CHAPTER 2

REVIEW OF LITERATURES

2.1 Solid phase microextraction

Sample preparation methods are typically time consuming. Employing multi-step procedure has high risk for loss of analytes and use extensive amounts of organic solvents. Most of the analysis time is consumed by sampling and sample preparation. Extensive use of organic solvents in analytical laboratories is no longer tolerated because of the associated health risks and disposal concerns. As a result, many solvent-free extraction methods have been described. These can be classified according to the nature of the extraction phase such as gas, membrane and sorbent. One solvent-free extraction approach is solid phase microextraction which is a sorbent extraction technique similar to solid phase extraction(SPE). SPME can be used in liquid or gaseous matrices and equilibrium extraction of the analyte (45).

2.1.1 General description of solid phase microextraction

Solid phase microextraction was invented and first described by Pawliszyn and co-workers in 1990 (38 : 217). SPME is an extraction technique for organic compound in aqueous samples, in which analytes are adsorbed directly from the sample onto a fused-silica fiber coated with an appropriate stationary phase. While the fiber is inserted in the sample, the analytes partition from the sample

matrix into the stationary phase until equilibrium is reached. The fiber is then inserted into the injection port of a GC or HPLC. In HPLC, solvents are used to desorb the analytes from the fiber. In the injection port of a GC where it is heated, the analytes are rapidly thermally desorbed into a capillary GC column for analysis (Figure 2.1). SPME is based on a modified syringe which contains a stainless steel microtubing within its syringe needle (Figure 2.2). This microtubing has a small fused silica fiber tip about 1 cm in diameter coated with an organic polymer (extraction phase). Each fiber can be used for 50 to 100 analyses or more, depending on the particular application and the care taken. The coated silica fiber can be moved between two positions, inside and outside the needle, with a plunger as in the case of a normal syringe. During the extraction and desorption periods, the fiber is thus exposed by setting outside of the needle. During transfer of the SPME unit to a desorption apparatus, the polymeric end of the fiber is inside the protective needle. This simple equipment requires several steps of sample preparation combined in one device. Extraction and enrichment of the analyte is completed by the coating in the position outside the syringe needle. SPME is the use of a small volume of extracting phase, usually less than 1 µl. The extracting phase can utilized a high molecular weight polymer or a solid sorbent. A small fused silica fiber is mounted in a syringe for ease of handling (26 : 169).

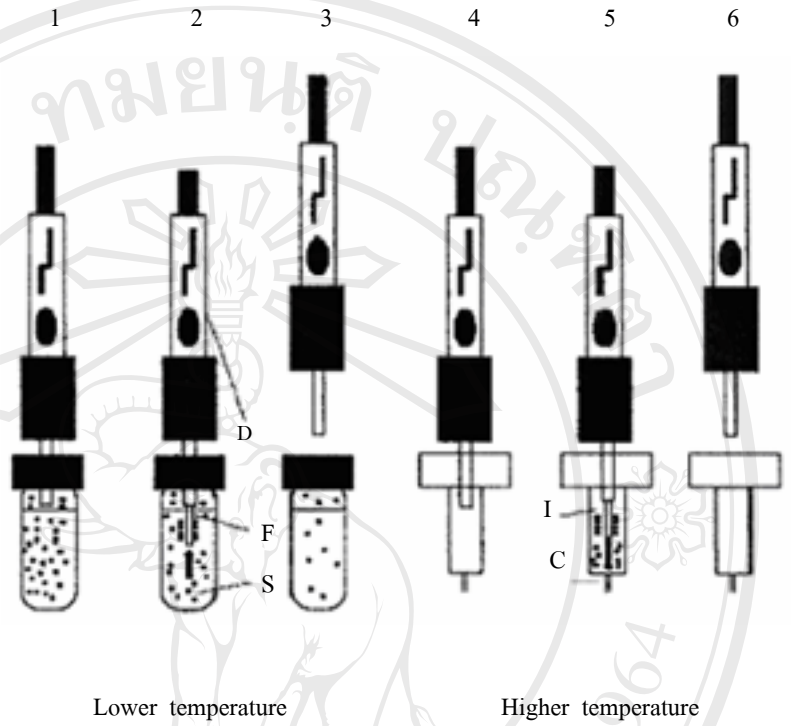

Figure 2.1 Diagram of extraction and analysis using SPME and GC :

1. Introduction of syringe needle of the SPME device (D) into the sample vial, close to the sample (S)
2. Moving the fiber(F) into the position outside the syringe and into the sample(extraction)
3. Moving the fiber back into the syringe needle and subsequent transfer the device to the GC injection port (I) and capillary head (C),
4. Penetration of the septum with syringe needle,
5. Moving the fiber into the position outside the syringe (desorption),
6. Moving the fiber back into the syringe needle, and then withdrawing the syringe needle (26 : 170).

