1 INTRODUCTION

1.1 Polychlorinated Biphenyls (PCBs)

1.1.1 Introduction to polychlorinated biphenyls

Polychlorinated biphenyls (PCBs) are a group of compounds derived from biphenyl by substitution one to ten atoms of hydrogen with chlorine atoms[1] as shown in Figure 1.1.

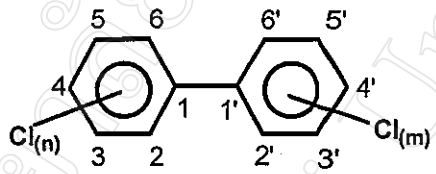


Figure 1.1 Common structure of polychlorinated biphenyls (PCBs) ;
m, n = No. of chlorine atoms in molecule.[2]

This class of compound consists of 209 components called "congener". Each congener differs from the others in substitution pattern and/or in number of chlorine atoms. In order to simplify the nomenclature of PCBs, the systematic numbering has been introduced (see Appendix A) and widely used now. The synonyms of PCBs are biphenyl chlorinated, chlorinated biphenyl, diphenyl chlorinated, chlorobiphenyls and polychlorobiphenyls.

1.1.2 Production[2]

PCBs were first synthesized in 1881 by Schmidt and Schulz. The commercial PCBs were produced as complex mixtures beginning in 1929 through the mid 1970s. Commercial products of PCBs were the mixtures of a large number of congeners because of reaction of gaseous chlorine with the molten biphenyl under given conditions. The reaction is controlled by thermodynamics thus the certain

substitution patterns are favoured (but not pure) and, consequently, some congeners are more abundant than the others.

1.1.3 Physical properties[2]

Most of PCB congeners are colorless, odorless crystals. The commercial mixtures are clear viscous liquid and the more highly chlorinated mixtures are more viscous. PCBs have low solubility in water, low vapor pressures, soluble in most of organic solvents, oil and fats.

PCBs are difficult to degrade due to their thermodynamic stability. These cause the environmental problems. However, under the certain conditions, they may be destroyed by chemical, thermal and biochemical processes. According to their chemical and thermal stability, low flammable, low vapor pressure and electrical insulating properties, they were used in many industries such as dielectric fluid in capacitors and transformers, heat transfer fluids, hydraulic fluids, additive in pesticides and paints, plasticizers etc. Some important physical properties of PCBs are shown in Table 1.1.

1.1.4 Environmental problem and toxicity

PCBs have entered to the environment through both legal and illegal disposal and using. PCBs do not readily degrade and lipophilic properties thus they tend to be persistent and bio-accumulate in the environment. PCBs have low volalitility and highly lipophilic with the consequence that more than 99% of the environmental PCBs mass are found in soil. The environmental transport of PCBs is complex and global.

The atmospheric transport is the primary mode of global distribution of PCBs. Environmental levels of PCBs are high near the source of PCBs such as urban areas and indoor areas. PCBs may be carried long distances in the air.

Table 1.1 Some physical properties of PCB homologs[2]

PCBs Isomer group	Melting point (°C)	Boiling point (°C)	Vapor pressure at 25°C (Pa)	Water solubility at 25°C (g/m^3)	Log K_{ow}*
Biphenyl	71	256	4.9	9.3	4.3
Mono-CB	25-77.9	285	1.1	4.0	4.7
Di-CB	24.4-149	312	0.24	1.6	5.1
Tri-CB	28-87	337	0.054	0.65	5.5
Tetra-CB	47-180	360	0.012	0.26	5.9
Penta-CB	76.5-124	381	2.6×10^{-3}	0.099	6.3
Hexa-CB	77-150	400	5.8×10^{-4}	0.038	6.7
Hepta-CB	122.4-149	417	1.3×10^{-4}	0.014	7.1
Octa-CB	159-162	432	2.8×10^{-5}	5.5×10^{-3}	7.5
Nona-CB	182.8-206	445	6.3×10^{-6}	2.0×10^{-3}	7.9
Deca-CB	305.9	456	1.4×10^{-6}	7.6×10^{-4}	8.3

*K_{ow} = octanol-water partition coefficient

They can remain in the air for approximately ten days.[3] This is one of the major routes that PCBs can spread over the world. PCBs are easily absorbed by most animals through ingestion, inhalation or dermal contact. The highly chlorinated congeners tend to accumulate in body fat due to their lipophilic properties, while the lower chlorinated congeners may be metabolized via hydroxylation to excretable forms. However, these hydroxy forms may be more toxic than the original parent molecule.

PCBs are considered ubiquitous pollutants. They have been found in nearly all marine plants and animal specimens, fish, mammals, birds (especially fish-eating birds), bird eggs and humans.

The following are some of toxic effects of PCBs in animals and human.[1]

- High dose of PCBs can effect on animals include body weigh loss, lesions dysfunctions of skin (chloracne), liver, bile duct, gall bladder, urinary tract, reproductive system and also teratogenesis and carcinogenesis.

- On the human effects, these can be investigated in individuals exposed to PCBs either occupationally or due to poisoning. There have been two accidents of mass PCBs poisoning caused by ingestion PCBs contaminated edible oil. The first called "Yusho" happened in Japan in 1968 and the second called "Yucheng" in Taiwan in 1979. In the poisoned humans many effects can be observed such as liver damage, dermal lesions, respiratory disorders, severe ocular signs, various neurological system, immuno deficiency and reproduction disorders.

- The effects are more seriously in children who are exposed to PCBs during foetal development and after birth they may be fed with breast milk which may contain significant levels of PCBs. It has been found that infants from mothers who had high level of PCBs in blood had significantly decreased weights and gestational ages. The children who have fed breast milk that contained higher level of PCBs for longer periods were less active than the less exposed children.

- Carcinogenicity

In human, this effect has not been proved. However, it has been found that the workers exposed to PCBs significantly were found to have increased levels of some oncogene proteins and chromosomal aberrations of human peripheral blood

lymphocytes. The Department of Health and Human Services (DHHs)[3] U.S.A., EPA and International Agency for Research on Cancer (IARC)[4] have determined that PCBs may reasonably be anticipated to be carcinogens.

