CHAPTER II

THEORY

2.1 Liquid phase microextraction

Liquid phase microextraction (LPME) has been developed as a sample preparation technique for complex samples to overcome many drawbacks of conventional liquid-liquid extraction (LLE). LPME is a miniaturization of LLE, which uses less organic solvent for preconcentration of trace analytes. The advantages of LPME are rapid, simple, inexpensive, environmental friendly and easy to set for automation [4].

2.1.1 General principle

Liquid phase microextraction shares the same principle with liquid-liquid extraction but LPME is non-exhaustive extraction. There are immiscible phases; sample matrix as aqueous phase and organic solvent as organic phase. The analyte transfers via partition of the solute between immiscible phases based on equilibrium phenomenon as shown below and its property such as hydrophobicity called "Like dissolves like".

$$A_{aq} \rightleftharpoons A_{org} \qquad \text{Equation 2.1}$$

Where A_{aq} is the analyte in aqueous phase and A_{org} is the analyte in organic phase. The distribution constant (K_d) is the ratio of activities of analyte in two phases, which can be written as

$$K_d = \frac{(a_A)_{org}}{(a_A)_{aq}} \approx \frac{[A]_{org}}{[A]_{aq}} \qquad \text{Equation 2.2}$$

Where $(a_A)_{org}$ and $(a_A)_{aq}$ are the activities of the analyte in each phase while $[A]_{org}$ and $[A]_{aq}$ are the molar concentrations of the analyte in each phase.

Because LPME uses less organic solvent, the analyte is preconcentrated after extraction. The parameter that indicates its preconcentration ability is enrichment factor (EF), which can be written as

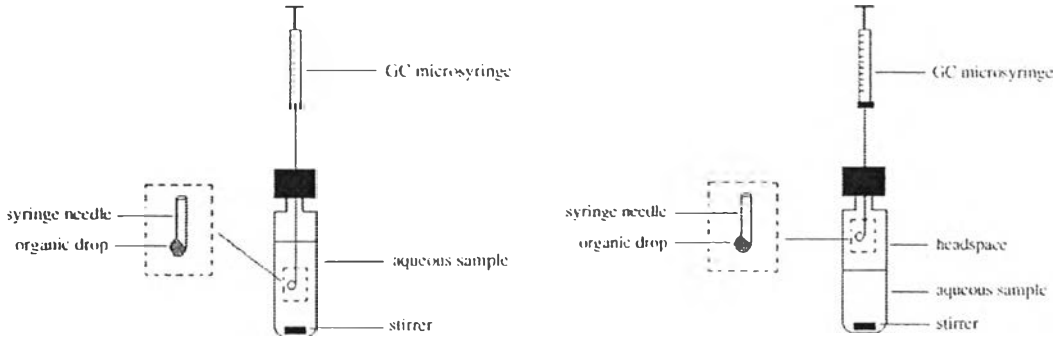
$$EF = \frac{C_{org}}{C_i} = \frac{K_d}{K_d V_{org}/V_{aq}+1} \qquad \text{Equation 2.3}$$

Where K_d is distribution constant; C_{org} and C_i are the concentrations of the analyte in each phase; and V_{org} and V_{aq} are the volumes of the organic phase and the aqueous phase, respectively. LPME is available in many formats, which can be described in the next paragraphs.

2.1.2 Single drop microextraction (SDME)

SDME is one of the forms of LPME, where the organic solvent is in a microliter drop suspended at the tip of a microsyringe. SDME is devided into two main modes; direct immersion (DI)-SDME and headspace (HS)-SDME. Direct immersion SDME uses a droplet of a water-immiscible solvent suspended at the tip of the syringe needle that is immersed in a stirred aqueous sample as shown in Figure 2.1 a). After extraction, the organic solvent is retracted into the microsyringe and then it is directly injected into the analytical instrument. The properties of analytes that are suitable for (DI)-SDME are medium polarity and non-polar. Besides, the organic solvents should be immiscible with water. Headspace SDME is similar to (DI)-SDME but the organic solvent drop is suspended in the headspace of the sample solution, which is thermostated at a given temperature for a pre-set extraction time as shown in Figure 2.1 b). This technique is suitable for volatile and semi-volatile analytes. From the procedure, the analytes are distributed among three phases (water sample, headspace, organic drop). The advantages of this technique are the flexibility of choosing solvent, where water can be used as a solvent for volatile and water-soluble analyte, providing a variety of extractable analytes. In addition, HS-SDME offers relatively clean extraction for complicated samples. However, there are some limitations. There are small variety of solvents can be used because the vapor pressure of the solvent should be low enough to avoid evaporation during sampling

and it should be compatible with GC analysis. Furthermore, the drop size should not be large avoiding falling from the needle. Thus, many techniques are developed to overcome these problems including hollow fiber membrane liquid phase microextraction (HFM-LPME) [4, 32].