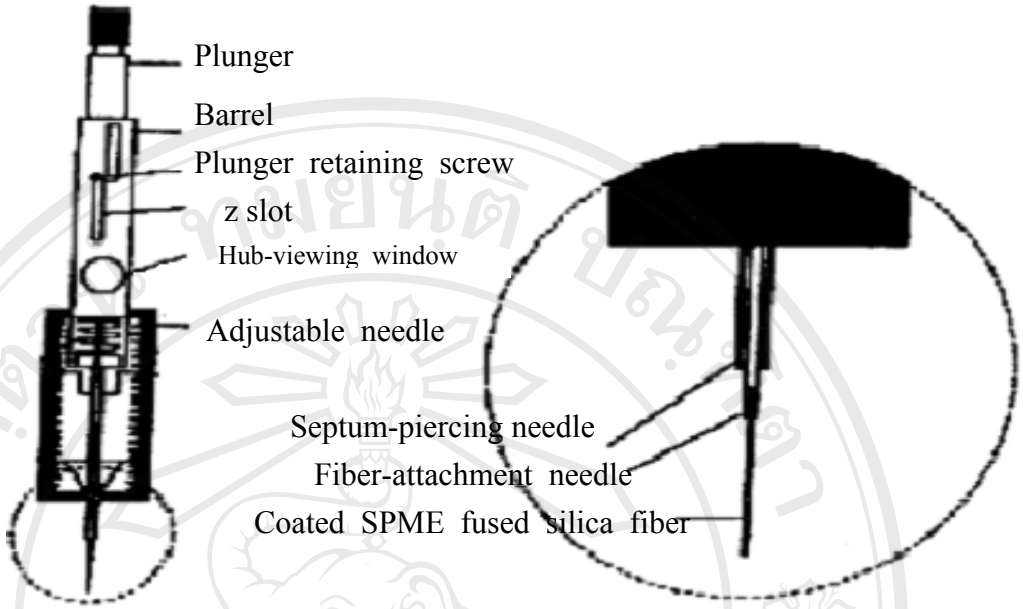

Figure 2.2 Structure of a SPME holder of Varian AutoSampler (46)

2.2 Theory of SPME

2.2.1 Thermodynamics (26 : 171)

PDMS is most often applied in SPME, and the extraction obeys the rules of liquid-liquid equilibrium:

$$\text{analyte}_w \xrightarrow{K^{fw}} \text{analyte}_{fiber}$$

$$K^{fw} = \frac{C_f}{C_w} \qquad [1]$$

Where K^{fw} is the equilibrium constant of liquid-liquid equilibrium,

c_f is the equilibrium concentration of the analyte in the coating

c_w is the equilibrium concentration of the analyte in the aqueous matrix.

Eq. [1] can also be written as:

$$K^{fw} = \frac{n_f V_w}{n_w V_f} \qquad [2]$$

and because $n_0 = n_f + n_w$ is a rearrangement it is possible to:

$$n_f = \frac{K^{fw} V_f n_0}{(K^{fw} V_f + V_w)} \qquad [3]$$

where n_f is the number of molecules in the fiber, in equilibrium,

n_w is the number of molecules in the aqueous phase in equilibrium,

n_0 is the number of molecules in the aqueous phase prior to SPME,

V_w is the volume of aqueous phase

V_f is the volume of the coating.

