Chapter 1

Introduction

1.1 Liquid chromatography

High performance liquid chromatography (HPLC) is a form of column chromatography. The acronym HPLC is often shortened to LC. Of all the chromatographic techniques whose mobile phase is a liquid, HPLC is perhaps the best known. The analyte is carried through a stationary phase in a packed column by a liquid mobile phase at high pressure, during which time the components separate from each other on the column. One of the aspects particular to HPLC is that of the partition mechanisms between analyte, mobile phase and stationary phase. They are based on coefficients of adsorption or partition. Traditionally, HPLC columns were polar, e.g. silica, and the mobile phases used were relatively nonpolar in nature. This mode of HPLC was called normal phase chromatography. More common today is reversed phase HPLC where the columns are nonpolar (hydrophobic), e.g. C-18 bonded phase, and the mobile phases used include any miscible combination of water and various organic modifiers such as methanol and acetonitrile. On a reversed phase column, hydrophobic analytes are better retained, eluting more readily as the proportion of the hydrophobic component of the stationary phase is increased. Following separation, the analytes are detected by the detector as they elute from the column. There are many types of LC detectors; some of the most common ones are conductivity detector, UV detector, fluorescence detector and mass spectrometric detector. A HPLC instrument consists of the components shown in Figure 1.1. Moreover, reversed phase HPLC has many application include micellar liquid chromatography (MLC) and ion interaction chromatography (McMahon, 2007; Rouessac & Rouessac, 1994).

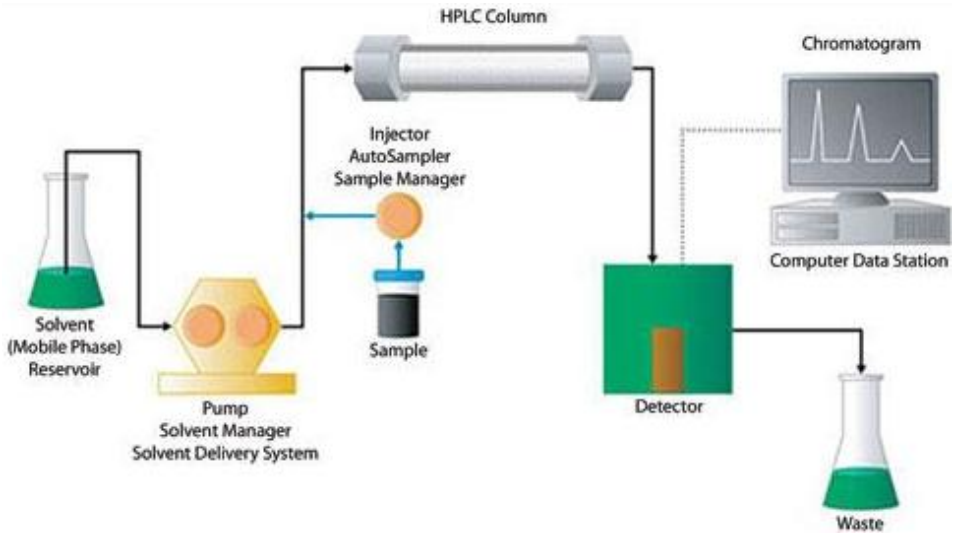


Figure 1.1 Schematic diagram of a high performance liquid chromatograph (Lambertson, 2010)

1.1.1 Micellar liquid chromatography

Micellar liquid chromatography (MLC) is a mode of reversed phase liquid chromatography, which uses aqueous solution of surfactants (ionic or non-ionic) above its critical micellar concentration (CMC). Several advantages of micellar mobile phase in comparison with classical aqueous-organic mobile phase are presented below: (1) the possibility of simultaneous separation of charged and uncharged solutes; (2) direct injection of physiological fluids due to the capability of some micellar solutions (anionic or nonionic) to solubilize the protein matrix of samples; (3) compatibility of mobile phases with salts and water-insoluble compounds; (4) unique separation selectivity that is due to microheterogeneity of micellar mobile phase and dynamic modification of stationary phase; (5) robustness of results that is caused by stabilization of surfactant monomer concentration in the presence of micelles; (6) rapid gradient capability (shorter equilibration times); (7) enhanced luminescent detection that is due to the solubilization effect on solutes; (8) low cost of micellar mobile phase; (9) safety versus expensive and flammable solvents of chromatographic grade. For all reasons, the popularity of micellar-

mediated separation techniques has grown rapidly (Ruiz-Ángel, Carda-Broch, Torres-Lapasió, & García-Álvarez-Coque, 2009).

1.1.1.1 Micelles

Surfactants are an amphiphilic molecule that possesses both a hydrophobic group and a hydrophilic group in the molecule itself. The hydrophobic group is derived from the property of affinity for oil, since it is mainly made up of longer alkyl chains. While, the hydrophilic group is ascribed to polar groups which have affinity for water, such as hydroxyl group, ether type oxygen atom and carboxylic group etc. Thus, the surfactant has affinity for both water and oil. In aqueous solution, with an increase in concentration of surfactant, a spontaneous association of molecules makes aggregates termed “micelles”. The concentration at which surfactants start forming micelle is called the “critical micellar concentration (CMC)”. With the aid of micelles, surfactants are used as emulsifiers, dispersants, bubbles, antifoams, and detergents. As shown in Figure 1.2, these aggregates of amphiphiles assemble such that the tails of the molecules are packed together in the core of the micelle while the polar head groups form a boundary zone between the nonpolar core of the micelle and the polar aqueous solution beyond.

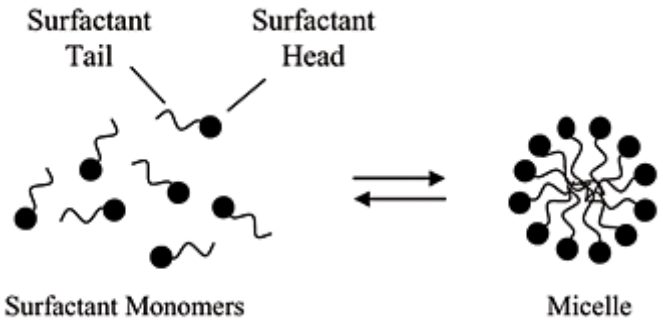


Figure 1.2 Micelle formation and the open circles represent polar head groups which may be anionic, cationic, nonionic or switterionic
(Loginova, Samokhina, Boichenko, & Kulikov, 2006)

Figure 1.3 showed the surfactants can form micelle at higher concentration than the critical micellar concentration. At concentration above the CMC, the phenomenon can be observed by changing in any of several physical properties (i.e., absorbance maxima, surface tension and conductance) of the solution with increasing concentration of amphiphile. The concentration of free amphiphiles remains fairly constant with added surfactant. It is important to realize that the concentration of free amphiphile does slowly increases above the critical micellar concentration although the change is often small enough so as to be unobstructive to the analytical technique at hand. This feature has important consequences in the use of micellar mobile phase in separations (Armstrong & Henry, 1980; Khaledi, 1997).

