CHAPTER II

THEORY

2.1 Membrane Extraction Technique

Membrane extraction techniques are used to extract the analyte molecules from the sample phase (often called donor or feed solution) to the acceptor phase (or strip phase). The analyte pass through the membrane which absolutely separates the two phase of solutions. This process is sometimes called pertraction (permeation-extraction). In the literature, membrane extraction technique can be divided into two main categories, porous and non-porous membrane techniques as demonstrated in Table 2.1 (34).

Table 2.1 Classification of membrane separation techniques.

Porous Membrane Technique		Non-Porous or Micro-Porous Membrane Technique	
Filtration	Dialysis	Two-Phase system	Three-Phase system

2.1.1 Porous Membrane Technique

The porous membrane technique, which can call one-phase techniques, is the technique that a liquid (or gas) contact moves through the pores between donor and acceptor phases. Both phases are similar in chemical composition. The two examples of this technique are filtration and dialysis. The flux in the two techniques is similarly dependent on the surface area of the membrane and the sample viscosity as can be shown by Hagen-Poiseuille equation for filtration:

$$J_v = -H_p A(dP/dx) \quad \text{(Eq. 1)}$$

which J_v = volume flux

dP/dx = pressure gradient across the membrane

A = surface area of the membrane

H_p = hydrodynamic permeability defined by sample viscosity and the resistance of membrane which depends on pore size.

And by Fick's law for dialysis:

$$J_m = -D_m A(dC/dx) \quad \text{(Eq. 2)}$$

which J_m = mass flux

D_m = diffusion coefficient in sample matrix

dC/dx = concentration gradient

From Eq.1 and Eq.2, it can be noted that flux is directly proportional to the membrane surface area in one-phase technique. High membrane surface area will enhance the transfer of analyte from donor to acceptor phase. Other properties such as solvent viscosity and analyte diffusion coefficient in each solvent can also influence the extraction efficiency.

2.1.1 Non-Porous Membrane Technique

Non-Porous membrane technique involves two or three different phases separated by a polymeric material or a liquid between donor and acceptor phase. Non-porous membrane techniques can be divided into two types (34).

2.1.1.1 Two-Phase Membrane Technique

This system has the same surrounding phase as the membrane. For example, the membrane phase and the acceptor contain organic solvent but the donor is aqueous or gaseous. The system which a membrane

separates one aqueous and one organic phase has been called microporous membrane liquid liquid extraction (MMLLE). For hydrophobic membrane, the organic solvent fills the pores of membrane and contact directly to the donor phase. In another principle, the membrane could be hydrophilic and is filled by an aqueous phase instead, but the approach has not yet been tried for analytical purposes.

The MMLLE technique uses organic solvent as an acceptor and the same solvent is also impregnated in the membrane pores. The maximum extraction efficiency is directly given by the partition coefficient in the same way as in liquid liquid extraction (LLE).

$$P = [A]_{octanol}/[A]_{water} \qquad \text{(Eq. 3)}$$

which $[A]_{octanol}$ = concentration of analyte in octanol

$[A]_{water}$ = concentration of analyte in water

If the partition coefficient of the analyte is high, it is possible to enrich the analyte to organic phase. Consequently, high rate of extraction efficiency can be obtained.

The typical types of membranes used in this technique are polytetrafluoroethylene (PTFE) or polyproplylene (PP) membranes because of their hydrophobicity and commercial availability. Thus the extractant from MMLLE is generally organic that is compatible to gas chromatography (GC) and normal-phase high-performance liquid chromatography (normal-phase HPLC).

2.1.1.2 Three-Phase Membrane Technique

In this system, a separating membrane phase is surrounded by two different phases (donor and acceptor) forming a system with two phase boundaries. The most popular used three-phase liquid membrane format is called supported liquid membrane (SLM) where a porous

membrane is soaked in an organic solvent, filling the pores of the membrane, and keep the two different phases (donor and acceptor) apart. The ideal organic solvent used in the pores of the membrane has to be immiscible with water, non-volatile and can be easily immobilized in the membrane. Because of the non-volatility of the acceptor phase, three-phase extraction system always interfaces with reverse-phase HPLC, capillary electrophoresis (CE), and high-performance liquid chromatography mass spectrometry (HPLC-MS)

Table 2.2 Schematic overview of different membrane extraction techniques.

Type	Donor	Membrane	Acceptor
Dialysis	aqueous	porous	aqueous
MMLLE	aqueous	microporous with solvent impregnate or organic non-porous	organic
SLM	aqueous	microporous with solvent impregnate	aqueous

2.1.1.3 Extraction Modules of Micro-Porous Membrane Techniques

Both two and three-phase membrane extraction techniques can be performed on the same extraction modules. As shown in Table 2.2, these two techniques are different in types of acceptor phase. The technique and module used in real sample depend on properties of target analyte, volume of sample, compatibility with available instrument and other requirement such as time constraint, cost, etc. Figure 2.1 shows the development of extraction modules. Each module has specific design and performance. The first picture (a) is a two compartments module with flat sheet membrane that uses motor to stir large volume (200 mL) of donor and acceptor solutions. Figure (b),

(c), and (d) show modules for different volumes of donor and acceptor solutions such as in microliters or larger. These modules require mechanical pump to maintain the flow across the membrane. They can be made from many types of materials such as PTFE, polycarbonate, polyvinylidene difluoride (PVDF) etc.