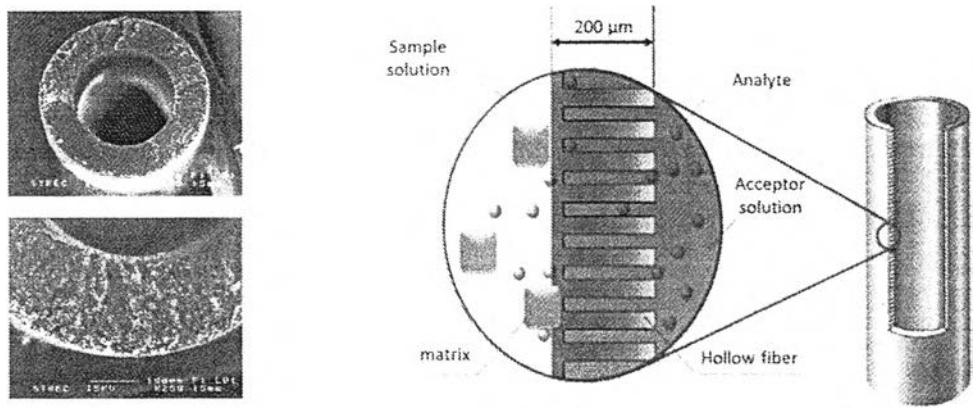
a) b)

Figure 2.1 Scheme of single drop microextraction a) direct immersion SDME and b) headspace SDME [4]

2.1.3 Hollow fiber membrane-liquid phase microextraction (HFM-LPME)

Hollow fiber membrane is a porous membrane whose geometry likes tube, containing many pores in the wall. They are made of different types of polymer materials. Materials can be classified based on their hydrophobic or hydrophilic nature. For hydrophobic membranes such as polytetrafluoroethylene (PTFE), polypropylene (PP) and polyvinylidenedifluoride (PVDF) membranes are applied for supported liquid membrane field. While hydrophilic membranes such as polysulfone (PS) and polycarbonate are applied for dialysis field. These membranes are chemically resistant in wide range of pH and thermally stable [33, 34].

In this study, polypropylene hollow fiber membrane was used. Typical dimension of the membrane are 600 μm internal diameter; 200 μm wall thickness; and 0.2 μm pore size as shown in Figure 2.2. These pores in the wall provide high contact area between the sample solution and the organic solvent that is supported in the wall of the membrane resulting in high extraction efficiency.



a) b)

Figure 2.2 Hollow fiber membrane in a) cross section and b) transportation of analyte. [35]

Hollow-fiber membrane liquid phase microextraction (HF-LPME) has been developed for overcoming the instable forms of liquid phase microextraction. A piece of hollow fiber membrane is soaked in an organic solvent to fill the pores with the organic solvent. The lumen contains an acceptor solution approximately 2-30 µL. Then, the fiber is held at the tip of syringe needle and the end of it is sealed. After that, the fiber is immersed in the sample solution. Finally, after extraction, the acceptor solution is drawn by a syringe and is injected into the analytical instrument. For mass transfer, the analyte in the aqueous sample solution (donor phase) is transferred through the immobilized organic solvent in the pores at the wall of the membrane so called supported liquid membrane (SLM) into the acceptor solution in the lumen of the membrane.

The criterion of choosing organic solvent is immiscible with aqueous, strongly immobilized in the pores of the fiber wall to prevent leakage as well as appropriate extraction selectivity [36].

As seen in Figure 2.3 HFM-LPME can be devided into two modes. First is two-phase HFM-LPME, where the analyte is extracted from the aqueous sample (donor solution) through the organic solvent (SLM) into the same organic solvent (acceptor

solution) in the lumen of the membrane. The other is three-phase HFM-LPME, where the analyte is extracted from the aqueous sample (donor solution) through the organic solvent (SLM) into another aqueous solution (acceptor solution) in the lumen of the membrane.