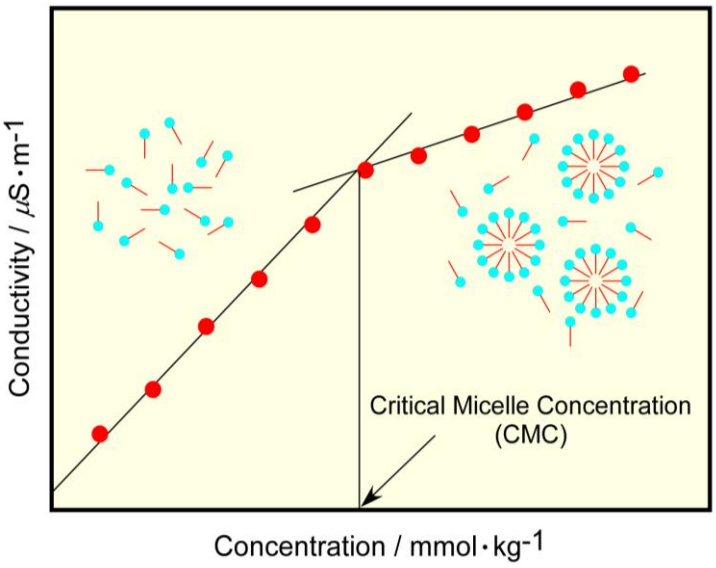


Figure 1.3 Detection of critical micellar concentration (CMC) by the electrical conductance method (Shimoaki, 2010)

Micelles have a dynamic structure that is the results of the rapid exchange of surfactants in the aggregated and monomeric forms. The number of monomer surfactants in the aggregate form which is called aggregation number (AN) and the size of micelles vary greatly between surfactants. The critical micellar concentration and aggregation number greatly depend on a number of factors such as

ionic strength, presence of a co-solvent and temperature. Another property of surfactants is the Kraft point, which is defined as the temperature at which the solubility of surfactant is equal to its critical micellar concentration (Khaledi, 1997). The critical micellar concentration and aggregation number of some common surfactants are shown in Table 1.1.

For practical purpose in HPLC, a suitable surfactant should have a low critical micellar concentration and Kraft point. A high critical micellar concentration would mandate operating at high surfactant concentrations. This condition is not desirable in HPLC because of its high viscosity. The Kraft point should preferably be much lower than the ambient temperature. Moreover, since absorbance detectors are the most commonly used in HPLC, a proper surfactant should have small molar absorptivity at the operating wavelength. Since the size of micelles is a few nanometers, they do not cause any light scattering in the operating UV-visible range.

Surfactants are classified into four types due to the hydrophilic group; anionic surfactant, cationic surfactant, amphoteric surfactant (zwitterionic surfactant) and nonionic surfactant.

(1) Anionic surfactant

When dissociated in water, anionic surfactants become surfactant anions and metalic cations, just like electrolytes such as sodium dodecyl sulfate, SDS (Figure 1.4). Hydrophobic group of anionic surfactant adsorbs on a hydrophobic surface of substance, generating high detergency and foaming power. Thus, the anionic surfactant is usually used as detergents for clothes and body.

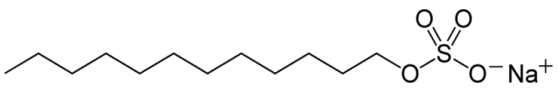


Figure 1.4 Structure of sodium dodecyl sulfate (Kyriacos, 2009)

Table 1.1

Lists some common surfactants used to form micelles along with their critical micellar concentration (CMC) and aggregation number (AN) where available

Type	Name	CMC (mM)	AN
Anionic	Cholic acid, sodium salt	14	2-4
	Deoxycholic acid, sodium salt	5	4-10
	Glycocholic acid, sodium salt	13	2
	Sodium dodecyl sulfate (SDS)	8.27	62
	Taurocholic acid, sodium salt	10-15	4
Cationic	Cetyltrimethylammonium bromide (CTAB)	1.3	78
	Dodecyltrimethlyammonium bromide (DTAB)	14	50
	Hexadecyltrimethylammonium bromide	0.026	169
Zwitterionic	3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate (CHAPS)	8	10
	3-[(3-cholamidopropyl)dimethylammonio]-2-hydroxy-1-propanesulfonate (CHAPSO)	8	11
	N-Dodecyl-N,N-dimethylammonio-3-propane sulfonate	3.3	
Nonionic	n-Decyl-b-D-glucopyranoside	2.2	
	Triton X-100	0.24	140
	Polyoxyethylene (23) dodecanol (BRIJ 35)	0.1	
	Polyoxyethylene [20]-sorbitane monooleate (Tween 80)	0.01	
	Polyoxyethylene [20]-sorbitane monolaurate (Tween 20)	0.059	

(Baker, 1995; Khaledi, 1997)

(2) Cationic surfactant

When dissociated in water, cationic surfactants become surfactant cations and small anions such as cetyltrimethylammonium bromide, CTAB (Figure 1.5). Since many substances have negative charges on their surfaces, cationic surfactants are adsorbed near there. Thus, cationic surfactants are used as lubricant agents, antistats, fabric care liquid and conditioners.

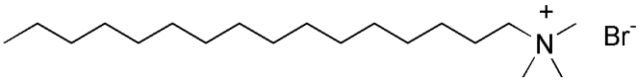


Figure 1.5 Structure of cetyltrimethylammonium bromide (Shimoaki, 2010)

(3) Amphoteric surfactant (zwitterionic surfactant)

Amphoteric surfactant, such as alkylamidopropyl betaine, is the type of amino acid and changes its charge depending on the pH of aqueous solution. When pH of solution is basic (pH>7), hydrophilic groups are cationic. On the other hands, when pH of solution is acidic (pH<7), hydrophilic groups are anionic. Amphoteric surfactant can be used with other types of surfactant; especially, the use with anionic surfactant enhances detergency and foaming power. Amphoteric surfactant is also used as shampoos, fungicides, antistats, fabric softeners and corrosion inhibitors for metal.