1.1.5 Regulation[1]

The limit concentrations of PCBs used for regulatory purposes are based either on the "total PCBs" or more recently on "standard individual congeners" (PCBs No. 28, 52, 101, 138, 153 and 180). They were chosen in order to cover the wide range of chlorination (3-7 chlorine atoms), high level of these congeners in sample and chromatographic resolution. U.S. Food and Drug Administration (FDA,1972) limited the level of total PCBs in milk and milk products at 1.5 mg kg^{-1} milk fat, in poultry at 3.0 mg kg^{-1} fat, in fish (1978) at 5.0 mg kg^{-1} of edible fractions and in food stuffs for infants at 0.2 mg kg^{-1}.

In Germany (1988) limited the level of PCBs congeners in milk and milk products at 40 μg kg^{-1} milk fat for PCBs No. 28, 52, 101 and 180 at 50 μg kg^{-1} milk fat for PCBs No. 138 and 153, in edible animal fat at 80 μg kg^{-1} for PCBs No. 28, 52,101 and 180, at 100 μg kg^{-1} for PCBs No. 138 and 153.

EU limited the maximum level of PCBs at 1-2 μg l^{-1} for natural water and 0.5 μg l^{-1} for the sum of all isomers and 0.1 μg l^{-1} for each isomer in drinking water.[4]

Environmental Protection Agency (EPA) currently limited the maximum level of PCBs at 0.5 μg l^{-1} in drinking water.[5]

1.1.6 Analytical chemistry of PCBs

In most instance analytical chemistry of PCBs consists of four steps, namely 1) sampling 2) extraction 3) cleanup 4) determination and evaluation. In this work, extraction, cleanup and determination steps were focused.

1.1.6.1 Extraction and cleanup steps

In each type of sample, extraction and cleanup may be different thus this topic is separated according to sample type.

- Soils, sediments and sewage sludges

PCBs in these samples are normally extracted with the organic solvents which could penetrate into the micro-cavities.[6] Then the solutions were

further cleaned up additionally with sulfur removal step in some samples. The examples of extraction, cleanup and determination methods in soils, sediments and sewages analysis are summarized in Table 1.2.

Table 1.2 Some examples of extraction, cleanup and determination methods in soils, sediments and sewage sludges analysis

Sample	Extraction method	Cleanup method	Determination method
Soil	Liquid extraction with dichloromethane or methanol	Florisil and alumina	GC/MS[7]
Soil	Supercitical fluid extraction with carbondioxide	No further cleanup	GC/ECD[8]
Soil	Soxhlet extraction with acetone : hexane (1:1 v/v)	No further cleanup	GC/ECD[8]
Soil	Subcritical water extraction	Solid phase microextraction	GC/ECD[9]
Soil	Soxhlet extraction with acetone : hexane (1:1 v/v)	No further cleanup	GC/ECD[9]
Soil	Subcritical water extraction	Tenax solid phase extraction	GC/ECD[10]
Sediment	Solvent extraction with acetone : hexane (1:1 v/v)	Florisil	GC/ECD[11]
Sediment	Sonication with acetone	Liquid-liquid extraction with isooctane and sodium chloride solution then passed through alumina column	GC/ECD[12]

Table 1.2 (continued)

Sample	Extraction method	Cleanup method	Determination method
Sediment	Soxhlet extraction with acetone : hexane (1:1 v/v)	Alumina	GC/ECD[12]
Sediment	Steam distillation	Alumina	GC/ECD[12]
Sediment	Sonication with dichloromethane : methanol (2:1 v/v)	Alumina : silica gel (7:8 w/w) and permeation chromatography	GC/ECD GC/MS[13]
Sediment	Supercritical fluid extraction with carbon dioxide and carbon dioxide with methanol as modifier	No further cleanup	GC/ECD[14]
Sediment	Soxhlet extraction with dichloromethane : methanol (2:1 v/v)	Neutral alumina	GC/ECD[14]
Sediment	Microwave-assisted extraction with toluene: water (10:1 v/v)	Florisil and removed sulfur by copper	GC/MS, GC/ECD[15]
Sediment	Soxhlet extraction with acetone : hexane (1:1 v/v)	Florisil and removed sulfur by copper	GC/MS, GC/ECD[15]
Sediment	Sonication with toluene	Florisil and removed sulfur by copper	GC/MS, GC/ECD[15]

Table 1.2 (continued)

Sample	Extraction method	Cleanup method	Determination method
Sulfur-containing sediment	Supercritical fluid extraction with carbon dioxide	Trapped the eluate with C_{18} or florisil and removed sulfur with copper	GC/ECD[16]
Sulfur-containing sediment	Soxhlet extraction with acetone : hexane (59:41 v/v)	Silica impregnated with sulfuric acid and removed sulfur with tetrabutyl ammoniumsulfite	GC/ECD[16]
Sediment	Supercritical fluid extraction with carbon dioxide	C_{18} trapping and florisil to cleanup, removed sulfur with mercury	GC/MS[17]
Sediment	Soxhlet extraction with acetone : hexane (1:1 v/v)	Florisil and removed sulfur with mercury	GC/MS[17]
Sewage sludge	Supercritical fluid extraction with carbon dioxide	Florisil or C_{18} trapping	GC/ECD[18]
Sewage sludge	Soxhlet extraction with acetone : hexane (1:1 v/v)	Silica impregnated with sulfuric acid	GC/ECD[18]
Sewage sludge	Soxhlet extraction with acetone : hexane (1:1 v/v)	Florisil and removed sulfur with copper	GC/MS GC/ECD[19]

Table 1.2 (continued)

Sample	Extraction method	Cleanup method	Determination method
Sewage sludge	Supercritical fluid extraction with carbon dioxide	Florisil trapping and removed sulfur with copper	GC/MS[20]
Sewage sludge	Soxhlet extraction with acetone : hexane (59:41 v/v)	Florisil and removed sulfur with copper	GC/ECD[20]

- Waters

PCBs are of very low concentration in water due to their lipophilic property. Therefore, the pre-concentration step is required for determination. The methods widely used for extraction and pre-concentration of PCBs in water are liquid-liquid extraction (LLE) and solid phase extraction (SPE).[4] The examples of extraction, cleanup and determination method in water analysis are summarized in Table 1.3.