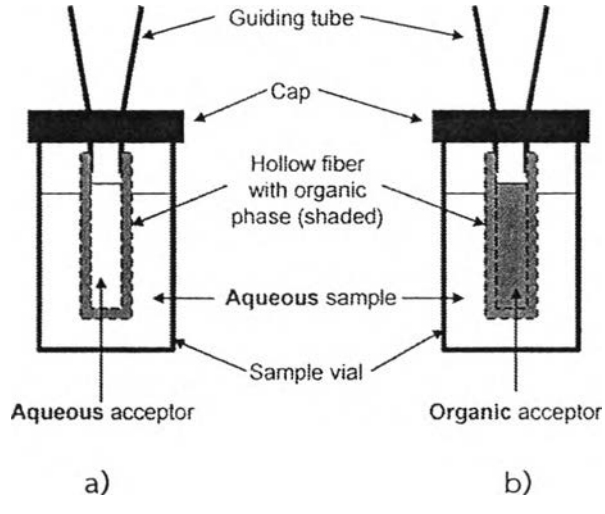


Figure 2.3 Scheme of HF-LPME; a) three-phase HFM-LPME; and b) two-phase HFM-LPME [37]

For two-phase HFM-LPME, the analyte moves between two phases; aqueous phase and organic phase, based on passive diffusion and partition coefficient ($K_{a/d}$), which is written below.

$$A_{donor} \rightleftharpoons A_{acceptor} \qquad \text{Equation 2.4}$$

$$K_{a/d} = \frac{C_{eq,\,a}}{C_{eq,\,d}} \qquad \text{Equation 2.5}$$

where A is the target analytes; $K_{a/d}$ is partition coefficient between the phase which is the ratio of the target analyte in two phases; $C_{eq,\,a}$ and $C_{eq,\,d}$ is the concentration of analyte at equilibrium in the acceptor and the donor solution, respectively. The analytes that are suitable for two-phase HFM-LPME are relatively non-polar organic compounds having high distribution constants in the organic solvent. In case of ionisable organic compounds such as acidic or basic organic compounds, pH adjustment in the donor solution in order to deionize the analytes is

necessary to decrease their distributions in the aqueous sample resulting in enhancing the extraction efficiency.

$$E = \frac{K_{a/d}V_{org}}{K_{a/d}V_{org}+V_d} \qquad \text{Equation 2.6}$$

Moreover, the extraction efficiency (E) can be written as the Equation 2.6 where V_a is volume of acceptor solution; V_{org} is volume of organic phase; and V_d is volume of donor solution.

For three-phase HFM-LPME, the equilibrium and partition coefficient can be written as Equation 2.7 - Equation 2.11 [38]

$$A_{\text{aqueous donor}} \rightleftharpoons A_{\text{organic phase}} \rightleftharpoons A_{\text{aqueous acceptor}} \qquad \text{Equation 2.7}$$

$$K_{o/d} = \frac{C_{eq,\,o}}{C_{eq,\,d}} \qquad \text{Equation 2.8}$$

$$K_{a/o} = \frac{C_{eq,\,a}}{C_{eq,\,o}} \qquad \text{Equation 2.19}$$

$$K_{a/d} = K_{o/d} \times K_{a/o} \qquad \text{Equation 2.10}$$

$$K_{a/d} = \frac{C_{eq,\,a}}{C_{eq,\,d}} \qquad \text{Equation 2.11}$$

where A is the target analyte; $K_{o/d}$ is partition coefficient of A between the organic phase and donor solution; $K_{a/o}$ is partition coefficient of A between the acceptor solution and the organic phase; $K_{a/d}$ is partition coefficient of A between the acceptor solution and the donor solution; $C_{eq,\,a}$, $C_{eq,\,o}$ and $C_{eq,\,d}$ is the concentration of the analyte at equilibrium in the acceptor solution, organic phase and the donor solution, respectively.