(4) Nonionic surfactant

Nonionic surfactant is the nonelectrolyte type such as alcohol ethoxylate (AE) and Triton X-100, etc. Generally, hydrophilic group is polyoxyethylene group. Nonionic surfactant is used as detergents for kitchen and clothes, emulsifiers for cosmetics and food additives.

1.1.1.2 Principle of micellar liquid chromatography

Micellar liquid chromatographic technique is the technique that uses surfactants as a part of mobile phase. The surfactants can form micelles at higher concentration than the critical micellar concentration. The liquid phase is then comprised of aqueous phase and micellar pseudo phase. In reverse phase chromatography, the surfactant becomes significantly adsorbed on the surface of alkyl-bonded stationary phase as shown in Figure 1.6 (a) sodium dodecyl sulfate, SDS and (b) cetyltrimethylammonium bromide, CTAB as mobile phases. Solutes can partition between the aqueous, the micellar and the stationary phase, which is the basis of micellar liquid chromatography (Khaledi, 1997).

(a) (b)

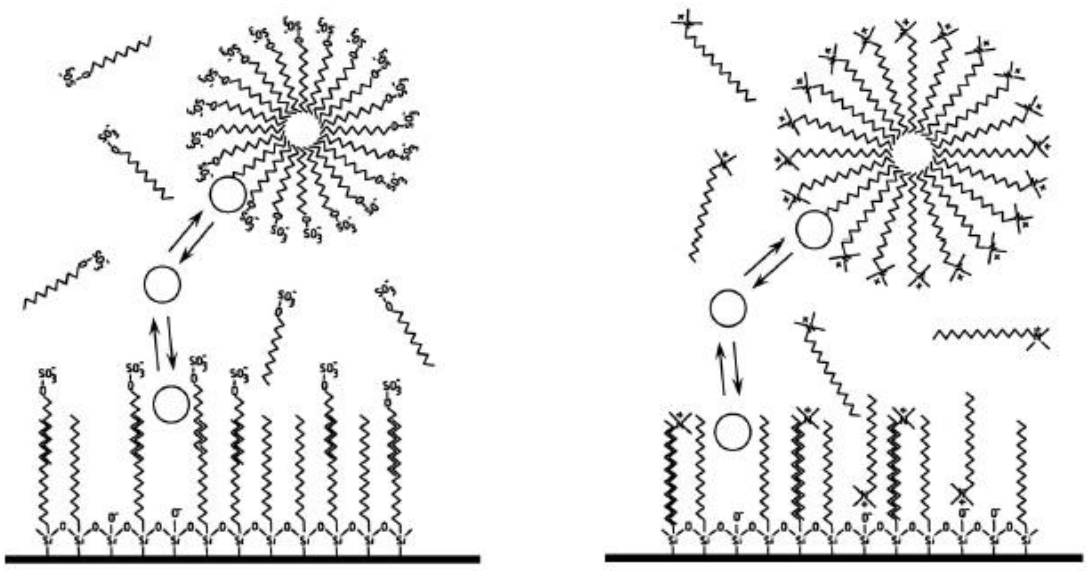


Figure 1.6 Equilibria between bulk solvent, micelle and surfactant-modified stationary phase (Solute environment in a chromatographic system using octadecyl-bonded and mobile phases containing (a) the anionic SDS and (b) the cationic CTAB) (Ruiz-Ángel, et al., 2009)

Partitioning of solutes from the bulk aqueous mobile phase into micelles has a large effect on retention and selectivity. According to the three-

phase model as shown in Figure 1.7, retention behavior in micellar liquid chromatography is controlled by the solute partitioning from bulk solvent into micelles (P_{mw}) and into stationary phase (P_{sw}) as well as by direct transfer from the micelles in the mobile phase into the stationary phase (P_{ms}). While retention of more polar compounds is illustrated by their partitioning from the bulk aqueous phase into micelle and alkyl stationary phase, the more hydrophobic compounds might be directly transferred from micelles in the mobile phase into the stationary phase (Fischer & Jandera, 1996; Jandera & Fischer, 1996; Khaledi, 1997).

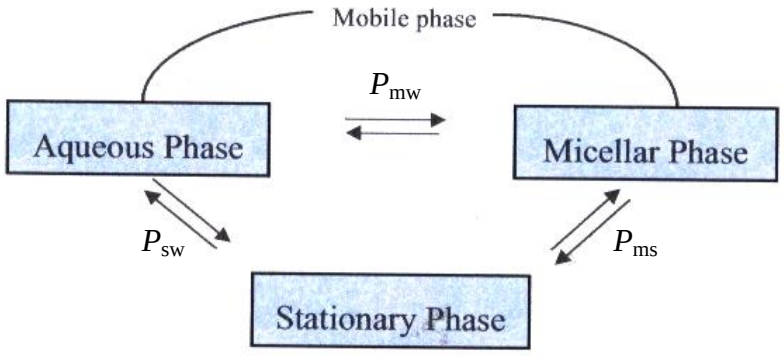


Figure 1.7 Three-phase equilibria in micellar liquid chromatography (Khaledi, 1997)

1.1.1.3 Chromatographic behavior in MLC

Retention behavior of solutes in micellar liquid chromatography have been studied by several groups and retention equations suggested to describe the retention of binding solutes in dependence on the concentration of surfactant as micelle foam in the mobile phase, [M], which is equal to the difference between total concentration of surfactant, [SDS], and the critical micelle concentration, CMC ([M] = [SDS] - CMC). The chromatographic behavior of solutes in micelle system with common analytical column C8 and C18 that proposed by Arunyanart and Cline-Love, as described below (Armstrong & Henry, 1980; Arunyanart & Cline-Love, 1984; Ruiz-Ángel, et al., 2009).