It is evident from Eq. [3] that the basis for a quantitative method is given because of the linear relationship between n_f and n_0. However, SPME is an equilibrium extraction but not an exhaustive extraction. A simple rearrangement of Eq. [3] gives an expression for the recovery of SPME in equilibrium, which is also the maximum recovery Eq. [4].

$$\text{Maximum recovery} = n_f / n_0 = \frac{K^{fw} V_f}{(K^{fw} V_f + V_w)} \qquad [4]$$

The values of K^{fw} are influenced by temperature, salt, pH and organic solvents. The dependence of K^{fw} on temperature is expressed by Eq. [5] where K_0^{fw} is the equilibrium constant at T_0 and ΔG^{fw} is the free enthalpy of the transfer of analyte between the two phases:

$$K^{fw} = K_0^{fw} \exp \frac{-\Delta G^{fw}}{R} \cdot \left(\frac{1}{T} - \frac{1}{T_0} \right) \qquad [5]$$

$$\Delta G^{fw} = G^f - G^w \qquad [6]$$

$$\ln \frac{K^{fw}}{K_0^{fw}} = \frac{-\Delta G^{fw}}{R} \cdot \left(\frac{1}{T} - \frac{1}{T_0} \right) \qquad [7]$$

Because of the interference of organic molecules with the intermolecular interactions of water, the free enthalpy in water (G^w) is always higher than in PDMS (G^f). Thus, according to Eq. [6] ΔG^{fw} should be negative except for, perhaps, rare cases with a high entropy term. It can be concluded that K^{fw} decreases when the temperature and, therefore, the amount of analyte extracted and the recovery of SPME should be decreased. The relationship between K^{fw} and concentration of salt (c_s) can be expressed with Eq. [8], where K_0^{fw} is K^{fw} at c_s = 0 and k_s is a specific constant. The higher concentration of the salt the more K,^{fw} and amount of analyte extracted is. However, this was not always confirmed in real samples. The relationship between K^{fw} and pH can be described in Eq. [9], if only the acid is extracted where K_0^{fw} is K^{fw} of the undissociated form. This was confirmed for short-chain fatty acids. The analyte is extracted better at low pH. Equation [10] can be used if only the basic form is extracted. The analyte can also be extracted at high pH. Finally, the presence of an organic solvent in the aqueous sample usually decreases K^{fw}.

$$\ln \frac{K^{fw}}{K_0^{fw}} = k_s c_s \qquad [8]$$

$$\log \left(\frac{K_0^{fw}}{K^{fw}} - 1 \right) = pH - pK_a \qquad [9]$$

$$\log \left(\frac{K_0^{fw}}{K^{fw}} - 1 \right) = pH - pK_a - 14 \qquad [10]$$

In HS-SPME Eq. [3] is extended to Eq. [13],

where K^{hw} is the equilibrium constant of HS and aqueous sample [Eq. 11],

K^{fh} is the equilibrium constant of fiber and HS [Eq. 12],

c_h is the equilibrium concentration of the analyte in HS,

V_h is the volume of HS:

$$K^{hw} = \frac{c_h}{c_w} \quad [11]$$

$$K^{fh} = \frac{c_f}{c_h} \quad [12]$$

$$n_f = \frac{K^{fh} K^{hw} V_f n_0}{(K^{fh} K^{hw} V_f + K^{hw} V_h + V_w} \quad [13]$$

K^{hw} and K^{fh} can be calculated with the Henry's Law constants of the analyte in water (H_w) and in the coating (H_f), respectively Eqs. [14a] and 14b]. The vapor pressures in aqueous sample (p_w) and coating (p_f) are given in Eqs. [15a] and [15b]:

$$K^{hw} = \frac{H_w}{RT} \quad [14a]$$

$$K^{fh} = \frac{RT}{H_f} \quad [14b]$$

$$P_w = H_w c_w \quad [15a]$$

$$P_f = H_f c_f \quad [15b]$$

Equation [16] and an alternative expression for the amount extracted (Eq. [17]) can be derived from Eqs. [1], [14a], [14b], [15a] and [15b], because the equation $p_w = p_f$ is valid in equilibrium. A similar rearrangement, as shown in Eqs. [3] and [4] , provides the recovery of HS-SPME (Eq. [17a]). Accordingly, the recovery of HS-SPME should be lower than that of direct SMPE (Eq. [17b]):