Typically, three-phase HFM-LPME is suitable for extraction of ionized analyte. The mechanism is similar to liquid extraction and back extraction. First, the analyte in the donor solution should be in the non-ionized form either by adjusting the pH. The analyte in non-ionized form, which has higher distribution constant in organic solvent can be extracted and transferred into the organic solvent (SLM). The analyte in non-ionized form is then back extracted into another aqueous solution (acceptor

solution) by turning the analyte back into the ionized form, which has higher distribution constant in aqueous solution. The extraction efficiency (E) can be written as the equation below

$$E = \frac{K_{a/d}V_a}{K_{a/d}V_a + K_{o/d}V_o + V_d} \qquad \text{Equation 2.12}$$

Where $K_{a/d}$ is partition coefficient between acceptor solution and donor solution, $K_{o/d}$ is partition coefficient between organic solvent and donor solution, V_a, V_o and V_d is volume of acceptor solution, organic solvent and donor phase, respectively.

Another way of transferring the ionized analyte is by forming an ion-pair that is added as a carrier in the organic solvent (SLM). The target analyte, which forms ion pair with the carrier, becomes highly hydrophobic molecules and can easily pass the organic solvent (SLM). Then, the analyte is released from the ion-paired complex into the acceptor solution at the contact region of the organic solvent (SLM) and the acceptor solution, by exchanging with the counter ions as illustrated in Figure 2.4 [39].

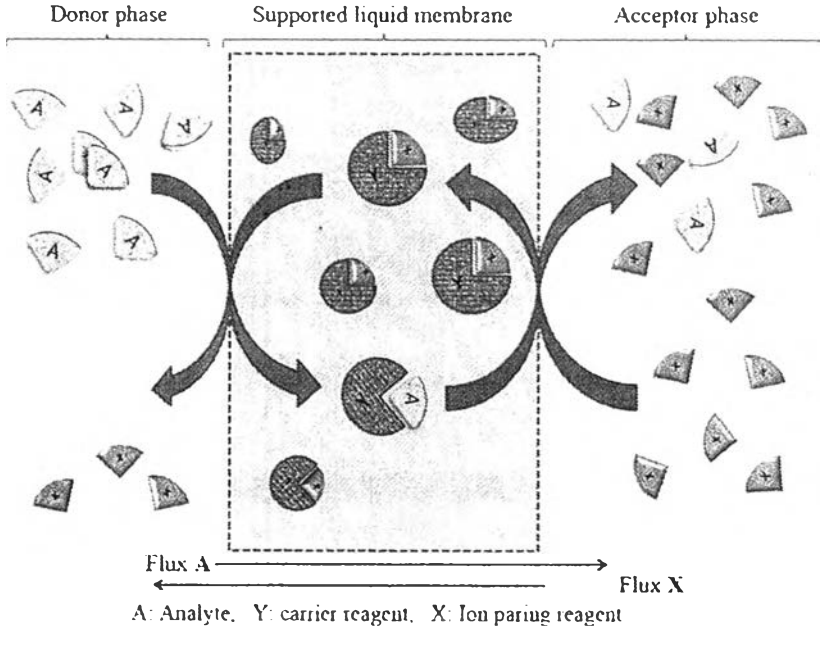


Figure 2.4 Active transport in HFM-LPME [39]

The advantages of HFM-LPME include reducing the consumption of hazardous organic solvent, excellent sample clean up, simple and inexpensive method, high enrichment, direct compatibility with HPLC, CE and MS as well as easy for automation. Although HFM-LPME provides many advantages, one problem of this technique is the long extraction time in the range of 15-45 min. So applying an electrical field to HFM-LPME was introduced in name of "Electromembrane extraction (EME)", where the analyte is transferred based on electrokinetic migration for solving the time-consuming problem [39].

2.1.4 Electrical field assisted hollow fiber membrane liquid phase Microextraction

Electrical filed assisted hollow fiber membrane liquid phase microextraction or electromembrane extraction (EME) was introduced by Pedersen-Bjergaard, S. and Rasmussen, K. E. [5] to reduce the extraction time of three-phase HFM-LPME that described in 2.1.3. Two platinum wires are inserted in the acceptor solution and the sample solution as electrodes. If the analyte is cation, the anode (+) is placed in the sample solution and the cathode (-) is placed in the acceptor solution and vice versa for the anion. These electrodes are connected to the power supply as seen in Figure 2.5 [5].