Arunyanart and Cline-Love assumed association equilibria of solute in bulk aqueous solvent (A) with the stationary phase binding sites (S) and with monomers of surfactant in the micelle (M), governed by the binding constants K_{AS} and K_{AM}, respectively. The capacity factor of the solute, k, is defined as

$$k = \phi \frac{[AS]}{[A]+[AM]} = \frac{\phi K_{AS}[S]}{1+K_{AM}[M]} \qquad \text{.....(1.1)}$$

The binding constant K_{AM} is referred to the association of the solute with a surfactant monomer in the micelle, and should be multiplied by the aggregation number to get a parameter referred to the whole micelle. Due to the stationary phase activity, [S], usually does not change by changing the concentration of surfactant, it can be combined with K_{AS} and ϕ in equation 1.1 to give

$$k = \frac{K_{AS}}{1+K_{AM}[M]} \qquad \text{.....(1.2)}$$

where the yield, $\phi K_{AS}[S]$, has been called again K_{AS} for simplicity. Equation 1.2 is usually rewritten to perform the linear dependence between the reciprocal of k and the concentration of surfactant as micelle foam in mobile phase:

$$\frac{1}{k} = \frac{1}{K_{AS}} + \frac{K_{AM}}{K_{AS}}[M] = c_0 + c_1 \cdot [M] \qquad \text{.....(1.3)}$$

where k is capacity factor, $k = (t_R - t_0)/t_0$. [M] is the concentration of surfactant as micelle foam in mobile phase. c_0 and c_1 are formal constants.

The advantages of micellar liquid chromatography over conventional HPLC are the unique selectivity from the three-phase equilibria (Khaledi, 1997), the ability to perform gradient elution separations with virtually no

column re-equilibrium, improved compatibility of electrochemical detection with gradient elution, better sensitivity with fluorescence and phosphorescence detection and the ability to perform direct injections of biological samples with no protein precipitation problems (Nishi, 1997). In addition, micelles (surfactants) are more eco-friendly to the environments than the conventional hydro-organic mobile phase, such as methanol and acetonitrile, because surfactants are biodegradable substances.

1.1.2 Ion interaction chromatography

Although the efficiency of the HPLC column is less than those used for GC, new stationary phases that can operate in several modes such as ion-pairing, which is the chromatographic approach of RP ion interaction chromatography (IIC). It has become a widely used separation mode in analytical HPLC because it provides a useful and flexible alternative to ion exchange chromatography. Better selectivity, enhanced resolution, and retention are usually gained by this separation strategy.

Unlike conventional ion exchange, ion interaction chromatography can be used to separate nonionic and ionic or ionizable compounds in the same sample, because retention of an analyte involves its transfer trough the electrical double layer and depends on both electrostatic interactions and adsorptive (RP) effects (Teresa Cecchi, 2010).

Under reversed phase (RP) HPLC conditions, ionic compounds are weakly retained. On the contrary, when an ion-interaction reagent (IIR), which is a large lipophilic ion, is added to the mobile phase, ionized species of opposite charge are separated on RP columns with adequate retention.

Ion pair chromatography (IPC) is one technique of ion interaction chromatography that use ion-paring reagent (IPR). The kind and concentration of the ion-paring reagent are key factors in ion pair chromatographic separations. The types of ion-paring reagents are classified as followed (Teresa Cecchi, 2010). Clearly the major role for positively charged ion-paring reagents is still played by classical organic ammonium ions, whereas for negatively charged ion-paring reagents, perfluorinated carboxylic acids (volatile IPR) and chaotropic lipophilic salts are outperforming traditional sulfonium organic ions.

(1) Traditional IPRs

Conventional ion pair chromatographic separations were achieved by adding organic amines and ammonium salts of varying chain lengths as cationic ion-paring reagents for anionic analytes and alkyl or aryl sulfonates and sulfates as anionic ion-paring reagents for cationic solutes.

(2) Volatile IPRs

Liquid chromatography mass spectrometry (LC-MS) is now routinely used in analytical laboratories. Traditional ion-paring reagents are non-volatile salts that are not compatible with MS techniques because they play a major role in source pollution that is responsible for reducing signals.

Small organic acids such as formic and acetic acid are effective volatile ion-paring reagents. They impact the retention behaviors of pH-sensitive compounds, changing their charge status and providing pairing anions that may easily interact with protonated solutes. The acidity of these ion-paring reagents facilitates the formation of the protonated molecular ion, $[M+H]^+$, measured by MS in the positive ion mode.

The use of perfluorinated carboxylic acids has increased greatly for peptide, amino acid and hydrophilic metabolite applications. Interestingly, other volatile ion-paring reagents are trifluoroacetic acid (TFA), heptafluorobutyric acid (HFBA), pentafluoropropanoic acid (PFPA), tetrabutyl ammonium acetate and bromide, etc.

(3) Chaotropic salts

Choatropic salts as novel ion-paring reagents are not strongly hydrated; they are known to disrupt the hydrogen bonding structure of water and increase disorder (“chaos”). The following series are order of increasing chaotropicity: $H_2PO_4^-$ < $HCOO^-$ < $CH_3SO_3^-$ < Cl^- < NO_3^- < CF_3COO^- < BF_4^- < ClO_4^- < PF_6^-. At variance with traditional ion-paring reagents that tend to stick very strongly

to the stationary phase and to impair the initial column properties also when their presence in the eluent is discontinued, chaotropic ion-paring reagents are quite hydrophilic and can be easily removed from the column. Among newly introduced ion-paring reagents, chaotropic salts achieved good recognition among chromatographers during the past decade, and proved particularly useful in the ion pair chromatography of basic compounds in low pH eluents.

(4) Unusual IPRs

Uncommon ion-paring reagents were tested recently. For example, polymerized acryl monoglycinate surfactant was found to be as effective as sodium dodecyl sulfate for the resolution of organic amines (Waichigo & Danielson, 2006). For the analysis of pyridine-based vitamins in infant formulas, dioctylsulfosuccinate produced a unique retention pattern (Woollard & Indyk-Harvey, 2002). Hexamethonium bromide, a divalent ion-paring reagent, was used successfully to separate sulfonates and carboxylates (Bruzzoniti, Mentasti, & Sarzanini, 1997). 3,5-Dinitrobenzoic acid proved capable of improving reversed phase retentions of chemical warfare agent derivatives (Papoušková, et al., 2007) and [S-(R,R)]-(-)-bis(-α-methylbenzyl)amine hydrochloride was an effective ion-paring reagent for zwitterionic analytes (T. Cecchi, Pucciarelli, & Passamonti, 2004; T. Cecchi, Pucciarelli, Passamonti, & Cecchi, 2001).

Nowadays, ion-paring reagents are more many kinds that are commercial ion-paring reagents and manual synthetic ion-paring reagents in laboratory, so they are many applications of ion pair chromatography. To achieve ion pair chromatographic separations, it also has many IPC modes and variations such as mixture of different ion-paring reagents in mobile phase, permanently coated columns, special additives in mobile phase and ion-paring reagents added only to sample solution and ghost peaks, etc. The essential role played by ion-paring reagents in ion pair chromatography is described.