$$K^{fw} = \frac{H_w}{H_f} = K^{hw} K^{fh} \quad [16]$$

$$n_f = \frac{K^{fw} V_f n_0}{(K^{fw} V_f + K^{hw} V_h + V_w)} \quad [17]$$

$$\text{Maximum recovery (HS-SPME)} = \frac{n_f}{n_0}$$

$$= \frac{K^{fw}V_f}{(K^{fw}V_f + K^{hw}V_h + V_w)} \quad [17a]$$

$$\frac{\text{Maximum recovery (HS - SPME)}}{\text{Maximum recovery (direct SPME)}}$$

$$= \frac{1}{1 + \frac{K^{hw}V_h}{K^{fw}V_f + V_w}} \quad [17b]$$

2.2.2 Kinetics

The relationship of the SPME with time as shown in Fig. 3, for example, was mathematically described in a model which used several prerequisites with regard to geometry, size of sample and access of analyte molecules to the fiber (26 : 10, 38 : 173). That is, if all analyte molecules have access to the coating, i.e. the perfectly agitated model, the time to equilibrium (t_e) is given by Eq.[18] with r_0 the outer radius of the coating, r_i the inner radius of the coating and D_f the diffusion coefficient of the analyte in the coating. Taking into account the experimental error, it can be assumed that t_e is reached when 95% ($t_{95\%}$) of the maximal amount is extracted (Figure 2.3). Otherwise, the theoretical t_e is infinitely long according to the model used:

$$t_e = t_{95\%} = \frac{2(r_0 - r_i)^2}{D_f} \quad [18]$$

Not all analyte molecules have simultaneous access to the coating. This is described in a more real approach using a model with a hypothetical boundary

layer of radius δ with no agitation. Perfect agitation occurs only in the sample outside the boundary layer. The radius δ of this static layer depends on the rate of agitation. The higher the rate of agitation the lower is δ. The time to maximal extraction can be calculated with Eq. [19] where D_w is the diffusion coefficient of the analyte in water:

$$t_e = t_{95\%} = 3.\frac{\partial K^{fw}(r_0 - r_i)}{D_w} \qquad [19]$$

It is concluded that when the time of extraction is increased K^{fw} is increased also when higher fiber thickness (r_0-r_i) and lower diffusion coefficients of the analyte molecule are present in the sample (D_w), the time of extraction may be decreased by using an improved agitation method, thus by decreasing δ. In the case of perfect agitation the minimal time of extraction is reached and t_e depends on the geometry of the fiber and the analyte's diffusion coefficient in the fiber only (Eq. [18]. However, it is emphasized that equilibrium is not a prerequisite for a quantitative method. The time of extraction, t_e , is independent from the concentration of analyte in the sample. The relative number of molecules extracted at a distinct time $\left(n_f^t / n_f\right)$ is also independent from the concentration of analyte. Finally, the absolute number of molecules extracted at a distinct time $\left(n_f^t\right)$ is linearly proportional to the concentration of analyte.

In HS-SPME, Eq. [18] is also valid for the estimation of t_e if the aqueous phase and the HS are perfectly agitated. Several variables have to be taken into account for the estimation of t_e in the case of practical agitation (Eq. [20]): thickness of coating, HS and aqueous phase (L_f, L_h and L_w, respectively), revolution rate of the stir bar (N), radius of the stir bar (R), D_w and diffusion coefficients of analyte in HS (D_h), as well as K^{hw} and K^{fw}. A simple model was

applied with the assumption that only one-dimensional diffusion and R are slightly smaller than the radius of the vial.

$$t_e = t_{95\%}$$

$$= 1.8 \cdot \left(\frac{L_h}{K^{hw} \cdot (D_h + 2 \cdot 10^{-5} NR^2)} + \frac{L_w}{1.6 \cdot (D_w + 0.03NR^2)} \right) \cdot K^{fw} L_f \quad [20]$$