Table 1.3 Some examples of extraction, cleanup and determination method in water samples analysis

Sample	Extraction method	Cleanup method	Determination method
Waste water	Liquid-liquid extraction with 15% diethylether in hexane	Sulfuric acid or KOH-ethanol treatment	GC/ECD[22]
Water	C_{18} disk and styrene-divinyl benzene disks	-	GC/ECD[23]
Water	Florisil-SPE	-	GC/MS[7]
Snow	C_{18} disk	-	GC/ECD GC/MS[24]

Table 1.3 (continued)

Sample	Extraction method	Cleanup method	Determination method
Tap water and seawater	Liquid-liquid extraction with dichloromethane	-	GC/ECD[25]
Tap water and seawater	C_{18} disk and microwave-assisted solvent elution	-	GC/ECD[25]
Water	Steam distillation-solvent extraction (SDE)	-	GC/ECD[26]
Natural water and seawater	C_{18}-cartridge	-	GC/ECD[27]
Snow and rain	Liquid-liquid extraction with hexane	-	GC/ECD[28]
Ocean wetland and leachate waters	Solid-phase microextraction (SPME)	-	GC/ECD[29]
Water	Headspace solid-phase microextraction (HSSPME) and solid-phase microextraction	-	GC/ECD[30]

- Fatty matrices

Considering the lipophilic properties of PCBs, the general analytical procedure for the fatty matrices samples involves the extraction of PCBs from the matrix often together with fat and other lipophilic matrix components. These components could interfere the analysis especially trace analysis. Furthermore, even small amounts of lipids can cause deterioration of gas chromatographic column and detector.[31] Thus the extensive cleanup is required. The examples of extraction,

cleanup and determination methods in fatty matrices sample analysis are summarized in Table 1.4.

Table 1.4 Some examples of extraction, cleanup and determination methods in fatty matrices samples analysis

Sample	Extraction method	Cleanup method	Determination method
Human milk	Soxhlet extraction with hexane	Florisil	GC/ECD[32]
Mineral oils and powdered milk	Mineral oils were dissolved in hexane Powdered milk was mixed with florisil and extracted with acetone : hexane (1: 2 v/v)	Sulfuric acid and then florisil Sulfuric acid and then florisil	GC/ECD[33]
Cows milk and powdered milk	C_{18}-SPE	No further cleanup	GC/ECD[34]
Human milk	Milk was adsorbed on fibrous cellulose plus florisil and soxhlet extraction with hexane	High performance liquid chromatography	GC/MS[35]
Milk fat	Liquid extraction with pentane then saponification with potassium hydroxide-ethanol	Basic alumina	GC/ECD[36]

Table 1.4 (continued)

Sample	Extraction method	Cleanup method	Determination method
Soybean infant formulas	Liquid extraction with acetone : hexane (1 :1 v/v)	Silica gel impregnated with sulfuric acid and then florisil column	GC/ECD[37]
Freeze-dried milk	Freeze-dried milk was mixed with florisil then extracted with supercritical fluid extraction with carbon dioxide	Supercritical fluid chromatography	GC/MS GC/ECD[38]
Bovine adipose tissue	Liquid extraction with hexane	Florisil-SPE	GC/ECD[39]
Butter fat	-	Dissolved butter fat in petroleum ether then cleaned up with sulfuric acid	GC/ECD[40]
Human and bovine adipose tissues	Liquid extraction with 8% benzene in hexane (v/v)	Florisil	GC/MS[41]
Fish tissues	Supercritical fluid extraction with carbon dioxide	Florisil trapping	GC/ECD[42]
Fish tissues	Soxhlet extraction with acetone : hexane (2:3 v/v)	Silica gel impregnated with sulfuric acid	GC/ECD[42]

Table 1.4 (continued)

Sample	Extraction method	Cleanup method	Determination method
Fish	Liquid extraction with petroleum ether	Florisil	GC/ECD[11]
Fish	Soxhlet extraction with ethylacetate	Gel permeation chromatography	GC/ECD GC/MS[13]
Fish	Matrix solid-phase dispersion with C_{18} coupled with silica gel impregnated with sulfuric acid	No further cleanup	GC/ECD GC/MS[13]
Mussels	Supercritical fluid extraction with carbon dioxide and florisil was added into the extraction cell	No further cleanup	GC/ECD GC/MS[44]
Fat	Dissolved fat in ethylacetate-cyclohexane (1:1 v/v)	Cleanup method was used in series gel permeation chromatography-high performance liquid chromatography (normal phase)	GC/ECD[45]
Human body fluids	No liquid extraction step	Florisil column	GC/MS GC/ECD[46]

Table 1.4 (continued)

Sample	Extraction method	Cleanup method	Determination method
Plasma	Plasma mixed with acetonitrile	Florisil SPE	GC/MS[7]
Human serum	Serum was denatured protein with formic acid	C_{18} disk and then sulfuric acid washed	GC/ECD GC/MS[47]
Human serum	Liquid extraction with hexane : ethylether (1:1 v/v)	Silica gel	GC/ECD[48]
Human blood	Sonication and protein precipitation with acetic acid : 2- propanol (4:1 v/v)	Lipid decomposition with sulfuric acid onto styrene-divinyl benzene SPE then cleanup further with aluminium oxide and silica gel impregnated with sulfuric acid in the same column	GC/ECD[49]

1.1.6.2 Determination step

Gas chromatography is widely used for determination of PCBs. It has several advantages over other techniques such as very sensitive detector, easy in operation, low cost in operation, very efficient in separation, good reproducibility and the possibility of coupling with some identification devices such as IR and MS.

In GC process includes injection, separation and determination. The injection techniques which widely used for determination of PCBs are splitless and on column injection. The separation is based on packed and capillary columns.

Bu t recently, capillary columns are widely used. Capillary columns were introduced in the early 1980s.[2] These column are easier to use, longer lasting, high resolution and many types of stationary phase available. The liquid stationary phase commonly used in PCBs analysis are polymethylsiloxane and phenylmethylpolysiloxane with 25-60 m long and 0.2-0.32 mm I.D..

The GC detectors most widely used in determination of PCBs are electron capture detector (ECD) and mass spectrometer (MS). Electron capture detector was invented in 1960 by Lovelock and Lipsky.[2] This provided the great revolution in environmental analysis especially organohalogens determination. The ECD give high response to high electron affinity components such as organohalogens, nitro compounds and pthalates. However, these are some disadvantages such as low linear dynamic ranges and its interference may be caused by a number of compounds, including fatty compounds and elemental sulfur. Besides, it is vulnerable to dirt and overloading. The second most frequently used detector in PCBs analysis is mass spectrometer (MS). This can provide the second dimension of data, i.e. mass spectrum. It permits confirmation of identification and also the use of labelled compounds as recovery surrogates. There are two modes which used in PCBs analysis : electron impact (EI) and negative-ion chemical ionization (NICI).