In RP-HPLC separations performed on non-polar stationary phases with polar hydro-organic eluents, polar compounds elute first and ionic solute

retention is usually poor because of their high affinity to the polar mobile phase. To achieve the adequate retention pre-requisite to good resolution, the mobile phase was supplemented with a specific ion-paring reagent which is a large organic ion oppositely charged to the analyte of interest. These additives were originally supposed to be effective because they form ion-pairs with the analyte in bulk eluent, and for this reason the technique was called ‘ion-pair chromatography’.

The most comprehensive survey of all equilibria supposed to occur in a chromatographic system, that is, the retention mechanism upon which the stoichiometric theory is structured, is depicted in Figure 1.8 (Teresa Cecchi, 2010). In a chromatographic system, the main equilibria are (1) the adsorption of the analyte (E) onto the stationary phase hydrocarbonaceous ligand site (L), (2) the adsorption of ion-paring reagent (H) onto L, (3) the ion-pair formation in the mobile phase (EH), (4) the ion-pair formation in the stationary phase (LEH), (5) and the displacement of H by E. The equilibrium constants for these equilibria are K_{LE}, K_{LH}, K_{EH}, K_{LEH}, and K_{LE}/K_{LH}, respectively. These models are termed “stoichiometric” because they use stoichiometric equilibrium constants instead of thermodynamic constants.

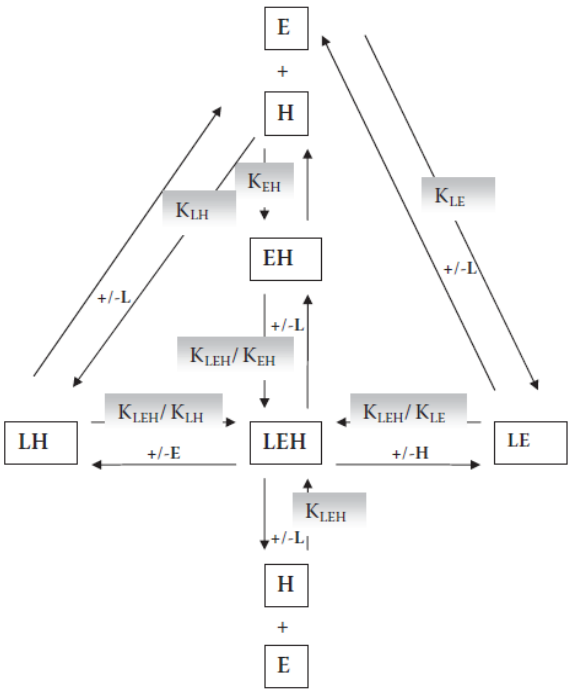


Figure 1.8 Comprehensive retention mechanism of IPC (Teresa Cecchi, 2010)

According to the qualitative retention model of Bidlingmeyer, the lipophilic ion-interaction reagent, flowing under isocratic conditions, dynamically adsorbs onto the alkyl-bonded apolar surface of the stationary phase, forming a primary charged ion layer. The corresponding counterions are found in the diffuse outer region to form an electrical double layer. This charged stationary phase can then more strongly retain analyte ions of the opposite charge (Bidlingmeyer, 1979, 1980).

Detections of ion pair chromatography are UV-visible detectors, fluorescence detectors, electrical conductivity detectors, electrochemical detectors, evaporative light scattering detectors, mass spectrometer, etc. Hyphenation techniques of ion pair chromatography are IPC-MS, IPC-ICP and other unusual hyphenations such as IPC-NMR and IPC-atomic fluorescent spectrometry. In this work, ion pair chromatography was hyphenated with mass spectrometric detection.

Joining ion pair chromatography to MS techniques probably provides the most valuable detection mode because it complements the chromatographic separation of complex mixtures with structural information and unequivocal identification of analyte along with good sensitivity. Moreover, this technique can be considered universal because all molecules can be sensed. Table 1.2 showed the contrasting requirements of ion pair chromatography and MS.

Table 1.2

Contrasting requirements of IPC and MS

IPC	MS
- Liquid phase process	- Vacuum process
- 1 mL min^{-1} eluent flow ≈ 500 mL min^{-1} of gas at flow STP	- Accept only 10 mL min^{-1} gas flow
- Inorganic buffers and IPRs	- Volatile mobile phase components
- 25 to 50 ^{o}C	- 200 to 350 ^{o}C
- All analytes	- Volatile analytes

IPC: ion pair chromatography; IPRs: ion-paring reagents; MS: mass spectrometry; STP: standard temperature and pressure (STP is 0 ^{o}C (32 ^{o}F or 273.15 K) and 1 atm (101.3 kPa, 14.7 PSI, 760 mmHg or 760 Torr))

From the analysis of the specifications in Table 12.2, IPC-MS hyphenation requires: (1) specific optimization of mobile phase composition, selecting only volatile components, (2) devising strategies to minimize eluent flow, and (3) an interface to make ion pair chromatography effluent amenable to MS detection. The goals of the interface are (1) separation of the analyte from the bulk eluent, (2) ion evaporation for ionic species or ionization of non-ionic solutes, and (3) fragmentation and quantitative transfer of analyte fragments to the mass analyzer. Common interfaces are electrospray ionization (ESI) and atmospheric pressure chemical ionization (APCI). Both are soft ionization techniques that cause little or no fragmentation.

In this method, electrospray ionization was used as interface. The advantages of ESI are good for non-volatile, thermally labile and high molecular weight analytes. It was occurred ion suppression in presence of high eluent ionic strength. It is not good for high water percentages in eluent, but it requires volatile mobile phase components. In contrast, ion pair chromatography is good for aqueous solution and is not good for organic modifier-rich eluents because both analyte retention and the ion-paring reagent adsorption isotherm are weakened when organic modifier is rich. Therefore, mobile phase compositions such as concentration of ion-paring reagent and organic modifier were optimized that will be good for both mass spectrometric detection and ion pair chromatographic separations.