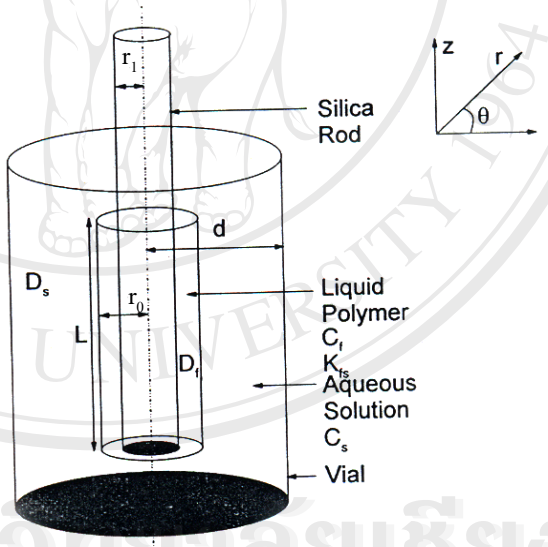

Figure 2.3 Graphic representation of the SPME / sample system configuration, with dimensions and parameters labeled;

r_0 the outer radius of the coating,

r_i the inner radius of the coating, and

D_f the diffusion coefficient of the analyte in the coating (36 : 11).

2.3 Extraction modes

Three basic types of extraction can be performed using SPME; direct immersion, agitation and headspace extraction (48,49). Figure 2.4 illustrates the difference between these modes.

2.3.1 Direct immersion solid phase microextraction

In the direct extraction mode, the coated fiber is inserted into the sample medium and the analytes are transported directly to the extraction phase. For gaseous samples, natural convection and diffusion in the medium is sufficient to facilitate rapid equilibration. Direct SPME was studied in several methods, for the assay of drugs and other analytes in plasma and urine.

2.3.2 Headspace solid phase microextraction

In the headspace mode, the analytes need to be transported through a layer of air before they can reach the coating. The outstanding advantage of HS-SPME, found in several forms of analysis, is the prevention of direct contact of the fiber with the sample and, therefore, prevention of contamination of the surface of the fiber with organic polymer. Temperature has a significant effect on the kinetics of the process, by determining the vapor pressure of analytes. The advantages of SPME can be completely and easily exploited in HS-SPME . The enrichment of analyte from the HS by SPME is unique in comparision to other HS sample preparation methods. It is considerably simpler than purge-and-trap techniques with cryofocusing of HS. It should be kept in mind that no

enrichment take place in the sampling from the HS by gas-tight syringes. On the other hand, HS-SPME is limited to special analytes because of the requirement of a high vapor pressure of the analyte. Furthermore, the transfer of fibers to the gas chromatograph and desorption should be performed immediately after extraction because of the high vapor pressure of analytes in the coating, and the risk of loss of analytes during storage of the loaded fiber also (26 : 181). An HS method should be applied whenever possible in SPME of body fluids. The burden of the fiber with proteins, for instance, is considerably decreased. A reversible change of extraction properties of the coating is also avoided and the precision of the method is improved. In addition, endogenous trace substances with molecular masses between 200 and 450 g/mol approximately are better separated from a volatile analyte in HS-SPME, too.

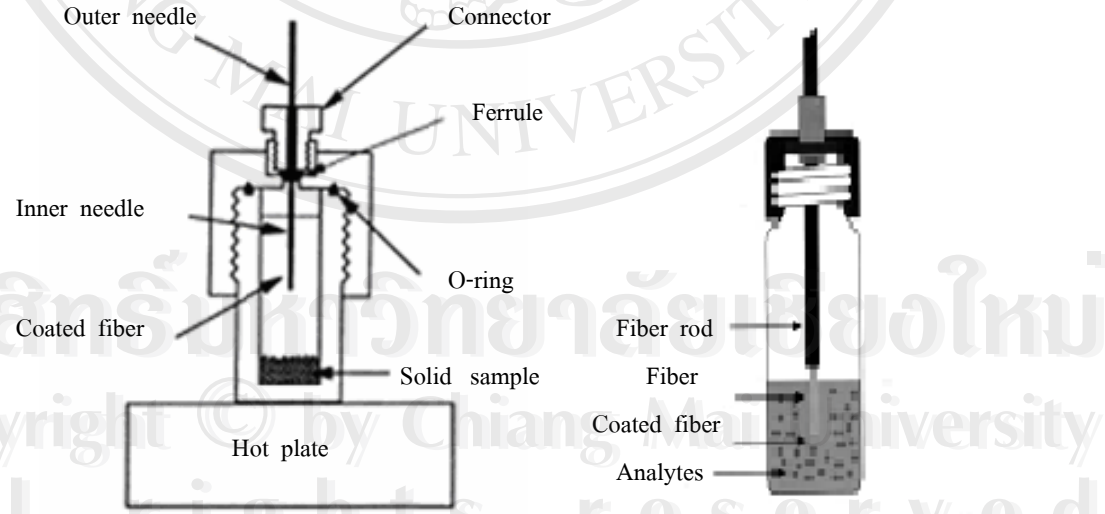

Figure 2.4 SPME operation mode (47 : 19), headspace (left), direct immersion (right)