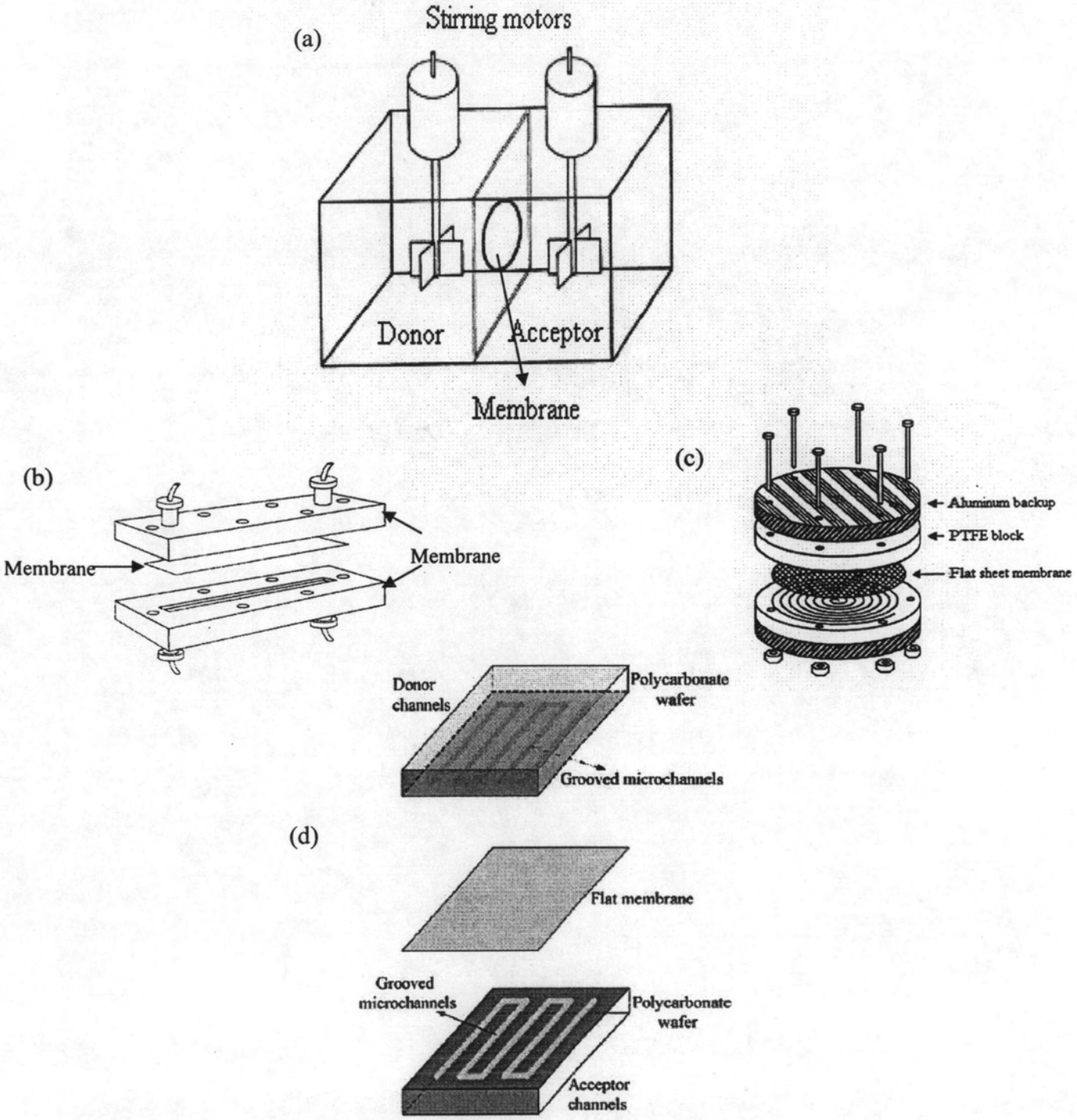


Figure 2.1 Extraction modules (19,29,35,36).

2.2 Hollow Fiber-Liquid Phase Microextraction

Hollow fiber-liquid phase microextraction (HF-LPME) was developed based on two-phase and three-phase extraction techniques. The concept is based on the use of a single, low-cost and disposable hollow fiber. This technique is easy and environmental friendly.

2.2.1 Technical Set-up and Extraction Principle

The technical set-ups of a u-shaped hollow fiber-liquid phase microextraction are demonstrated in Figure 2.2.

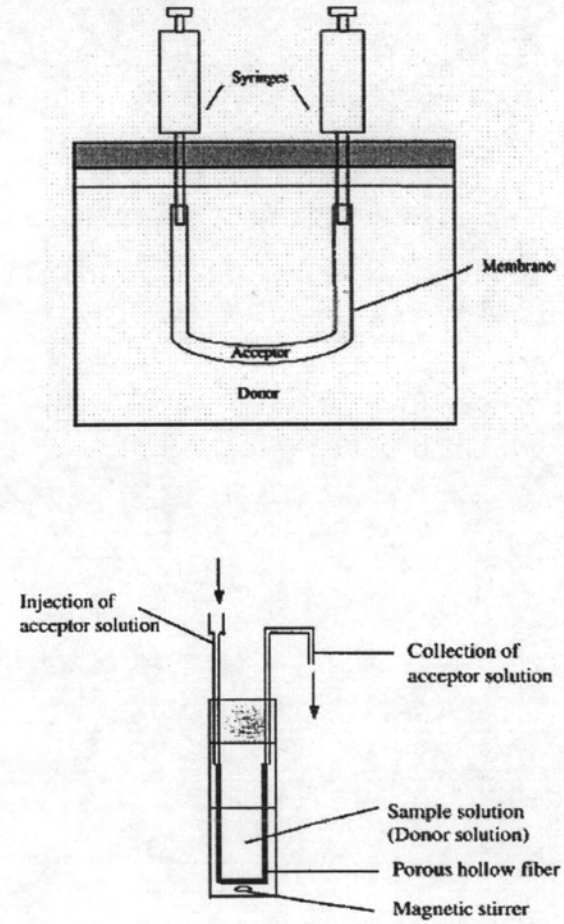


Figure 2.2 The schematic diagrams of hollow fiber-liquid phase microextraction (30,37).