1.1.3 Liquid chromatography/mass spectrometry

Liquid chromatography/mass spectrometry (LC/MS) combines the separating power of LC with the uniquely powerful detection capabilities of MS. The mass spectrometer has the potential to serve as the universal detector for the HPLC. Mass spectrometry (MS) is based on generating ions in the gaseous state, separating them according to their mass-to-charge ratio (*m/z*) and detecting them. Therefore, mass spectrometry is useful for identification as it can elucidate chemical and structural information about molecules from their molecular weights and distinctive fragmentation patterns. Moreover, mass spectrometry is also very important for the quantitative measurement of atoms or molecules. The sensitivity of mass

spectrometry for identifying molecules is in the 10^{-12} - 10^{-15} molar range. With LC/MS technique, it is now possible to separate, identify and quantify components in a mixture (Wilfried M. A. Niessen, 1999; Watson & Sparkman, 2007). A LC/MS instrument is schematically represented in Figure 1.9.

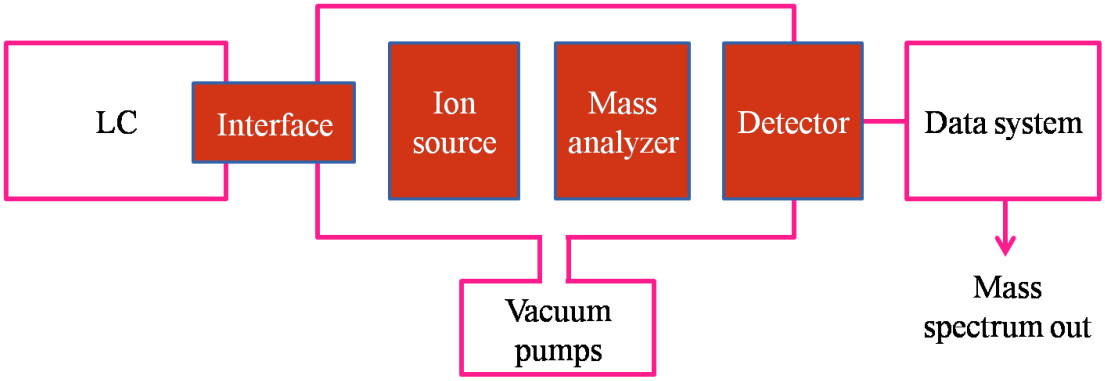


Figure 1.9 Schematic diagram of liquid chromatography/mass spectrometry (McMahon, 2007)

A mass spectrometer consists of a sample introduction system, an ion source (creating ions in the gaseous phase), a mass selective analyzer (separating the ions in space or time based on their mass-to-charge ratio), an ion detector (measuring the quantity of ions of each mass-to-charge ratio) and a computer. The ion source is where the sample of interest is both ionized with a positive or negative charge and converted into the gaseous phase. There are a number of ion sources available such as electron impact, chemical ionization, electrospray ionization, atmospheric pressure chemical ionization, fast atom bombardment, matrix assisted laser desorption ionization and surface enhanced laser desorption ionization. For LC/MS technique, the interface is the important issues: (1) LC effluent is liquid phase and at atmospheric pressure; (2) LC compounds are usually nonvolatile and thermally unstable which can be difficult to ionize; (3) LC flow rates are typically high; (4) MS works best with gases and low flow rates; (5) the MS is at high vacuum and needs to remove the solvent without removing analytes. Therefore, the interface must be able to get rid of the liquid mobile phase, convert the relatively nonvolatile and/or thermally labile analytes into a ‘gas’ and transfer the ‘gas’ from atmospheric conditions to a high

vacuum. The suitable ionization sources are the use of atmospheric pressure ionization (API) techniques which enables conversion of liquid phase to the gas phase analytes. They are including electrospray ionization (ESI) and atmospheric pressure chemical ionization (APCI) which offers ‘soft’ ionization that result in little or no fragmentation (De Hoffmann & Stroobant, 2007; McMahon, 2007; Wilfried M. A. Niessen, 1999).

1.1.3.1 Electrospray ionization

Electrospray ionization (ESI) is one of the two newer ionization techniques (the other being MALDI). ESI is a technique that takes place at atmospheric pressure and is considered to be a ‘soft’ ionization process (Boyd, Basic, & Bethem, 2008). Common interfaces used in liquid chromatographic techniques are electrospray ionization (ESI) and atmospheric pressure chemical ionization (APCI). In this work, the interface between the liquid chromatograph and mass spectrometer was ESI. The advantages of using ESI over using APCI were summarized in Table 1.3. Many reports have been presented the use of electrospray ionization as interface for determination of perchlorate such as electrospray LC-MS/MS and IC-ESI-MS/MS (Barron & Paull, 2006; Blount & Valentin-Blasini, 2006; Winkler, Minteer, & Willey, 2004).

In ESI, the sample solution is mixed with a nebulizing gas in the spray needle. This sample solution can be delivered by a syringe pump or as eluent from a separation technique such as HPLC. As shown in Figure 1.10, the sample arrives in the spray chamber as a fine mist of droplets or spray. Then, very mall droplets of solvent-containing analyte are formed at the end of a fine silica capillary whose surface has been metalized and which is hold at high positive potential (assuming a study of positive ions). A drying gas, e.g. nitrogen, at a fairly high temperature causes the evaporation of any solvent from the droplets. As the droplets evaporating, the charge density at their surface increases leading to coulombic explosion. A voltage gradient between the tip of the spray needle and the entrance to the transfer capillary, as well as a pressure difference from atmospheric pressure to vacuum, encourage appropriately charged ions to move into the capillary

and on towards the skimmer. Then, charged ions move into the mass analyzer and detect them by ion detector such as electron multiplier detector (Khandpur, 2006; Rouessac & Rouessac, 1994).

Table 1.3

Positive and negative features of most common interfaces used for IPC-MS combination

	ESI		APCI
+	Polycharged ions, good for high MW analytes	-	Not good for high MW analytes
+	Good for non-volatile and thermally labile analytes	+	Good only for volatile and thermally stable analytes
-	Scarce fragmentation	-	No fragmentation
-	Requires capillary column (eluent flow rate < 5 μL min^{-1}) or spit devices	+	Accepts eluent flow rates up to 1 mL min^{-1}
-	Ion suppression in presence of high eluent ionic strength	-	Eluent solvents should be able to give gas phase acid-base reactions with analytes
-	Not good for high water percentages in eluent, requires volatile mobile phase components	-	Requires volatile mobile phase components
+	Preferred for anionic (negative mode) and at ionic (positive mode) analytes or very polar analytes	+	Preferred for polar analytes (apolar analytes better analyzed via GC-MS)

IPC: ion pair chromatography; MS: mass spectrometry

ESI: Electrospray Ionization; APCI: Atmospheric Pressure Chemical Ionization

(+) means positive and (-) means negative features

(Teresa Cecchi, 2010)