1.2 Solid Phase Extraction

1.2.1 Introduction to solid phase extraction (SPE)

Solid phase extraction consists of bringing a liquid or gaseous solution in contact with a solid phase which is capable of selectively adsorbing analytes and/or matrix on its surface.[50] Synonyms of SPE include liquid–solid extraction, column extraction, bonded phase extraction or selective adsorption techniques. SPE is today one of the popular sample preparation methods. There are many formats of SPE available now[51] such as disposable cartridges which have been introduced for more than 20 years, precolumns for the on-line coupling with liquid chromatography in the early 1980s, and recently in the disk format.[52] In the disk SPE, the sorbent particles are tightly held within an inert matrix of polytetrafluoroethylene (PTFE) at the ratio of 90% sorbent and 10% PTFE by weight. The dense particles packing and uniform

distribution within the disks provide a great improvement in the extraction efficiency and reproducibility. Thus, high flow rate and large amount of samples can be extracted efficiently. For purposes of convenience, a disposable cartridge for SPE is shown in Figure 1.2. Variation of extraction disks is illustrated in Figures 1.3 and 1.4. Some advantages of SPE can be summarized as follows.

- Reduce the organic solvent usage and the toxic waste
- Reduce cost and labor in analysis
- Large choice of sorbents
- Easy to operate in the on-line mode
- Ability to use on site

However, a key SPE problem remains the method development.

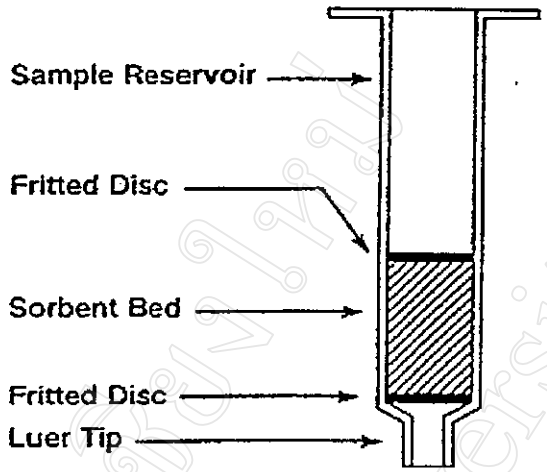


Figure 1.2 Disposable cartridge solid-phase extraction.[53]

Figure 1.3 Empore Extraction Disk, 3M, in 47 and 90 mm membrane diameters.[52]

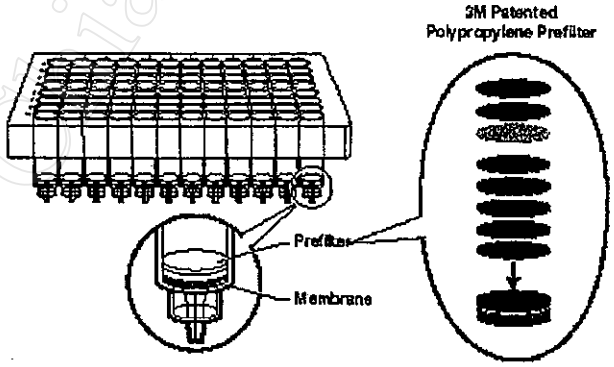


Figure 1. 4 Empore 96 well Extraction Disk Plates and the Filter Plates.[52]

1.2.2 Use of a SPE column

The SPE columns can be operated in two different ways.

1.2.2.1 Batchwise or static method

This method is performed by mixing the sorbent and test solution in a tube and both phases are separated by centrifugation or filtration.

1.2.2.2 Dynamic method

This method is performed by passing the test solution through the packed column filled with a suitable sorbent. The test solution can be drawn through the cartridge by using a vacuum device, as shown in Figure 1.5, or by means of centrifugation.

1.2.3 Operation steps

The operation of SPE consists of five steps,namely wetting, conditioning, adsorption, washing and elution.

1.2.3.1 Wetting

This step is used to ensure a good contact between the analyte and sorbent in the adsorption step. It is important that the sorbent remains wet in the following two steps. Polar and ion-exchange sorbents can be prewetted with water miscible solvents such as methanol or acetonitrile. As for normal phase sorbents, they can be prewetted with nonpolar solvents capable of dissolving the analytes.

1.2.3.2 Conditioning

Solvents or buffers similar to the test solution are used. For the ion-exchange sorbents, a buffer must be used with a pH at which the analyte and the functional ionic sites have an opposite charge.

1.2.3.3 Adsorption (loading)

The test solution is passed through the sorbent at a controlled flow rate. There fore, the analyte is sorbed by the sorbent.

1.2.3.4 Washing

A suitable solvent is passed through the sorbent to remove interfering matrix components, while the analyte still remains sorbed. This step is optional.

1.2.3.5 Elution

The analyte is desorbed from the sorbent with an appropriate solvent.

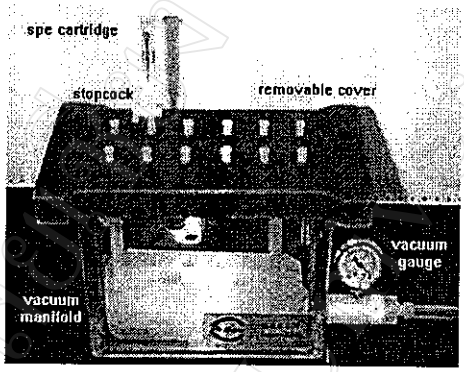


Figure 1.5 Vacuum manifold.[54]

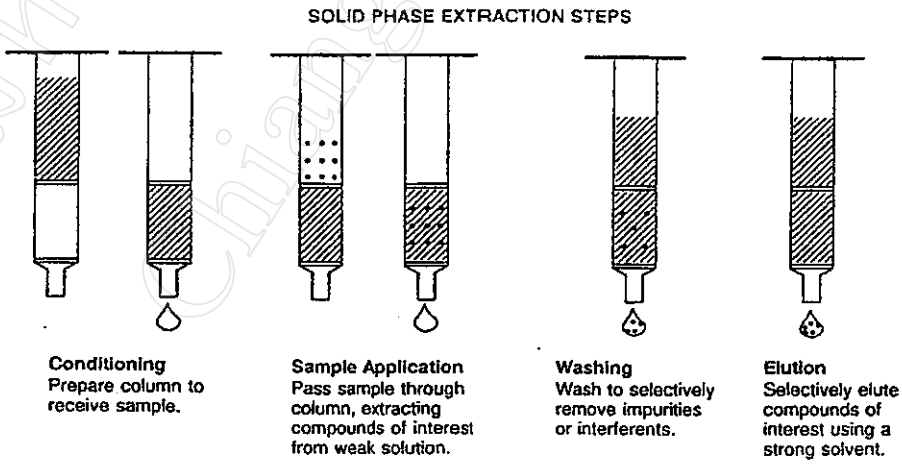


Figure 1. 6 Solid – phase extraction steps.[53]