The aqueous sample solution is filled into a vial or a bottle depends on the required volume. A u-shaped hollow fiber soaked with organic solvent is placed within the sample. After soaking the membrane with organic solvent, excess solvent must be completely removed from the lumen of the membrane. The acceptor volume can simply vary by adjusting the fiber length.

The analytes in the sample solution are extracted from aqueous donor phase through the membrane organic phase into an aqueous solution of the acceptor phase. This extraction method is limited to ionic, basic, or acidic analytes. For basic analytes, the donor pH should be adjusted to alkaline region for effective suppression of the analyte in uncharged form. After passing through the membrane organic phase into the low-pH acceptor phase, the analyte is fully charged again. As a result, the analyte can be extracted into the acceptor phase without back-extraction. For acidic analytes, pH of the sample solution should be low and acceptor solution should be alkaline. The schematic diagram of a SLM system for basic and acidic analytes is illustrated in Figure 2.3.

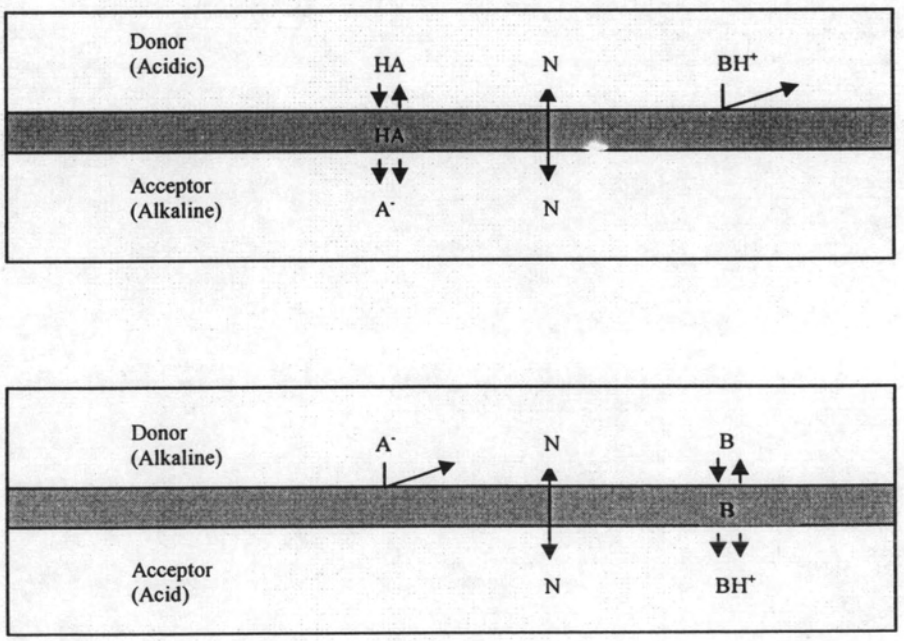
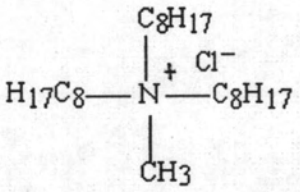


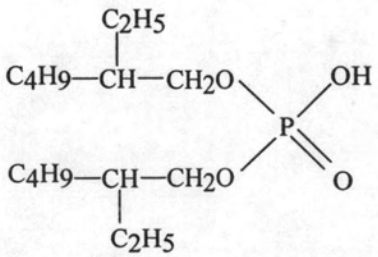
Figure 2.3 Schematic diagram of a SLM system for basic and acidic analytes: acid (HA), neutral (N) and alkaline (BH^+) species.

2.2.2 Carrier-Mediated Transport Principle

Because the transport in the three-phase membrane extraction technique is based on diffusion, the analyte of high partition coefficient in the organic solvent can be extracted in high amount leading to large enrichment factor. Due to the chemical nature of some analytes, they may diffuse poorly in common organic solvents. Examples of these compounds are hydrophilic and amphoteric compounds. Hydrophilic compounds prefer aqueous solution where they can be ionized whereas amphoteric compounds are ionizable within a specific pH range. This can result in ineffectual extraction of these compounds in organic solvent which will result in poor extraction efficiency of a SLM system. In these cases, a carrier or an ion-pairing reagent must be incorporated to enhance selectivity and mass transfer resulting in improved enrichment. Carrier-mediated transport membranes incorporated a reactive carrier in the membrane which reacts or complexes with the analyte and helps transport the analytes across the membrane.

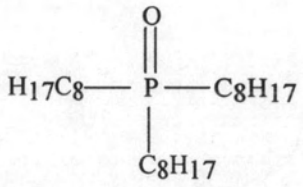


Aliquat 336 (methyltrioctylammonium chloride)



Diethylhexyl phosphoric acid (DEHPA)

Figure 2.4 Examples of common carrier.