2.3.3 Agitation

To facilitate rapid extraction, some levels of agitation are required to enhance transport to the analytes from the bulk of the solution to the vicinity of the fiber. For aqueous matrices, more efficient agitation techniques, such as fast sample flow, rapid fiber or vial movement, stirring or sonication are required. The following agitation methods can be applied; vortex mixing and flow through agitation. Magnetic stirring was mainly applied for SPME. The fiber movement agitation method, vibration of the fiber, is realized in a commercial autosampler. Sonication was expected to provide a better agitation than magnetic stirring.

2.4 Optimization of solid phase microextraction

A discussion on the theory of SPME and a survey of SPME methods were presented in the previous paragraphs. Thus, a discussion should be possible on how to perform optimization of these methods. Several variables have to be taken into account for this purpose, for example coating, temperature, agitation, pH, and addition of salt.

2.4.1 Coating

The coating should be resistant to extreme chemicals (pH, salt, additives) and physical(high temperature) conditions. Several types of coatings are commercially available now.They consist of one or two polymers e. g. plydimethyl siloxane (PDMS), polyacrylate (PA), polydimethylsiloxane / divinylbenzene

(PDMS/DVB), carboxen/polydimethylsiloxane (CAR/PDMS), andcarbowax ®/ divinylbenzene (CW/DVB). Coatings prepared with three polymers are available also e.g. DVB-Carboxen-PDMS. The simple rules should be applied for the selection of the coating in a first attempt for a new SPME method. The polarity of the coating should match the polarity of the analyte i.e., accordingly, "similar attracts similar" (Figure 2.5). The number of groups that form hydrogen bonds, e.g. NRH, NH_2, and OH, is a special property of analytes. These groups mainly determine the hydrophilicity of an analyte also the affinity to the coating. Many organic analytes investigated in samples are nonpolar molecules and they have a good affinity to the nonpolar PDMS (26 : 185). Table 2.1 summarizes the fibers that are commercially available (47 : 37).