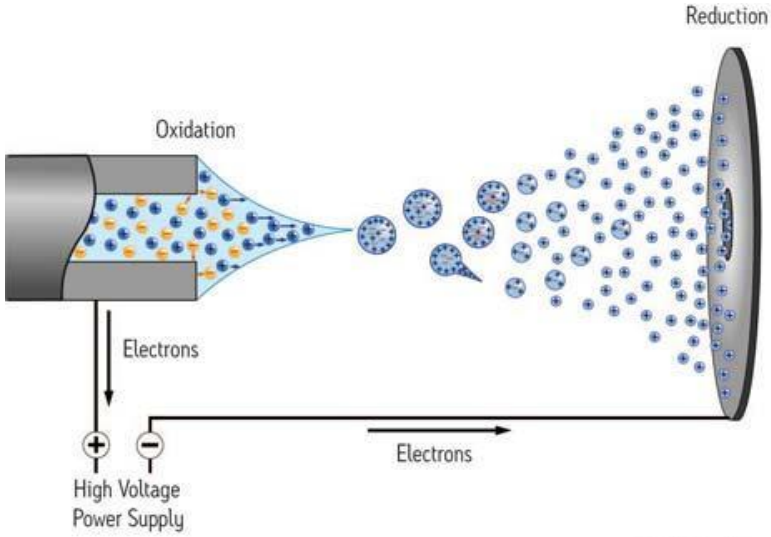


Figure 1.10 Schematic of the electrospray ionization process
(Watson & Sparkman, 2007)

1.1.3.2 Ion-trap mass spectrometer

The mass analyzer (sometimes called mass filter) is the discriminating element in a mass spectrometer and is where the ions are manipulated and sorted. They are many types of mass analyzer in MS such as transmission quadrupole, ion-trap, time-of-flight, magnetic sector and fourier transform-ion cyclotron resonance. However, in this thesis, an ion-trap mass analyzer was employed. Its principle is described below.

The ion-trap mass spectrometer is one of the most popular mass analyzer today. The ion-trap mass spectrometer uses three electrodes to trap ions in a small volume. The advantages of the ion-trap mass spectrometer include its compact size, the ability to trap and accumulate ions to increase the signal-to-noise ratio of a measurement, no transmission losses and the ability to carry out tandem mass spectral experiments (MS^n).

The mass analyzer consists of a ring electrode separating two hemispherical electrodes. Then, by changing the electrode voltages, the ions are sequentially destabilized, ejected from the trap and then detected to form the mass spectrum. The entire sequence of trapping, storing, manipulating and ejecting ions is

performed in continuous cycle. A schematic of an ion trap is shown in Figure 1.11 (Khandpur, 2006; McMahon, 2007).

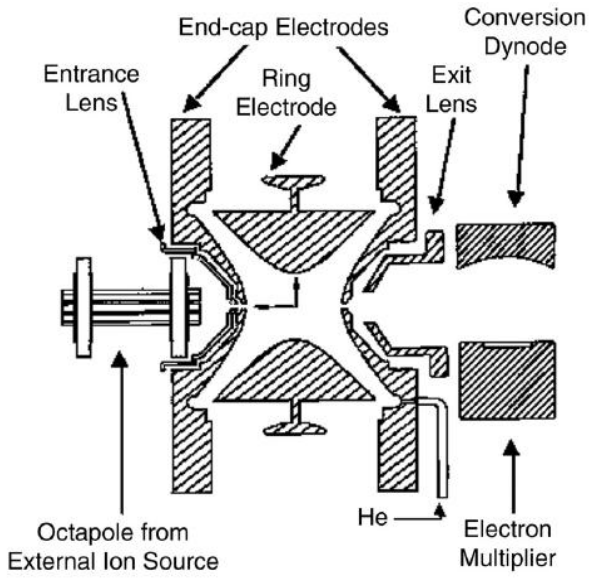


Figure 1.11 Schematic diagram of an ion-trap mass analyzer
(W. M. A. Niessen, 2006)

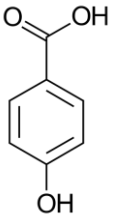
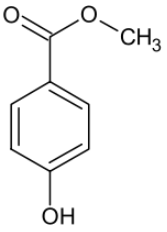
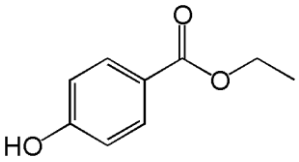
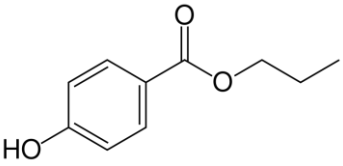
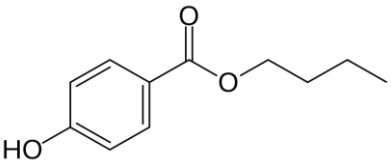
The one advantage of using ion-trap mass analyzer was its ability to carry out tandem mass spectral experiments or MS^n without complicated tandem mass spectrometer such as quadrupole mass analyzer or quadrupole-time-of-flight (TOF) tandem mass spectrometer that are high cost. In 2008, He et al. reported the use of ion-trap mass spectrometer for profiling and characterization of polyphenol polymers from cinnamon by characterizing of the compounds with MS^5 (He, et al., 2008).

1.2 The parabens

Parabens are a homologous series of hydroxybenzoic acid esterified at the C-4 position (*p*-hydroxybenzoic acid, PHBA), normally including methyl paraben (MP), ethyl paraben (EP), propyl paraben (PP) and butyl paraben (BP) (Wang, Cao, Fang, & Gong, 2010). The chemical structures of parabens are given in Table 1.4.

Table 1.4

Structures, molecular weight (MW) and pK_a of parabens

Compounds	Structures	MW (g mol^{-1})	pK_a
4- hydroxybenzoic acid (*p*-hydroxybenzoic acid, PHBA)		138.12	4.48
Methyl-4-hydroxybenzoate (methyl paraben, MP)		152.15	8.31
Ethyl-4-hydroxybenzoate (Ethyl paraben, EP)		166.17	8.4
n-Propyl-4-hydroxybenzoate (propyl paraben, PP)		180.20	8.23
Butyl-4-hydroxybenzoate (butyl paraben, BP)		194.23	8.24

(Kulikov & Verushkin, 2008; Yang, Tsai, Chen, Yang, & Lee, 2010)

Parabens and their salts are used primarily for their bacteriocidal and fungicidal properties. The antimicrobial activity of parabens seems to increase with the chain length of the ester group, but in practice, shorter esters are commonly employed because of their higher solubility in water. Among them, methyl paraben and propyl paraben are the most commonly used in cosmetics, since they are added together and have synergistic effects (Núñez, Tadeo, García-Valcárcel, & Turiel, 2008). Parabens commonly used as preservatives in foods, cosmetics, beverages and pharmaceuticals (Márquez-Sillero, Aguilera-Herrador, Cárdenas, & Valcárcel, 2010). In cosmetics, they can be found in shampoos, emulsion, moisturizing cream, lotion, hands cream, antiwrinkle cream, after sun, make-up and toothpaste (Zotou, Sakla, & Tzanavaras, 2010).