Tri-octyl phosphine oxide (TOPO)

Figure 2.4 *(continued)* Examples of common carrier.

A common carrier is Aliquat-336 (methyltrioctylammonium chloride) which is a quaternary ammonium salt that has permanently positively charges anionic ion-pairs with chloride. Aliquat 336 forms an organic-soluble complex with anionic analyte. On the donor side of the membrane, ammonium molecule pick up an analyte ion, liberating a chloride ion to the donor solution. The analyte-ammonium complex diffuses to the acceptor-membrane interface, where the reaction is reversed because of the higher concentration of chloride counter ions in the acceptor. The analyte ion is liberated to the acceptor solution, and the chloride ion is picked up. The re-formed ammonium molecules diffuse back to the donor-membrane interface where it can bind with a new analyte molecule. Extraction mechanism is shown in Figure 2.4.

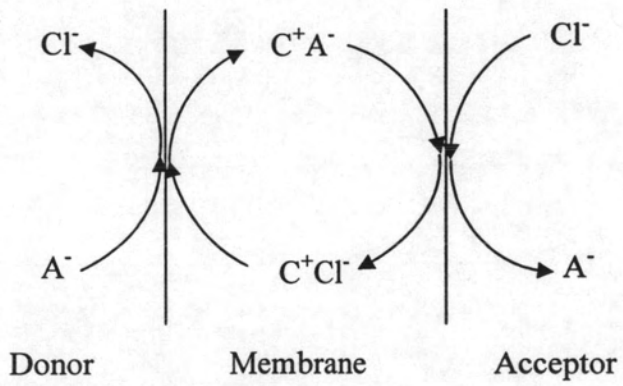


Figure 2.5 The extraction procedure of Aliquat-336 ion paring reagent (28).
(A^- = Anion analyte, C = Carrier)

Aliquat 336 often used in metals extraction (copper, cadmium, cobalt, zinc) because it can form negatively charged metal-thiocyanate complex by the

addition of thiocyanate. Amino acid can be efficiently extracted with Aliquat 336 as well (38).

Diethylhexyl phosphoric acid (DEHPA) can be used for the extraction of cationic metals too; the extraction procedure is shown in Figure 2.5.

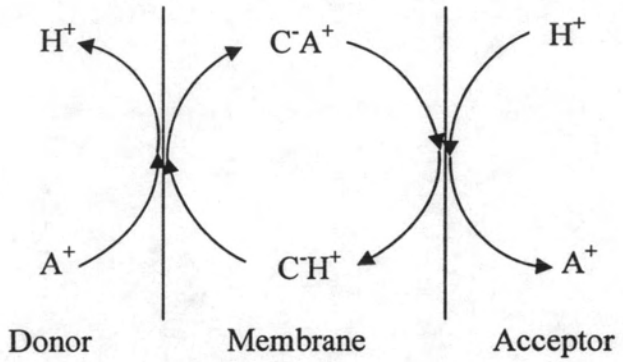


Figure 2.6 The extraction mechanism of DEHPA as ion paring reagent (39).
(A^+ = Cation analyte, C = Carrier)

DEHPA is deprotonated when the solution pH is greater than its pKa. At this pH, it can ion-pairing with cationic metals. The complexes move into the membrane interface where exchanges with hydrogen ions occur and releasing the metal ions.

There are other examples of carrier-mediated transport. For example, an ion-pair reagent tri-octyl phosphine oxide (TOPO) can be added to the membrane to improve the extraction of polar compounds. An aliphatic amine (hexyl amine) can be added to acidic donor solution permitted the formation of an ion pair which can readily be extracted. The examples discussed here demonstrated a carrier-mediated transport that improves the extraction efficiency, selectivity, and enrichment of target analyte.

2.3 Glyphosate

2.3.1 Basic Information

Common name:

- Glyphosate

Chemical name:

- N-(phosphonomethyl) glycine

Structure:

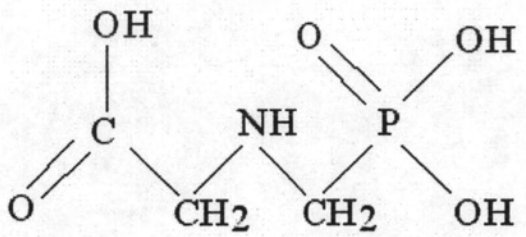


Figure 2.7 Chemical structure of glyphosate.

Pesticide classification:

- Herbicide

Trade names:

- Roundup, Rodeo, Accord, Honcho, Pondmaster, Protocal, Rascal, Expedite, Ranger, Bronco, Campain, Landmaster.

2.3.2 Herbicide Details

Target plants:

- Glyphosate is used to kill or suppress many grasses, deep rooted perennial weeds, brushes, shrubs, vines and some conifers.

Mode of action:

- Glyphosate is absorbed by leaf and kills plants by inhibiting the activity of the enzyme 5-enolpyruvylshikimic acid-3-phosphate synthase (EPSP), which is necessary for the formation of the amino

acids tyrosine, tryptophan, and phenylalanine. These amino acids are important in proteins synthesis of plants.

Method of application:

- Aerial spraying, spraying from a truck, backpack or hand-held sprayer.

Dissipation mechanism:

- Glyphosate is degraded primarily by microbial metabolism but it is unaffected by sunlight. The mainly breakdown product of glyphosate is aminomethylphosphonic acid (AMPA) as shown in Figure 2.7. Glyphosate is water-soluble, non-volatile and has an extremely high ability to bind to soil particles.