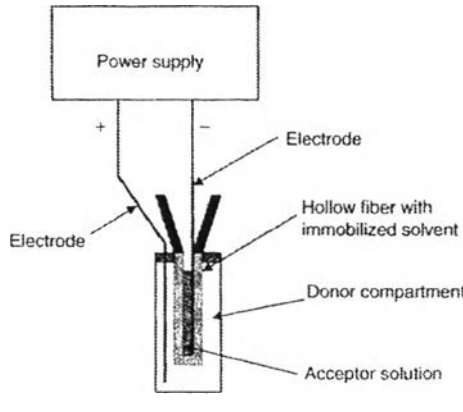


Figure 2.5 Scheme of electromembrane extraction [5]

After extraction, the extract is withdrawn into a microsyringe for analysis by a suitable instrument.

There are many parameters related to extraction efficiency. First parameter is the pH of the sample and the acceptor solution. The analyte that is suitable for EME should be ionic form so pH should be well controlled. For basic analyte, pH of both sample and acceptor solution is adjusted to be acid in order to protonate the basic compound to be charged analyte. In contrast, for acidic analyte, these solutions are adjusted to be alkaline in order to deprotonate the acidic analyte.

Second parameter is the composition of SLM that should be water immiscible, easily immobilized with the pores, appropriate electrically resistant to keep low electric current, suitable viscosity and high boiling point to avoid evaporation. Types of organic solvents are depending on polarity of analytes. In the case of extraction of hydrophobic analyte, nitro-substituted solvent such as 2-nitrophenyl octylether (NPOE) provides high extraction recoveries but low efficiency for highly hydrophilic compound [40]. To increase the solubility of the hydrophilic analytes in the organic solvent, ionic carrier is added to the SLM. Carrier is classified in many modes that are described in section 2.2.

The last parameter is the applied voltage. The extraction efficiency of EME can be described as the flux of ionic analyte (J_i) through the SLM in the modified Nernst-Planck equation below [41].

$$J_i = -\frac{D_i}{h}\left[1 + \frac{\nu}{ln\,\chi}\right]\left[\frac{\chi - 1}{\chi - exp(-\nu)}\right]\{c_{ih} - c_{i0}exp(-\nu)\} \qquad \text{Equation 2.13}$$

$$\nu = \frac{z_i e \Delta\phi}{kT} \qquad \text{Equation 2.14}$$

Where D_i is the diffusion coefficient for the charged analyte in the SLM; h is the thickness of the SLM; ν is the dimensionless driving force, which is defined by another equation above; χ is the ratio of total ionic concentration on the donor side to that on the acceptor side; C_{ih} and C_{i0} is the concentration of the analyte close to the membrane in the sample solution and in the acceptor solution, respectively; z_i is the charge of analyte; e is the elementary charge; k is boltzman's constant; $\Delta\phi$ is potential; and T is temperature.

The advantages of EME are highly sensitive sample preparation for ionic substances, simple instrument, high enrichment factor, short extraction time, compatible with a wide range of matrices, inexpensive and low sample size.

2.2 Ion-exchange mechanism

The transportation of ionic analyte or metal ion in supported liquid membrane (SLM) under three-phase HFM-LPME can be classified into two main modes; cation transport and anion transport. Each mode is devided into two minor types; symport and antiport transport that are shown in Figure 2.6 [42, 43].

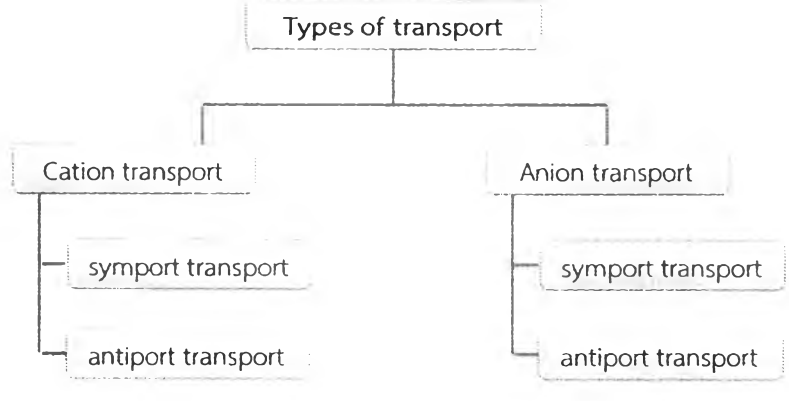


Figure 2.6 Schematic classification of transport of ionic analyte in three-phase HFM-LPME

To begin with cationic symport transport, the analyte (M^+) and the counter anion (X^-) are moved together through SLM by neutral carrier (I) such as Trioctylphosphine oxide (TOPO), Tris(2-ethylhexyl) phosphate (TEHP) and then the analyte (M^+) is released into aqueous acceptor solution while the carrier (I) diffuses back across the membrane as shown in Figure 2.7.