Although parabens were considered to have low toxicity, many studies demonstrated the endocrine disruption activity of parabens (Routledge, Parker, Odum, Ashby, & Sumpter, 1998). They reported allergic contact dermatitis and a relation with male infertility (Tavares, Martins, Oliveira, Ramalho-Santos, & Peixoto, 2009). Moreover some studies suggest a connection between parabens and the risk of breast cancer (Calafat, Xiaoyun, Lee-Yang, Bishop, & Needham, 2010; Darbre, et al., 2004). In fact, parabens have been detected in human breast tumor tissues at the low ng/g level (Harvey & Everett, 2004). In addition, they have some reports the perniciousness of propyl paraben and butyl paraben to cause DNA damage and the induction of chromosome aberrations together with sister-chromatid exchanges (Tayama, Nakagawa, & Tayama, 2008).

Because of the reproductive and endocrine toxicity, the withdrawal of Acceptable Daily Intake (ADI) of 0-10 mg per kg body weight for propyl paraben from food has been recommended. The above studies indicated that even though long-term, low-dose consumption maybe has side effects on humans and wildlife. Therefore, parabens are regulated by EU Cosmetic Directive 76/768/EEC, Annex VI, part 1 can therefore be used as preservative up to a maximum concentration of 0.4% (w/w) in the finished product for each one and up to 0.8% (w/w) for total maximum concentration, expressed as *p*-hydroxybenzoic acid (Núñez, Turiel, Martin-Esteban, & Tadeo, 2010). Therefore, it is necessary to develop some high selective and sensitive methods for determination of parabens in cosmetics to ensure human health.

1.3 The perchlorate

Perchlorate is the anion of an oxyhalide salt containing a single chlorine atom and four oxygens atom. These salts occur both naturally and through manufacturing (Snyder, Pleus, Vanderford, & Holady, 2006). The formula of the perchlorate ion is ClO_4^-, which is the negatively-charged ion present in salts such as the common compounds sodium perchlorate ($NaClO_4$) and ammonium perchlorate (NH_4ClO_4). Another common commercial form is perchloric acid ($HClO_4$). Most perchlorate salts and perchloric acid are readily soluble in water. Such solutions contain ClO_4^-. This ion consists of the unique element, Cl, in the center of a tetrahedral grouping of the four oxygens; the ion is isostructural with the common compound methane (Figure 1.12). Table 1.5 showed oxidation state of perchlorate and related compounds. The advantage of perchlorate was used as a medicine for more than 50 years, which beginning in the 1950s, perchlorate was used as a drug at very high levels (equivalent to 70-300 mg L^{-1} in drinking water) to treat hyperthyroidism.

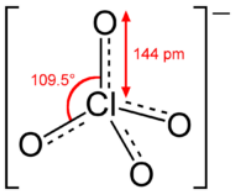


Figure 1.12 The structure of the perchlorate ion (Washington University, 2004)

Table 1.5

Perchlorate and its various oxidation states

Common name	Stock name	Oxidation state	Formula
Hypochlorite	Chlorate(I)	+1	ClO^-
Chlorite	Chlorate(III)	+3	ClO_2^-
Chlorate	Chlorate(V)	+5	ClO_3^-
Perchlorate	Chlorate(VII)	+7	ClO_4^-

(Environmental Working Group, 2007)

Therapeutic use was discontinued in the US because reported fatal cases of agranulocytosis and aplastic anemia (Martinelango, Gümüş, & Dasgupta, 2006). Moreover, perchlorate is also used as an oxidizer in rocket fuel and solid fuel engines (e.g., US Space shuttle, missiles, etc.) and can be found in explosives, automobile air bags and fireworks (Li & George, 2005). The presence of perchlorate in water, soil, agriculture irrigation and food supplies have become focal issues and poses a considerable human health risk, even at trace levels, as it can interfere with the thyroid gland's ability to produce thyroid hormones. Perchlorate is known to inhibit the transport of iodide by the sodium-iodide symporter (NIS), present e.g., in the thyroid and mammary glands (Jackson, Laikhtman, & Rohrer, 1999). In the human body, perchlorate inhibits production of thyroid hormones, essential to normal organ development in babies, especially brain development. Figure 1.13 described (1) iodide from foods, such as salt, enters the body; (2) iodide that used to produce thyroid hormones is transported into the thyroid by the sodium-iodide symporter (NIS) as sodium is transported out; and (3) if perchlorate is digested, it blocks the symporter, disrupting the uptake of iodide.

Therefore, the U.S. Environmental Protection Agency's Candidate Contaminant List (CCL) is considering a regulatory limit for perchlorate of 4 µg L^{-1} in water (U.S. Environmental Protection Agency, 2005; Winkler, et al., 2004). Therefore, the effective method to determine perchlorate is required. In this work, liquid chromatographic with mass spectrometric detection was applied to determine perchlorate in environmental samples such as soil and water samples in the north of Thailand.

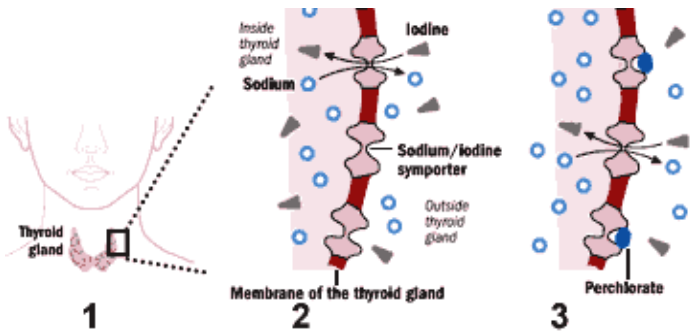


Figure 1.13 Inhibition of the NIS by perchlorate (Waldman, 2002)