Behavior in the environment:

- Glyphosate binds strongly to soil particles. It is unlikely to enter water through surface or subsurface runoff except when the soil itself is washed away by runoff. Most glyphosate found in waters likely results from runoff from vegetation surfaces and spray drift.

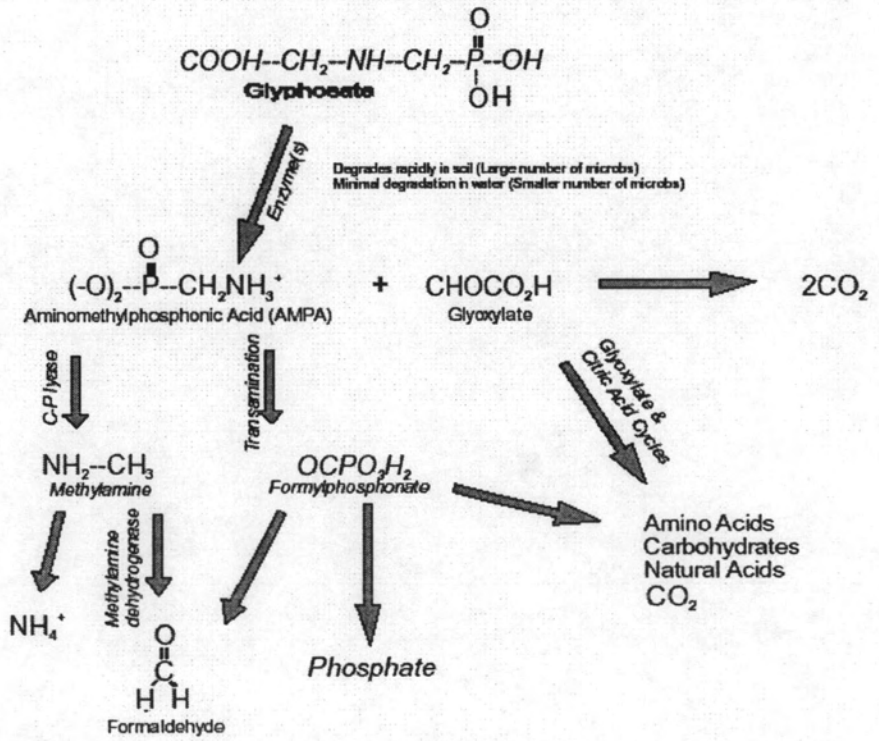


Figure 2.8 Glyphosate degradation pathways (3).

2.3.3 Physical and Chemical Properties

Molecular weight:	169.08
Water solubility:	11,600 ppm (at 25 °C)
Soil adsorption coefficient (K_a):	61 g/m^3
Octanol-water coefficient (log K_{ow}):	-3.5
Melting point:	189.5 °C
pK_a:	0.8, 2.3, 6.0, 11

2.3.4 Use of Glyphosate in Thailand

Glyphosate is the most imported pesticide in Thailand based on the information on imported agro-chemical list. The Table 2.3 shows imported quantities of herbicide glyphosate from 1998 to 2003.

Table 2.3 Thailand glyphosate imported quantities during 1998-2003 (1).

Year	Imported quantities (kg)
1998	7,969,446
1999	6,808,479
2000	15,775,829
2001	16,501,077
2002	18,661,994
2003	24,812,105

2.4 Enrichment Factor and Parameters Affecting HF-LPME Efficiency

Extraction efficiency (EE) and enrichment factor (EF) equations are commonly used to assess the effectiveness of the extraction process. The equations were earlier proposed by Kou (30) as followed:

$$EE = \frac{n_A}{n_D} \qquad \text{(Eq. 4)}$$

where n_A and n_D are the number of moles of analyte in the donor and the acceptor solutions.

$$EF = \frac{C_A}{C_D} = \frac{n_A V_D}{n_D V_A} \qquad \text{(Eq. 5)}$$

where C_A and C_D are the concentrations of analyte in the acceptor solution and the donor solution, respectively. V_A and V_D are the volume of the acceptor solution and the donor solution, respectively.

Both equations can be rearranged and EF can be expressed as the ratio of the concentration in the donor solution to the analyte concentration in the acceptor solution. EE and EF are now related:

$$EF = EE \frac{V_D}{V_A} \qquad \text{(Eq. 6)}$$

According to equation (6), EF is proportional to EE and volume ratio of the donor solution to the acceptor solution. This volume ratio is called phase ratio and govern the extraction process. Because lower detection limit can be achieved at increasing EF, this research will focus on optimizing extraction parameters to achieve the optimum EF.

HF-LPME technique consists of four major influencing parts: the donor solution, the membrane solution, the acceptor solution, and extraction time.

2.4.1 The Donor Solution

2.4.1.1 pH and Type of Donor Solution

As described in section 2.2.1 acidic or basic analyte can be extracted by adjusting the pH of the donor solution to an appropriate value that keeps the anlyte in uncharged form. Glyphosate and AMPA are amphoteric, therefore they can be either acidic or basic depend on the bulk solution pH. The pK_a values of glyphosate (pK_a = 0.8, 2.3, 6.0, 11.0) suggest that it will deprotonate and bears three negative charges (no positive charge) in alkaline solution (pH > 9.0). It was reported that Aliquat 336 is an effective carrier for an extraction of glyphosate (19,28,29). Aliquat 336 is a quaternary ammonium salt and permanently bears positive ion. Therefore, it is predictable that good EF can be obtained when extract glyphosate in alkaline solution. A range of pH was varied to evaluate the optimum extraction condition for glyphosate and its metabolite AMPA.