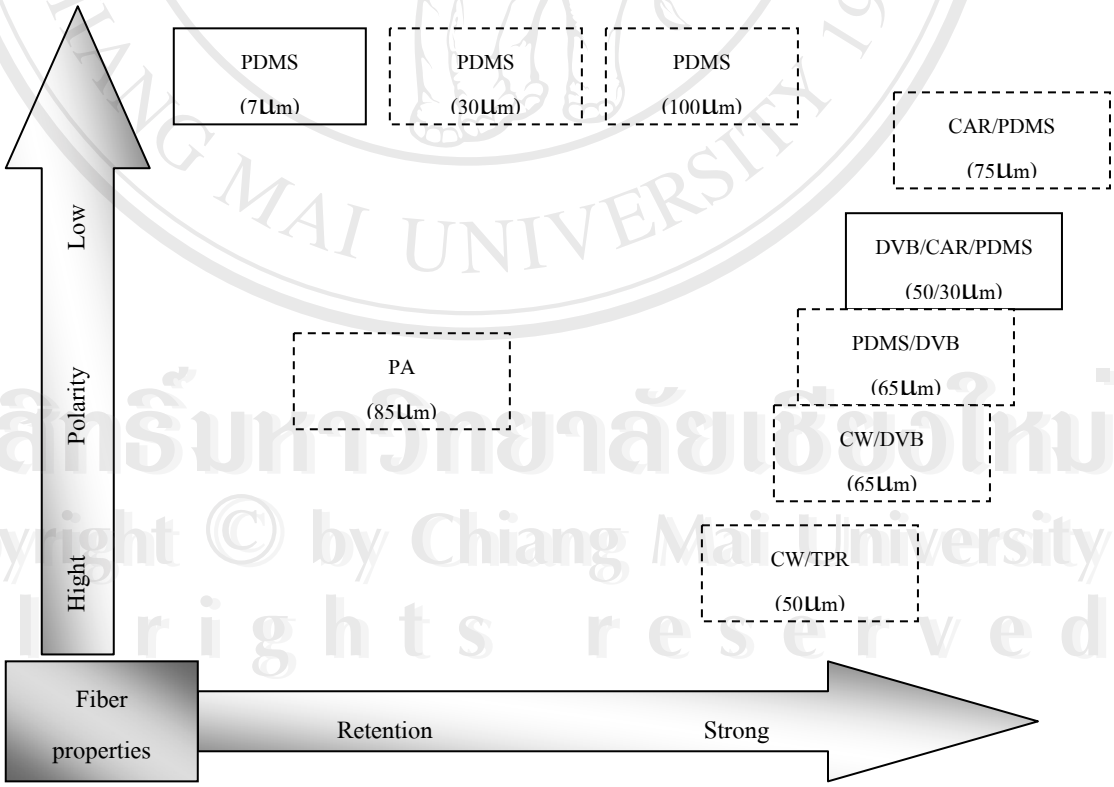

Figure 2.5 Polarity SPME operation mode (48).

Table 2.1 Commercially available SPME fibers for GC and GC/MS.

Phase	Application
Polydimethylsiloxane Three film thicknesses are available: -7 μm -30 μm -100 μm	Non-polar phase(for many semi-polar compounds: aromatics, esters, many pesticides). 100 μm used for relatively volatile compounds the thinner phases are for non-polar and polar compounds of low volatility
85-μm Polyacrylate	Polar compounds such as phenols, esters.
65-μm Carbowax / divinylbenzene	More polar than polyacrylate, for alcohols.
75-μm PDMS / divinylbenzene	Moderately polar, for amines.
65-μm Carboxen / PDMS	Highly volatile compounds including vinylchloride, sulfur gases

2.4.2 Extraction time

The extraction time can be known when the increase of peak areas is detected with increased time. The dependence of the extracted amount of analyte on the extraction time gives valuable information for SPME method development and allows the experimental determination of the equilibration time. At non equilibrium conditions, the extraction yield strongly depends on the

extraction time. Precise determinations are still possible at shorter times, if the extraction time is kept precisely constant.

2.4.3 pH

The pH of the sample is crucial for the SPME of acids and bases. Such compounds may only be extracted quantitatively by SPME, if they are present in the neutral form.

2.4.4 Salt and other additives

The effect of salt addition to enhance the extracted amount of an analyte by SPME is well known to improve extraction of organics from aqueous solutions. The effect of salt addition increases with the polarity of the compound.

2.4.5 Temperature

Extraction by SPME is an exothermic process. Thus, lowering of the temperature increases the distribution constant and the extraction yield at equilibrium.

2.4.6 Desorption

The time of desorption should be as short as possible and carryover effects must be excluded. Thus, the highest temperature obtained without causing

damage to the selected coating and the smallest diameter of the injection insert should be applied. The maxiumum temperatures are about 340ºC (7µm) and 280 ºC (30 and 100µm) for PDMS, 270 ºC for PDMS-DVB, 320 ºC for PA and Carboxen-PDMS, 265 ºC for Carbowax-DVB and 270 ºC for DVB-Carboxen-PDMS (26, 46). After the needle is introduced, the fiber should be exposed as quickly as possible because the partial desorption within the needle can result in split peaks. This applies, for instance, to volatile analytes and the temperature of desorption may need to be decreased considerably below the maximum temperature.

2.5 Advantages of solid phase microextraction

The advantages claimed for SPME were; no use of solvents, easy handling, little equipment necessary, fast method, ease of automation, as well as, good linearity, good accuracy, good precision and high sensitivity. SPME is a dirty sample preparation. The most dramatic advantages of SPME exist at the extreme of sample volumes, and because the set-up is small and convenient, coated fibers can be used to extract analytes from very small samples. SPME allows rapid sample extraction and transfer of analyte to the analytical instrument (29 : 233). SPME can be used for the assay of both , less volatile and highly volatile analytes, with high precision.