1.2.4 Method development in SPE

The development of a SPE method requires the selection of the correct sorbent, optimum adsorption, washing and elution conditions. To achieve this object, it is necessary to consider a number of analyte and matrix characteristics, interaction between the analyte, matrix and sorbent :

- Analyte characteristics : pKa, solubilities, molecular mass, polarity, structure and their functional groups.
- Matrix characteristics : polarity and the presence of co-extractable compounds.
- Elution order : relevant information on the elution order can be obtained from HPLC system.
- Interaction mechanisms : type of binding force, van der Waals force, hydrogen bonding, dipole–dipole interaction, induced dipole-dipole interaction, electrostatic interaction, and coordination bond.

To develop a method in SPE, there are many parameters which can be optimized, as described in the following.

1.2.4.1 Adsorption

The analyte-sorbent interaction must be enhanced, while the matrix-analyte and matrix-sorbent interactions must be reduced. In the ion-exchange system, this can be performed by adjusting pH, ionic strength, pretreatment of the test portion, such as dilution, precipitation. In the reversed phase system, this can be improved by increasing the polarity of the adsorption medium e.g. the addition of water or salt. In the normal phase system, this can be improved by decreasing the polarity of the adsorption medium e.g. removing excess of water, adding low polarity organic solvents.

1.2.4.2 Washing

The conditions must be chosen to retain the analytes of interest but remove the interfering matrix components. This requires a good knowledge about the properties of analytes and matrix. For the C_{18} cartridge, the solvents are restricted to polar solvents to prevent co-elution of analyte. For a polar cartridge, they can be washed with appropriate apolar organic solvents. In using an ion-exchange sorbent,

whenever the pH and ionic strength of solution are adjusted to retain the analytes, polar as well as apolar solvents can be used to remove both polar and apolar interferences.

1.2.4.3 Elution

The elution solvent must desorb the analyte in as small a volume as possible to avoid co-elution of interferences and further preconcentration. In a normal phase system, polar solvents such as water, methanol, isopropanol and acetic acid can be used. In a reversed phase system, solvents with low polarity such as methanol, hexane and isooctane can be used. In an ion-exchang system, pH modification to uncharge the analytes may be required. Additionally, the ionic strength can be increased or counterions added so that analytes are replaced from the sorbent. However, the solvent must be chosen on the basis of appropriateness to further analysis or changing the solvent may be required.

1.2.4.4 Selectivity of sorbents

The selectivity of a sorbent is determined by the difference in affinity for the analyte compared to the matrix compounds for lipophilic analytes, instead of C_{18} sorbent, C_2 or intermediate polarity sorbents such as cyano bonded phase can be used. For ionizable analytes, the ion-exchange sorbents can be used. The highest selectivity can be obtained from the sorbents forming covalent bond with the analytes such as platinum-silica bonded phase. Such sorbents are selective to phenylurea herbicides and the interferences, anilines, can be removed. If insufficient selectivity is obtained from one sorbent, a dual cartridge (tandem SPE) could be used. Each sorbent should have a different primary interaction mechanism, such as in the combination of an apolar phase with a normal phase. Schenck *et al.*[55] used three columns, namely diatomaceous earth, C_{18} and alumina to cleanup vegetable oil and butter fat samples for determination of organochlorine and organophosphorus pesticides.

1.2.4.5 Selectivity of the test solution systems

For small molecular mass and apolar sorbents, the retention can be increased by increasing hydrophobic properties of analytes. These can be done in several ways such as ion-pair formation or complex formation. Ma *et al.*[56] used on-line SPE in conjunction with flame atomic absorption spectrometry to determine

cadmium, copper and lead in the digested solutions of solid environmental samples. C_{18} was used as sorbent with diethylammonium-N,N-diethyldithio-carbamate (DDDC) or ammonium diethyldithio phosphate (DPPA) as complexing reagent.

1.2.4.6 Flow rate of loading

The importance of the flow rate and whether the flow rate needs to be strictly controlled during SPE is not always made clear in the literature. Some authors claimed that high flow rate did not effect on the recoveries due to small particles and high surface area of the sorbents. On the contrary, other authors claimed that flow rate can have an influence on the recoveries because both the adsorption and elution steps involve an equilibrium process. Thus at higher flow rates, non-equilibrium may exist.

1.2.4.7 Capacity of SPE columns

The capacity of a SPE sorbent can be defined as the maximum amount of analyte and matrix, retained by a given mass of sorbent from a specific test solution. The capacity is primarily controlled by many factors such as wetting and conditioning of the sorbent, the solvent and pH of the test solution, type and mass of the sorbent. In the optimization to increase the capacity, the amount of sorbent is the last possibility to attempt.

1.2.5 Types of sorbents

1.2.5.1 Silica–bonded phase sorbents[50]

The silica–bonded phases used in SPE are irregular particles with size between 30-60 μm. They can be synthesized by a reaction of silanol groups on the silica surface with chloroalkyl or alkoxy alkyl silanes, as shown in Figure1.7. A reaction with monofunctional silanes gives only monolayer while di- or tri-monofunctional silanes can give mono- or polymer layer depending on the reaction condition. In synthesization, the residual silanol groups which are polar and acidic sites still remained. These sites have interaction with the analyte and can interfere in the analysis. Therefore, some silica bonded phases are "end-capped", i.e. modified with a further reaction with trimethylchlorosilane to protect some residual silanol groups as shown in Figure1.8. These smaller chlorosilanes can react with surface silanol groups even in steric conditions. However, after end capping process about

30% of the silanol groups still remain and can possibly interfere the extraction process. But recently, nonendcapped C_{18} silicas and monofunctional C_{18} silicas have been developed[51] with an aim is to increase the number of non-modified silanol groups at the bonded silica surface in order to provide secondary polar interactions with analytes. By varying types of organochlorosilane reagents, many types of sorbents are obtained as shown in Table 1.5. There are also many types of silica-bonded phase. Some of them are shown together with corresponding analytes and samples in Table 1.6.