2.4.1.2 Donor Solution Volume

Equation (6) expressed the relationship between the donor volume and EF. When the phase ratio of the donor solution and acceptor solution were high, it is expected that EF will increase. The volume of donor solution depends on sample type and available quantity. For sample of great abundance such as river water sample, volume as high as hundreds milliliters can be used. The technique can be adjusted to a few milliliter of donor volume for limited quantity sample such as human plasma.

2.4.1.3 Agitation

HF-LPME technique requires a mass transfer system between the aqueous phases (both the donor and acceptor solution) and membrane phase. As the mass transfer at donor-membrane interface reaches

equilibrium, an increasing analytes mole in acceptor-membrane interface occurs. Simultaneously, the mole of analyte at donor-membrane interface is depleting. An extraction system is required to refresh the interface boundary with fresh bulk solution so that the next equilibrium can take place continuously until optimum EF is reached. Agitation can facilitate the diffusion of the analyte at HFM-aqueous interface and supports fast equilibrium. This experiment avoids interference from magnetic stirrer by using vortex.

2.4.2 The Membrane Solution

2.4.2.1 Aliquat 336 Concentration

Aliquat 336 is an effective ligand for glyphosate and AMPA. Because the analytes complex with the carrier, number of carrier moles are directly proportional to EF. As described in section 2.2.2, when the carrier pairs with anionic analyte and becomes complex and diffuses through the membrane under a concentration gradient and unpairs from the analyte at the acceptor-membrane interface. The carrier is recycled throughout the extraction and its optimum concentration should be observed.

2.4.2.2 Immersion Time of Hollow Fiber in Organic Solvent

Hollow fiber must be fully soaked with membrane solution prior to the extraction. This process was optimized in order to find the time that the membrane solvent completely filled into the membrane pores. The hydrophobic polypropylene membrane is easily filled with organic solvent by capillary force. However, sufficient time must be allowed for the pores to be completely filled to obtain full carrier dissipation in the membrane that maximizes the EF.

2.4.3 The Acceptor Solution

2.4.3.1 Type of the Acceptor Solution

Because chloride ion is the driving force in the carrier-mediated mechanism of Aliquat 336, salts such as sodium chloride, ammonium chloride and potassium chloride were evaluated as acceptor solution. Only monovalent cation salts are selected to prevent irreversible damage of cation-exchange glyphosate separation column. Hydrochloric acid was also selected to evaluate the acceptor pH effect. Formic acid was tested for chloride counter ion efficiency.

2.4.3.2 The Acceptor Solution Concentration

The concentration of chloride counter ion in acceptor concentration is very important. Because there are two major equilibria going on during extraction, the analytes and the counter ion equilibria, chloride ion and the analytes in the acceptor solution can compete with each other causing back extracting into the donor solution and thus lowering the EF.

2.4.3.3 The Acceptor Solution Volume

Because increasing EF can be obtained by increasing the phase ratio, the acceptor volume should be adjusted to obtain the maximum EF. The acceptor solution volume in microliters-scale was varied by using different hollow fiber lengths.

2.4.4 Extraction Time

Because HF-LPME technique is not an exhaustive extraction and its maximum efficiency depends mainly on system equilibrium, sufficient time must be allowed for the equilibrium to attain. Therefore, the extraction time must be

optimized. When equilibrium is reached, EF levels off due to diminish mass transfer gradient between the two interfaces. The optimized extraction time does not have to be exactly at equilibrium point but should be selected for timeliness, reproducibility, and good EF.

2.5 Ion-Exchange Chromatography

Ion-exchange chromatography is one of the most useful techniques of liquid chromatography. It has been used since the 1970s for the separation of both organic and inorganic substances. At that time, detection was normally performed with conductivity measurement, but nowadays, other detectors are also available. The separation procedure of ion-exchange chromatography can occur to both ionized or ionizable compounds. The chemical equilibria and ion-exchange packing play dominant roles in the distribution process of the compounds.

2.5.1 Ion-Exchange Equilibria

Ion-exchange processes are based on competitions between solute ions present in the mobile phase and the counter ions to pair with the oppositely charged fixed functional group on the solid matrix. The matrix bears fixed negatively or positively charged functional acidic or basic groups. The solute ions present in the mobile phase have to displace one or more of the counter ions that are paired with the fixed functional groups, in order to adhere to the stationary phase. If the solid matrix bears negatively charged functional groups, such as sulfonate ($-SO_3^-$), then it is described as a cation exchanger; if it bears a basic group, such as a quaternary amine ($-N(CH_3)_3^+$), then it is called an anion exchanger.

