber is immersed in the donor solution. The acceptor solution is then injected into the lumen of the hollow fiber with the help of the microsyringe. After the extraction is completed, the acceptor solution is collected by either pushing the solution into the vial or withdrawing the solution into the microsyringe for further analysis. The hollow fibers utilized are inexpensive and disposable which eliminates all problems related to cross contamination problems known as "carry-over effect".

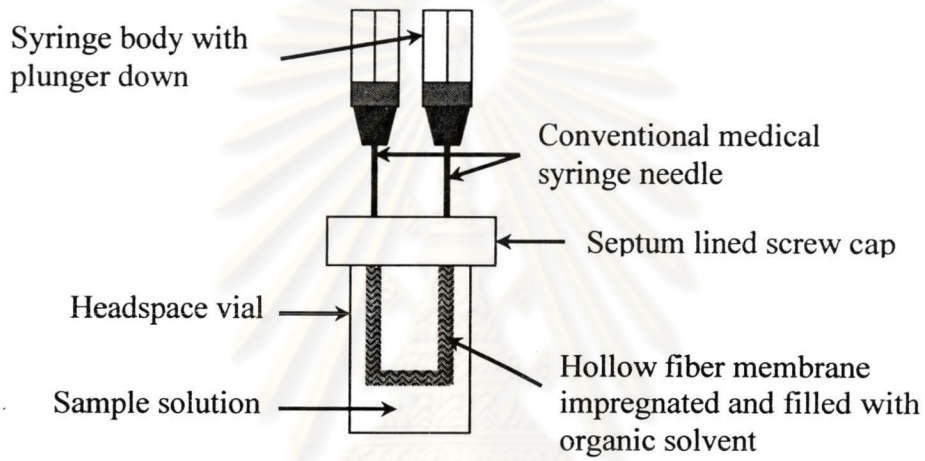


Figure 2.6 Schematic representation of the liquid-phase microextraction based on U-shaped. Adapted from Pedersen-Bjergaard and Rasmussen [52].

Since the volume ratio of the organic solvent to the sample is so small, LPME provide very high analyte enrichment factor. LPME also present excellent sample clean-up from biological, environmental, food and industrial analysis [52-57]. The Advantage of LPME is simplicity of the extraction units which is inexpensive to built and many samples may be processed in parallel providing a high sample throughput. In addition, LPME are environmental friendly because of small consumption of organic solvent (microliter).

2.9 Extraction efficiency and recovery of liquid-phase microextraction [87]

LPME can be used as directly extraction or headspace extraction. In directly extraction, the analytes are extracted from the aqueous sample solution (donor phase)

into the organic solvent (acceptor phase) present in the pores and inside the lumen of the hollow fibre. This process may be illustrated with the following equation:

$$A_{\text{sample (donor phase)}} \rightleftharpoons A_{\text{organic solvent}} \quad (2.9)$$

Where A represents the analyte of interest. At equilibrium, the partition coefficient $K_{org/d}$ is:

$$K_{org/d} = \frac{C_{eq,\,org}}{C_{eq,\,d}} \quad (2.10)$$

Where $C_{eq,org}$ is the concentration of A in the acceptor phase and $C_{eq,d}$ is the concentration of A in the donor phase at equilibrium. The initial amount of analyte, n_i is equal to the sum of the amounts of analyte present in two phases during the whole extraction process:

$$n_i = n_d + n_{org} \quad (2.11)$$

Where n_d is the amount of analyte present in the donor phase and n_{org} is the amount of analyte present in the acceptor phase. At equilibrium, Equation 2.11 can also be written as:

$$C_i V_d = C_{eq,d} V_d + C_{eq,org} V_{org} \quad (2.12)$$

Where C_i is the initial analyte concentration in the sample, V_d is the donor phase volume or the sample volume and V_{org} is the acceptor phase volume or the organic phase volume. At equilibrium, the amount of analyte extracted into the acceptor phase, $n_{eq,org}$ can be expressed as [87-90]:

$$n_{eq,\,org} = \frac{K_{org/d} V_{org} C_i V_d}{K_{org/d} V_{org} + V_d} \quad (2.13)$$

The extraction efficiency (*EE*) is defined as the percentage of the amount of analyte extracted ($n_{eq,org}$) relative to the amount of analyte originally present in the sample (n_i). At the end of the extraction *EE*:

$$EE = \frac{n_{eq,\,org}}{n_i} \cdot 100 = \frac{K_{org/d} V_{org}}{K_{org/d} V_{org} + V_d} \cdot 100 \quad (2.14)$$

The enrichment factor (*EF*) is defined as the ratio between the final analyte concentration $C_{eq,org}$ in the sample extraction (acceptor phase) and the initial concentration of analyte C_i in the sample, which can be expressed as:

$$EF = \frac{C_{eq,\,org}}{C_i} = \frac{V_d EE}{100 V_{org}} = \frac{K_{org/d} V_d}{K_{org/d} V_{org} + V_d} \quad (2.15)$$

Where $C_{eq,org}$ is the concentration of analyte (A) in the acceptor phase at the end of extraction. Equation 2.14 and 2.15 can use with LLE in two-phase extraction.

For headspace LPME mode, the amount of analytes needs to be transported through the barrier of air before absorbed by the organic solvent. This process may be illustrated with the following equation:

$$A_{\text{sample (donor phase)}} \rightleftharpoons A_{\text{gas phase}} \rightleftharpoons A_{\text{organic solvent}} \quad (2.16)$$

If we define the gas/sample partition coefficient $K_{gas/d}$ as:

$$K_{\text{gas/d}} = \frac{C_{\text{eq, gas}}}{C_{\text{eq, d}}} \quad (2.17)$$

Where $C_{eq,gas}$ and $C_{eq,d}$ are the analyte concentration in the gas phase and the analyte concentration in the sample, respectively. I define organic solvent/gas partition coefficient $K_{org/gas}$ as:

$$K_{\text{org/gas}} = \frac{C_{\text{eq, org}}}{C_{\text{eq, gas}}} \quad (2.18)$$

The overall partition coefficient for the extraction process $K_{org/d}$ may be calculated by:

$$K_{\text{org/d}} = \frac{C_{\text{eq, org}}}{C_{\text{eq, d}}} = K_{\text{gas/d}} \bullet K_{\text{org/gas}} \quad (2.19)$$

The amount of the analyte absorbed by the organic phase can be expressed as:

$$n_{\text{eq, org}} = \frac{K_{\text{org/d}} V_{\text{org}} C_{\text{i}} V_{\text{d}}}{K_{\text{org/d}} V_{\text{org}} + K_{\text{gas/d}} V_{\text{gas}} + V_{\text{d}}} \quad (2.20)$$

The extraction efficiency (EE) for headspace LPME can be expressed as:

$$EE = \frac{n_{\text{eq, org}}}{n_{\text{i}}} \bullet 100 = \frac{K_{\text{org/d}} V_{\text{org}}}{K_{\text{org/d}} V_{\text{org}} + K_{\text{gas/d}} V_{\text{gas}} + V_{\text{d}}} \bullet 100 \quad (2.21)$$

The enrichment factor (EF) can be calculated by the formula:

$$EF = \frac{C_{\text{eq, org}}}{C_{\text{i}}} = \frac{V_{\text{d}} EE}{100 V_{\text{org}}} = \frac{K_{\text{org/d}} V_{\text{d}}}{K_{\text{org/d}} V_{\text{org}} + K_{\text{gas/d}} V_{\text{gas}} + V_{\text{d}}} \quad (2.22)$$