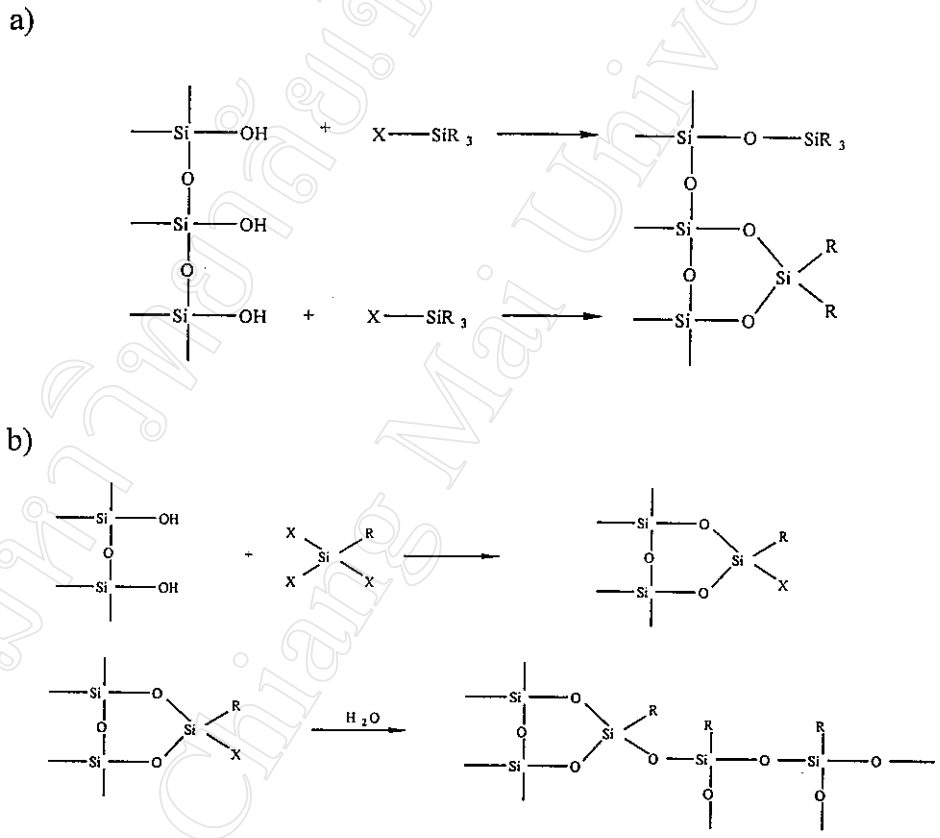


Figure 1.7 Reaction of silica surface silanols with mono-, di- or trialkyl chlorosilanes : a) Reaction with mono-, di- or tri- functional silanes resulting in monolayer, and b) Reaction with di and trifunctional silanes resulting in polymer layers.[50]

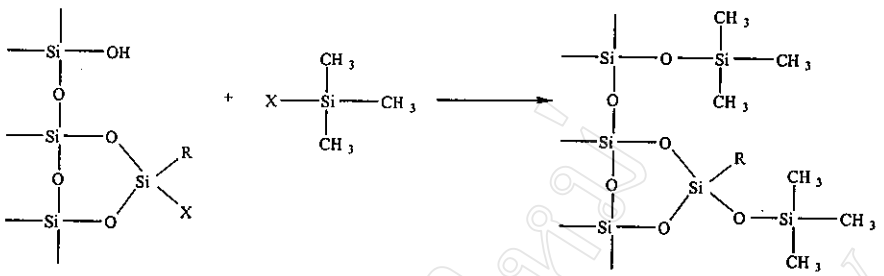


Figure 1.8 Endcapping processes.[50]

Table 1.5 Alkyl–bonded functionalities of commercially available silica bonded phases[50]

Phase	Bonded moiety
Apolar phases	
C_1 methyl	Si-CH_3
C_2 ethyl	Si-CH_2CH_3
C_3 propyl	Si-$(CH_2)_2CH_3$
C_4 butyl	Si-$(CH_2)_3CH_3$
C_6 hexyl	Si-$(CH_2)_5CH_3$
C_8 octyl	Si-$(CH_2)_7CH_3$
C_{18} octadecyl	Si-$(CH_2)_{17}CH_3$
CH cyclohexyl	Si-⬡ Si-CH_2CH_2-⬡

Table 1.5 (continued)

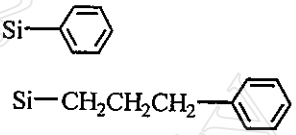
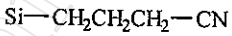
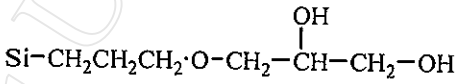
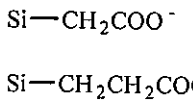
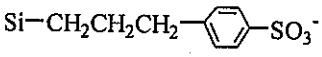
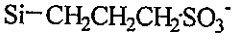
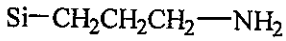
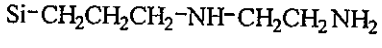
Phase	Bonded moiety
Ph phenyl	
Polar phases	
Si silica	
CN cyanopropyl	
2OH diol	
Ion exchange phases	
CBA carboxylic acid	
SCX benzenesulphonic acid	
PRS propanesulphonic acid	
NH_2 aminopropyl primaryamine	
PSA N-propylethylenediamine primary/secondary amine	

Table 1.5 (continued)

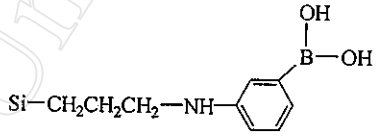
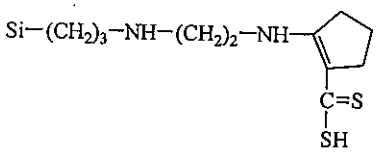
Phase	Bonded moiety
DEA diethyl ammoniopropyl tertiary amine	$Si-CH_2CH_2CH_2-NH^+-(CH_2CH_3)_2$
SAX trimethyl ammoniopropyl quaternary amine	$Si-CH_2CH_2CH_2-N^+-(CH_3)_3$
Covalent binding	
PBA phenylboronic acid	
Metal-loaded phases	
ACDA 2-amino-1-cyclopentene-1-dithiocarboxylic acid modified silica	

Table 1.6 Application of silica–bonded phases with different types of analytes and samples