If we used cation exchanger or a sulfonic acid group, we can describe the ion-exchange equilibria by:

$$xRSO_3^-H^+ + M^{x+} \longleftrightarrow (RSO_3^-)_xM^{x+} + xH^+ \qquad \text{(Eq. 7)}$$

where $RSO_3^-H^+$ represents one of many sulfonic acid groups attached to a stationary phase and M^{x+} represents a cation. At the same time, basic exchange equilibria can be expressed as:

$$xRN(CH_3)_3^+OH^- + A^{x-} \longleftrightarrow [RH(CH_3)_3^+]_xA^{x-} + xOH^- \quad \text{(Eq. 8)}$$

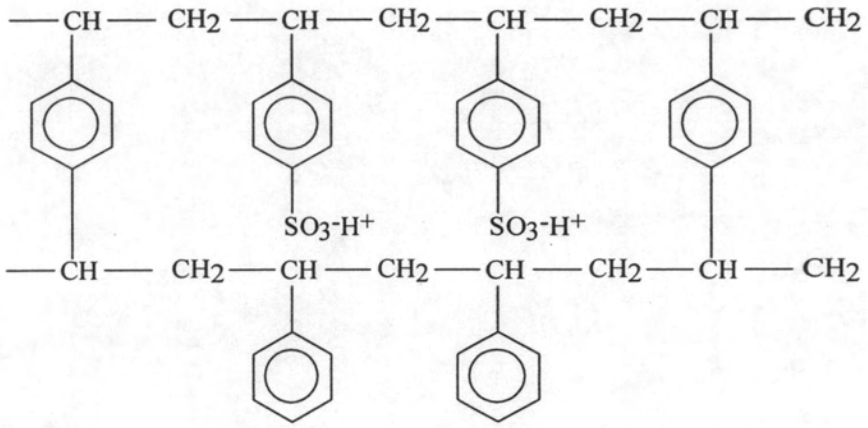
The equilibrium constant K_{ex} for the reaction shown in equation (7) takes the forms:

$$K_{ex} = [RSO_3^-M^{x+}]_{solid}[H^+]_{aq.}/[RSO_3^-H^+]_{solid}[M^{x+}]_{aq.} \quad \text{(Eq. 9)}$$

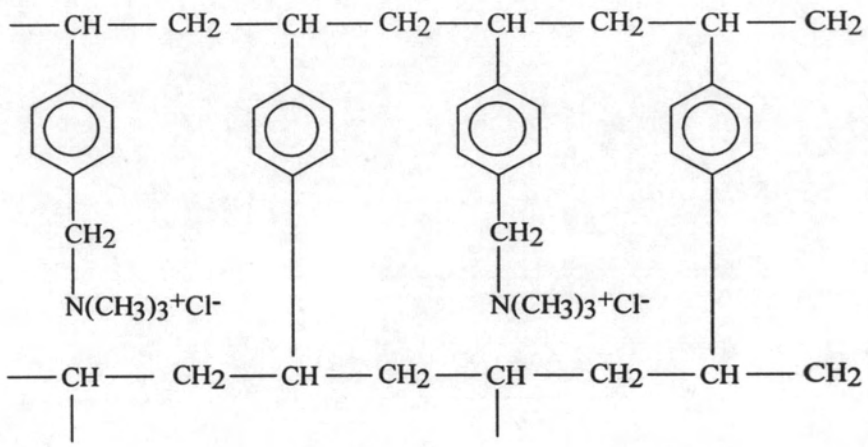
The K_{ex} in equation (9) represents the affinity of the resin for the ion M^{x+} relative to H^+ ion. If K_{ex} is large, a strong tendency exists for the solid phase to retain M^{x+}; but when K_{ex} is small, the reverse acquires.

2.5.2 Ion-exchange Packing

Ion-exchange resins are regularly synthesized from divinylbenzene or styrene by emulsion copolymerization. The product presents divinylbenzene in cross-linking small stable porous beads. Then, the basic or acidic functional groups such as sulfonic acid and quaternary amine are bonded chemically to the structure of divinylbenzene. These functional groups can be replaced by other groups (carboxylic groups, primary amine groups).



(a)



(b)

Figure 2.9 Ion-exchange packing chemistry of (a) sulfonic acid cation-exchange resin and (b) quaternary amine anion-exchange resin.

2.6 Post Column Derivatization

After separation by HPLC, the analytes must be detected for qualitative or quantitative determination. But many analytes are difficult to detect because they lack chromophore and/or fluorophore. Post column derivatization is a technique that attached fluoresce functional group to the analyte. When the analytes can be easily detected, the sensitivity of the detection improves. Most post column reagents are selective for class of substances, making detection easy against the background. Therefore, post-column derivatization is commonly used to increase sensitivity and selectivity in HPLC analysis.

2.6.1 Post Column Derivatization Procedure and Hardware

After separation by HPLC column, the analytes streams are mixed with a stream of post column solution. The mixture is flown through a heated reactor and is allowed the chemical reactions to complete. In some cases, another reactor is required for completing the reaction at ambient temperature. Eventually, the mixed streams pass into the detector, where UV/vis absorbance or fluorescence is measured. The hardware system is shown in Figure 2.10.