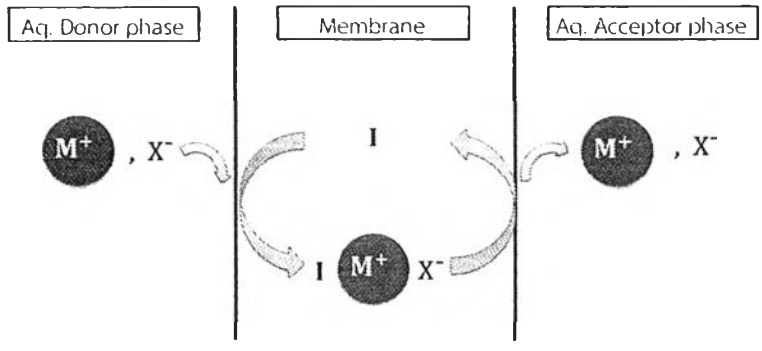


Figure 2.7 Cationic symport transport

Cationic antiport transport is the transportation of target cation (M^+) by complexing with anionic carrier (I^-) such as bis-(2-ethylhexyl) phosphate (DEHP) and dodecylbenzenesulphonic acid, moving into the SLM, releasing the target cation at the interface between the membrane and the acceptor solution, exchanging with the counter ion (N^+) and moving back to the donor phase as shown in Figure 2.8. This mechanism is generally called "cation-exchange". If proton is counter ion, the transport may be called "proton driven or counter cation driven".

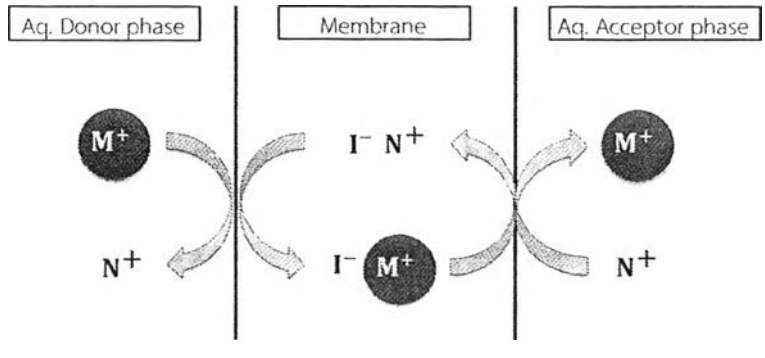


Figure 2.8 Cationic antiport transport

For anionic symport transport, the mechanism is the same as the cationic symport transport, where the neutral compound is a carrier. Finally, anionic antiport transport, the mechanism is similar to the cationic antiport transport. The cationic carrier such as methyltrioctylammonium chloride (Aliquat 336) is used instead of

anionic carrier as shown in Figure 2.9. Anionic antiport transport may be called "counter anion driven."