The main advantage of SPME is its simplicity and automation and is anticipated to be considerably easier than using other sample prearation methods. The SPME compares very favorabily to other sample preparation methodology (Table 2.2). The SPME unit can be used with split- splitless or on-column injectors (46 : 356).

Table 2.2 SPME provides advantages over other sample preparation techniques (46 : 356)

Technique	Detection limit (LOD)	Precision (%RSD)	Expense	Time (min)	Solvent use	Simplicity
Purge & Trap	ppb	1-30	high	30	none	no
Thermal Stripping (heated Purge & Trap)	parts per trillion (ppt)	3-20	high	120	none	no
Headspace	ppm	-	low	30	none	yes
Liq-Liq Extraction	ppt	5-50	high	60	1000mL	yes
Solid Phase Extraction	ppt	7-15	medium	30	up to 100mL	yes
SPME	ppt	<1-12	low	5	none	yes

2.6 Precision of the method

The most important factors affecting precision in SPME are listed below:

- Agitation condition
- Sampling time (if non-equilibrium conditions are used)
- Temperature
- Sample volume
- Headspace volume
- Vial shape
- Condition of the fiber coating (crack, adsorption of high MW species)
- Geometry of the fiber (thickness and length of the coating)
- Sample matrix component (salt, organic material, humidity, etc.)
- Time between extraction and analysis
- Analyte losses (adsorption on the walls, permeation through Teflon, absorption by septa)
- Geometry of the injector
- Fiber positioning during injection
- Condition of the injector (pieces of septa)
- Stability of the detector response
- Moisture in the needle

To ensure good reproducibility of the SPME measurement, the experimental parameters listed above should be kept constant (38 : 20).

2. 7 Applications of solid phase microextraction

Table 2.3 SPME method for the determination of pesticide and organic compound

Sample	Analyte	Preparation	Analytical method	Detection limit	Recovery	Reference
Biomedical	Drugs,metabolites	LLE, SPE, SPME	SPME/GC-MS, SPME/GC-NPD	1 ng/ml	-	(26)
Water	Polar organophosphorus	SPME	LC-MS	0.01-0.03 μg/ml	-	(6)
Water	Pesticide:organophos-phorus,organochlorine nitrogen containing, herbicides	SPME	CG-MS	0-100 μg/ml	-	(20)
Human fluids	Organophosphorus	SPME	GC-ECD-FPD	0.1-0.4 ng/ml	>70%	(28)
Water	Fire ant pesticides	SPME	SPME/GC-MS, SPME/HPLC-MS	10 ng/ml-1μg/ml	-	(30)
Soil samples	Organochlorine	SPME	HS-SPME-GC	0.06-0.65 ng/g	68-127%	(31)

Table 2.3 (continued)

Sample	Analyte	Preparation	Analytical method	Detection limit	Recovery	Reference
Spiked water solution	Semi-volatile compound	SPME	GC	10ng/l-µg/l	-	(40)
water	0rganochlorin	SPME	GC-ECD	5-20ng/l	-	(27)
Air and water	Chlorinated hydrocarbon	SPME	GC-ECD	1-30 ng/l	-	(25)
Environmental water	Organophosphorus	SPME	GC	0.01-0.2 ng/ml	-	(32)
Food	Food additive,flavor, contaminant,pesticids	SPME	GC-MS,LC-MS,GC-FPD	0.05-3 µg/l	-	(51)
Water	Organic contaminants	SPME	SPME-IR	0.5-1.5 mg/l	-	(49)
Water and soil	Polycyclic aromatic	HS-SPME	GC	-	-	(31)

34

Table 2.3 (continued)

Sample	Analyte	Preparation	Analytical method	Detection limit	Recovery	Reference
Chinese herbal formulations	Organochlorine	SPME	GC-MS	ng/g	0.5-17.8%	(33)
Medical plants	Organochlorine	Microwave assisted extraction (MAE),SPME	GC-ECD	<0.13ng/g	-	(34)
Water	Pesticide	Supercritical fluid extraction (SFE)	HPLC	0.1mg/l	-	(35)
Rice	Profoxydim	SPME	HPLC-UV	5 μg/ml	-	(36)
Air	Organophosphorus	SPME	GC	mg/l	-	(37)
Spiked water	Organophosphorus	SPME	HPLC	60-600 μg/l	64%	(50)

35