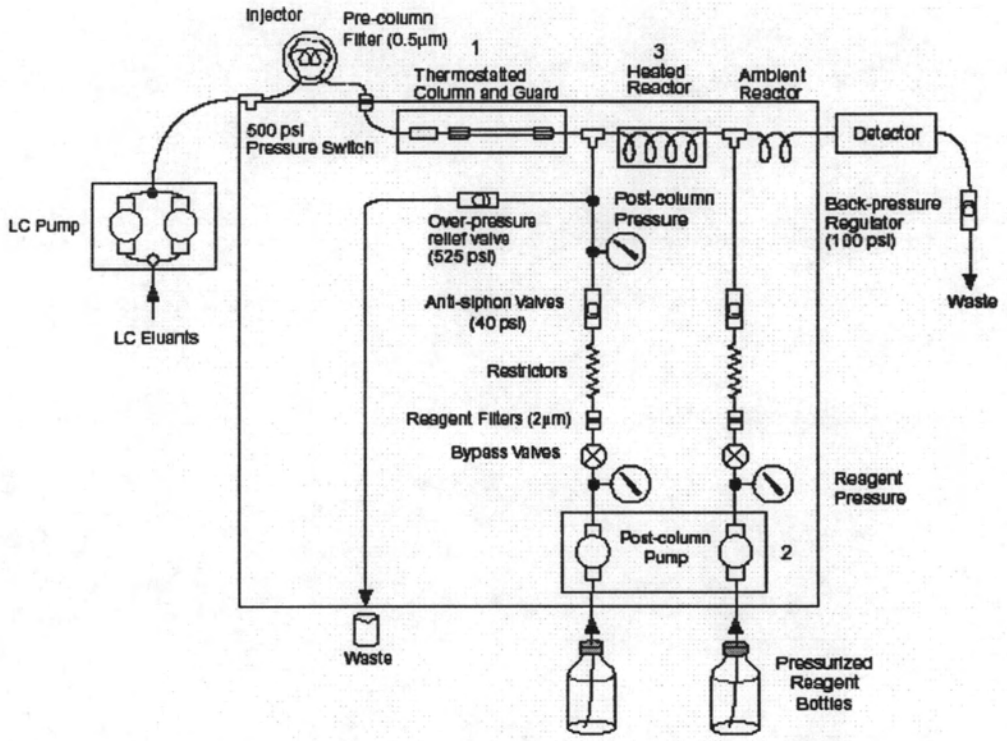


Figure 2.10 Pickering-post column derivatization system (14).

The Pickering-post column derivatization system uses a single-piston reagent pump to deliver the reagent. Pulses are eliminated by the combination of a gauge followed by a packed-bed restrictor. The mixing device consists of a mixing tee with a 0.010 inch bore. The use of pressurized reagent reservoirs allow the pump to operate more precisely at low flow rates and also provides an inert atmosphere to protect air-sensitive reagents. An over-pressure relief

valve manages the excessive pressure and diverts flow away from the reactor. Antisiphon valves prevent back-flow of caustic solution onto the analytical column by siphoning when the pump is off.

2.6.2 Glyphosate Post Column Derivatization

Glyphosate and AMPA can be separated on a strong cation-exchange column (fully sulfonated cross-linked polystyrene, K^+ form). The chromatogram followed a two-stage post column reaction is showed in Figure 2.11.

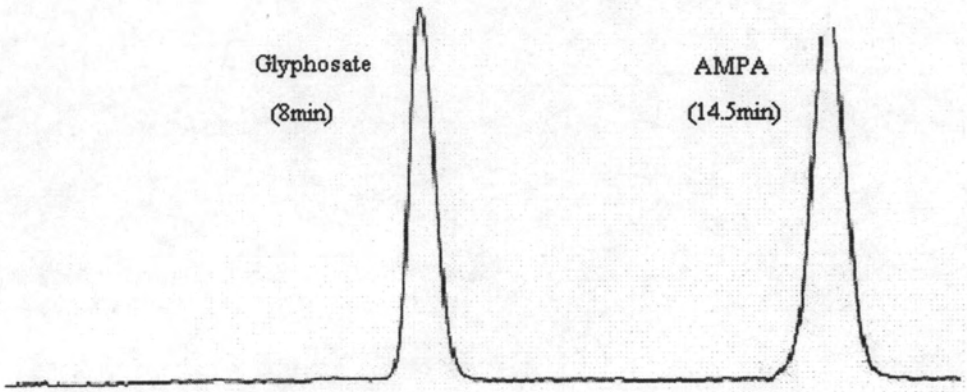


Figure 2.11 The chromatogram (FLD) of glyphosate and AMPA (14).

The detection is usually done by fluorescence detector (FLD) and the reactions can be described as follows. First, glyphosate is oxidized by hypochlorite to glycine. (Figure 2.12)

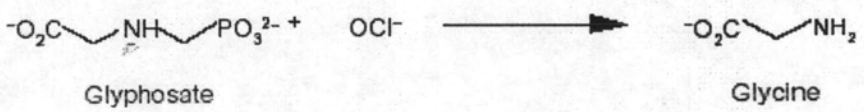


Figure 2.12 The oxidation reaction of glyphosate (14).

Second, glycine reacts with *o*-pthalaldehyde (OPA) and thiofluor at pH 9-10 to produce a highly fluorescent isoindole.

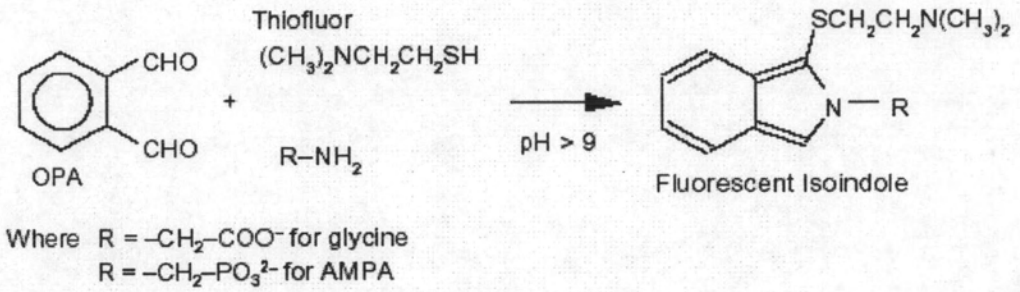


Figure 2.13 The Reactions of OPA and thiofluor with analytes (14).

AMPA does not need the oxidation reaction with hypochlorite because it is natively a primary amine compound and therefore can fluoresce naturally.