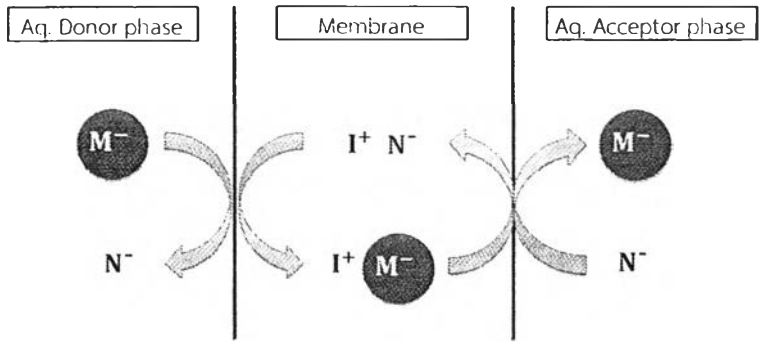
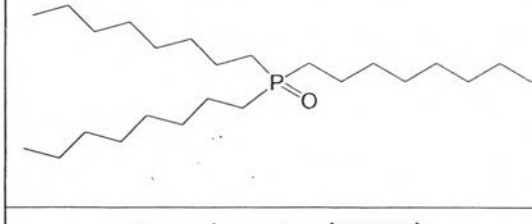
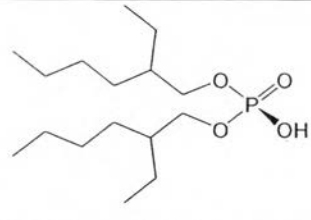
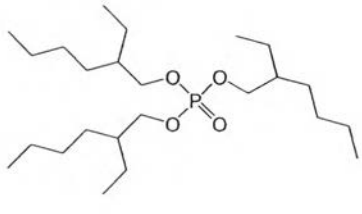
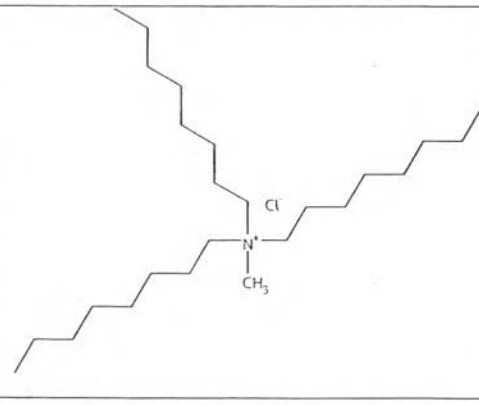


Figure 2.9 Anionic antiport transport

The types of carriers are classified into three modes that are neutral carrier, anionic carrier and cationic carrier as shown in Table 2.1.

Table 2.1 Types of carriers

	
Neural carrier (TOPO)	Anionic carrier (DEHP)
	
Neutral carrier (TEHP)	Cationic carrier (Aliquat336)

2.3 Chromium

Chromium in the form of hydro chromate ion is chosen for a model analyte in this work. Generally, chromium is present in the environment in two valence states that are trivalent chromium Cr(III) and hexavalent chromium Cr(VI). Cr(VI) is more toxic than Cr(III). According to the World Health Organization (WHO) [44] regulated Maximum Contaminant Level (MCL) for hexavalent chromium in drinking water under the total chromium at 50 $\mu g\ L^{-1}$.

2.3.1 Properties and toxicity

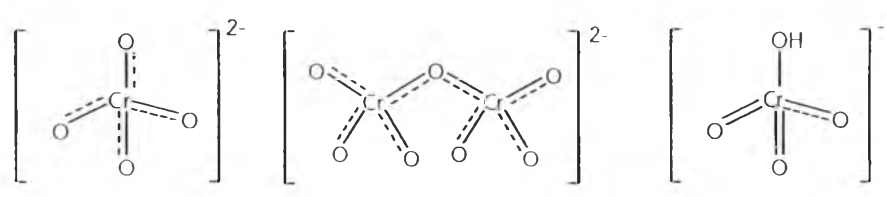
In solution, Cr(VI) is highly mobile and soluble while Cr(III) is more stable, less soluble and most immobile [45]. Normally, Cr(VI) is always in hydro chromate ($HCrO_4^-$), chromate (CrO_4^{2-}) and dichromate forms ($Cr_2O_7^{2-}$). Their structures are shown in Figure 2.10 [46].

The species distribution of Cr(VI) in a solution depends on pH of the solution as depicted in Figure 2.11. Chromate (CrO_4^{2-}) prevails in basic solution (pH > 7). Hydro chromate ($HCrO_4^-$) and dichromate ($Cr_2O_7^{2-}$) prevail in acidic solutions.

Moreover, species distribution of Cr(VI) in a solution also depends on the concentration. At concentration lower than $(1.26\text{-}1.74) \times 10^{-2}$ M, $Cr_2O_7^{2-}$ converts to $HCrO_4^-$, as at higher Cr(VI) concentration, the $HCrO_4^-$ turns to $Cr_2O_7^{2-}$ [47].

Hexavalent chromium are contaminated the environment mainly from the industrial processes including chrome planting, leather and wood preservation, the manufacture of dyes and pigments, treatment of cooling tower water as well as toner for copying machines [48].