Silica-bonded phase	Analytes	Samples
C_{18}, on-line SPE with FIA-AAS	Cadmium, copper and lead	Solid environmental samples[56]
C_{18}, automated SPE	Organochlorine pesticides	Drinking waters[57]
C_{18}, cartridge	Polychlorinated biphenyls	Water samples[27]
C_{18}, cartridge	Triazine herbicides, Organochlorine, carbamate and acidic pesticides	Surface and ground waters[58]
C_{18}, cartridge	Various pesticide residues	Wines[59]
C_{18}, disk	Oranochlorine pesticides and polychlorinated biphenyls	Human serum[47]
C_{18}, cartridge	Polar acidic herbicides	Surface waters [60]
C_{18}, partially non-endcapped, cartridge	Triazines and their Metabolites	Aqueous samples[61]
C_{18}, On-line SPE with LC-APCI/MS	Organophosphorus Pesticides	Ground waters[62]
C_{18}, cartridge	Organophosphorus pesticides	Oils and fats[63]
C_{18}, On-line SPE with HPLC	Aspirin and salicylic acid	Human plasma[64]
C_8 disk, ion-pair SPE	Quaternary ammonium Herbicides	Waters[65]
C_8, matrix solid phase dispersion (MSPD)	Carbamate residues	Fruits and vegetables[66]

Table 1.6 (continued)

Silica-bonded phase	Analytes	Samples
C_8, disk	Organochlorine Organophosphorus pesticides and triazines	Soil leachate[67]
Cyanopropyl – bonded silica, cartridge	Polar organic matter	Airborne particles[68]
Cyanopropyl – bonded silica	Papaverine, weakly basic drug[69]	

1.2.5.2 Polymeric resin sorbents

- Poly (styrene – divinylbenzene) sorbent, PS-DVB

This copolymer is normally used in SPE. This resin has high surface area in the range of 700-1200 $m^2 g^{-1}$. Structure of Poly (styrene–divinylbenzene) is shown in Figure 1.9. The characteristics of commercially available PS-DVB for LC and SPE are given in Table 1.7. For many organic compounds, especially in polar compounds, they are more retained on PS-DVB than C_{18} silica. Some applications of PS-DVB are given in Table 1.8.

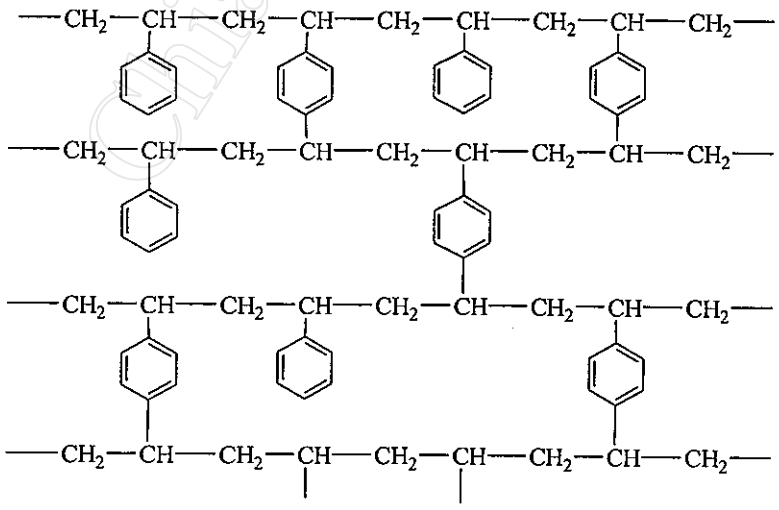


Figure 1.9 Structure of poly (styrene – divinylbenzene).

Table 1.7 Characteristics of commercially available PS-DVB used as LC and SPE sorbents[51]

Tradenames	Manufacturer	Structure	Porosity (°A)	Surface area ($m^2 g^{-1}$)
Bond – Elut ENV	Varian	PS – DVB	450	500
Bond – Elut PPL	Varian	Functional	300	700
SDB	J.T. Baker	PS-DVB-EVB[a]	300	1060
Speedisk–DVB	J.T. Baker	PS-DVB	150	700
Empore disk	J.T. Baker	PS-DVB	-	350
Li Chrolut EN	Merck	PS-DVB	80	1200
Isolute ENVT	IST	PS-DVB	100	1000
Envichrom P	Supelco	PS-DVB	140	900
Chromabond HR – P	Machery – Nagel	PS-DVB	-	1200
Porapak RDX	Waters	PS-DVB-NVP[b]	55	550
OASIS HLB	Waters	PS-DVB-NVP	55	800
PRP – 1	Hamilton	PS-DVB	75	415
P LRPS	Polymer Labs	PS-DVB	100	550
Hysphere – 1	Spark Holland	PS-DVB	-	>1000

[a]EVB : ethylvinylbenzene [b]NVP : N-vinylpyrlolidone

Table 1.8 Applications of PS-DVB sorbents with different types of analytes and samples

Sobents	Analytes	Samples
PS-DVB, cartridge	Herbicides	River waters, dissolved and suspended phases[70]
PS-DVB, on-line with GC/FID	Phenols	Waters[71]
PS-DVB, on-line and off-line with HPLC	Phenyl ureas, triazines and Organophorus pesticides	Waters[72]
PS-DVB, on-line with HPLC-diode array detection	Phenolic compounds	Sherry wine[73]
PS-DVB, cartridge	Phenolic compounds	Effluent from sewage treatment plants[74]

- Chemically modified polymeric resin sorbents

The modified polymeric resins are made to prepare the sorbent surface suitable for specific analytes. Many functional groups are introduced to the polymeric based. Masque *et al.* introduced the acetyl[75] and benzoyl groups[76] to the PS-DVB. These resins are used for determination of some phenolic compounds and pesticides in water which show a higher breakthrough volume than the unmodified PS-DVB. Merdivan *et al.*[77] synthesized phosphonate–PSDVB resin which had high selectivity to uranium (VI).

1.2.5.3 Carbon–based sorbents

The carbon sorbents consist of activated carbon, molecular sieves, graphitized carbon black (GCB) and porous carbon.[78] They differ in their physicochemical characteristics, pore size, shape, surface area, volume of pores, functional groups of surface and chemical inertness. In solid phase extraction, activated carbon, porous carbon and graphitized carbon black are widely used. They

have some unique characteristics which provide better results in some type of analytes such as polar pollutants.