For the toxicity, Cr(VI) is strong irritant to skin, mucosal tissue, acute tubular necrosis, metabolic acidosis and kidney failure. By oral, it leads to oral ulcers, diarrhea, abdominal pain, vomitting, indigestion and presence of immature neutrophils, Including teratogenic and mutagenic effect [46].



a) b) c)

Figure 2.10 Structures of a) chromate ion (CrO_4^{2-}), b) dichromate ion ($Cr_2O_7^{2-}$) and c) hydro chromate ion

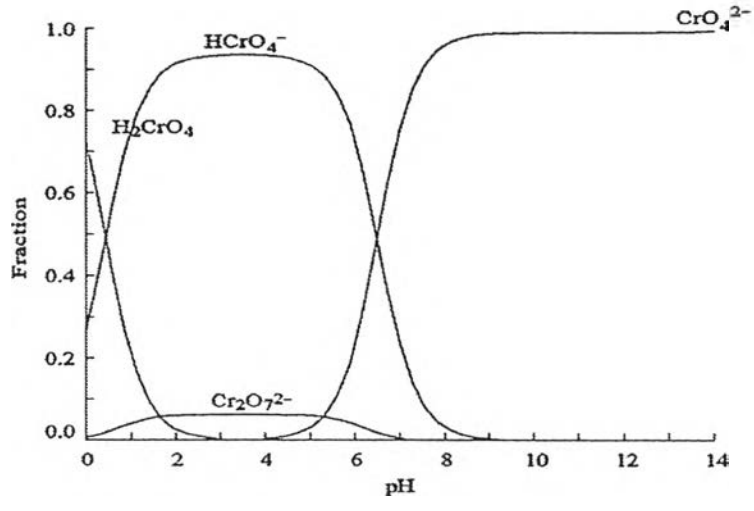


Figure 2.11 Species distribution diagram of Cr(VI) at different pH values [47]

2.3.2 Cr(VI)-DPC colorimetric method

Chromium (VI) can be quantitatively and selectively determined by colorimetric method via complexation with 1,5-diphenylcarbazide (DPC). The reaction is summarized in

Figure 2.12. First, Cr(VI) is reduced to Cr(III) by DPC. Then, the DPC in oxidizing form or 1,5-diphenylcarbazone reacts with Cr(III) and becomes Cr-DPC complex as shown in

Figure 2.12. The complex is violet color that can be determined by a spectrometer. This method has been adopted by the United States Environmental Protection Agency (EPA) as a standard method (METHOD 7196A) for determination of Cr(VI) [49].

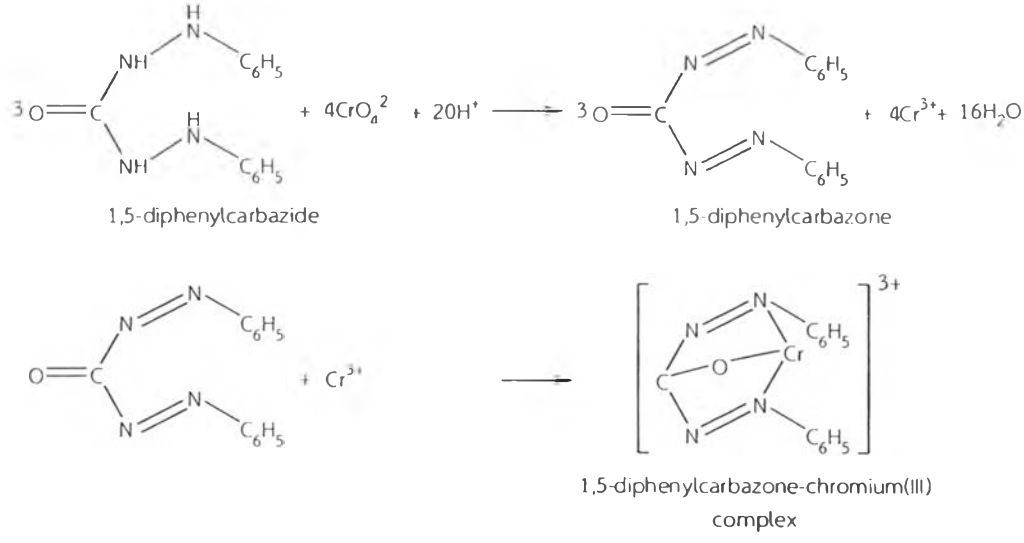


Figure 2.12 Cr(V)-DPC colorimetric method [50]