Activated carbon has a very complex structure containing a wide range of functional groups including phenolic, carboxylic, carbonylic, aldehydic, etheric, peroxidic, quinone and lactone. Thus, there are many binding mechanisms with analytes including hydrophobic interaction, charge transfer complexation, hydrogen bonding, ion exchange and another specific interaction.

Graphitized carbon black (GCB) is a material with a homogeneous surface and without micropores. Oxygen complexes with a chromene–like structure may be present as burnt–off residues originating from the heating of carbon black. In the presence of water these surface groups are rearranged to form benzpyrylium salts as shown in Figure 1.10.

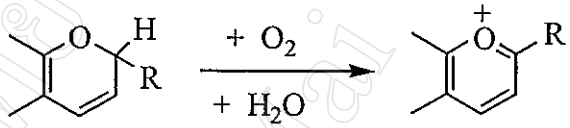


Figure 1.10 Forming of benzpyrylium salt in the presence of water.[78]

Benzpyrylium salts are responsible for binding anions via electrostatic forces. Thus, GCB can act as both an anion exchanger and a nonspecific sorbent.

Porous carbon sorbents are materials with a homogeneous hydrophobic surface. They are produced by impregnating a suitable silica gel or other porous template materials with a phenol–formaldehyde resin mixture, saccharose or another material. After polymerization within the pores of the template materials, the polymer is converted to glassy carbon by heating in an inert atmosphere to about 1000°C. The silica template is removed by alkali. Finally, the material is fired in an inert atmosphere at 2000–2800°C to anneal the surface, remove micropores and depending on the temperature, some degree of graphitization occurs. The particle size, shape, porosity and pore size are determined by template material. The surface

chemistry is determined by the final heat treatment and another subsequent chemical treatment. Some applications of carbon sorbents are shown in Table 1.9.

Table 1.9 Applications of carbon sorbents with different types of analytes and samples

Sorbents	Analytes	Samples
GCB, cartridge	Acidic organic compounds	Waters[79]
GCB, cartridge	Phenyl urea herbicides and their metabolites	Water[80]
GCB, cartridge	Benzene- and naphthalene sulfonates	Wastewaters[81]
Porous carbon, cartridge	Dicarboxyimide fungicide residues	Wines[82]
Activated carbon, Membrane	Pesticide multi-residues including organochlorine, organophosphorus and organonirogen compounds	Vegetables and fruits[83]
GCB, cartridge	Sulfonylurea herbicides	Waters[84]
GCB, cartridge	Imidazolinone herbicides	Waters and soils[85]

1.2.5.4 Ion-pair and ion-exchange sorbents

Ionic or ionizable analytes can be extracted by ion-pair and ion-exchange sorbents. Cation exchangers include weak ion exchangers, carboxylic acid, and strong ion exchangers, aromatic and non-aromatic sulfonic acids groups. Anion exchangers include weak ion exchangers, primary or secondary amino groups, and strong ion exchangers, quaternary amine forms. Ion-pair extraction is normally selected with a C_8 or C_{18} sorbent with some ion-pair reagents, tetramethyl, tetrabutyl- or cetyltrimethyl ammonium.

Nelieu *et al.*[86] used tandem SPE, coupled between C_{18} and cation exchanger (propylbenzene sulfonic acid), for determining atrazine degradation

products in water samples. By coupling these two sorbents, fractionation of atrazines according to their polarties was achieved.

Castro *et al.*[65] used C_{18} extraction disks with ion-pair on-line solid phase extraction to extract quaternary ammonium herbicides in water samples. Heptafluorobutyric acid was used as ion-pair reagent and separation was performed in ion-pair mode, using liquid chromatography-mass spectrometry detection.

Li and Lee[87] used the long chain ionic surfactant, cetyltrimethyl ammonium bromide, loaded into the C_{18} SPE. They called their procedure as dynamic ion-exchange solid phase extraction (DIE–SPE). The surfactant molecules can act as ion-exchange sites to extract ionized organic analytes from aqueous samples. At the same time, the C_{18} can act as reversed phase sorbent. The phenolic compounds are used as model compounds to evaluate many effects in the extraction.

1.2.5.5 Mixed–mode sorbents[51]

These sorbents provide more than one interaction with the analyte. In operation, firstly the sample solutions are adjusted to pH at which analytes are in neutral form. Thus, analytes and other matrix compounds are retained by hydrophobic interactions. After washing with buffer or water, the analytes are in ionic forms, thus they are retained by ionic interactions. Since the ionic interaction is much stronger than a hydrophobic one, a washing with methanol does not break the ionic interactions, but only the hydrophobic one. This is the cleanup step in which the matrix compounds are eluted from the cartridge. Finally, the desorption is obtained by using a basic solution in order to break the ionic interactions.

Mills *et al.*[88] used silica and styrene-divinyl benzene based mixed mode resins which contained C_8 or C_{18} and sulfonated cation exchange groups to isolation of neutral triazine compounds from water and of the basic drug, benzoylecgonine, from urine.

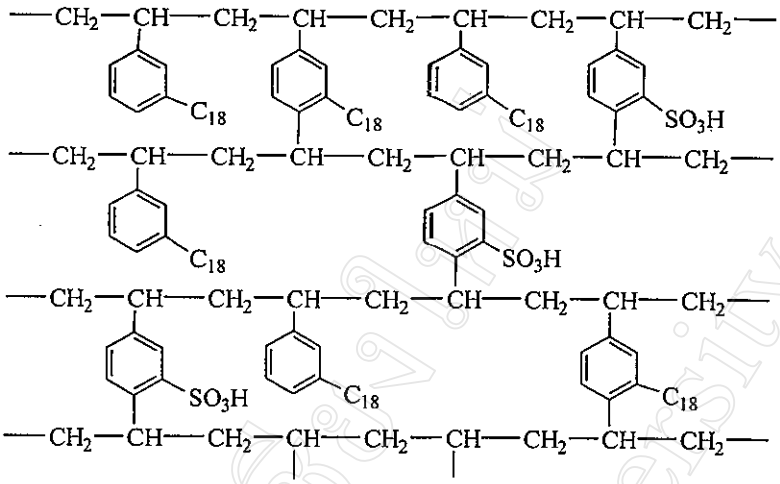


Figure 1.11 Structure of styrene-divinylbenzene based mixed mode resin containing which contained C_{18} and sulfonated cation-exchange groups.[88]

1.2.5.6 Normal phase solid-phase extraction sorbents[51]

Normal phase sorbents included bare silica, alumina, florisil and silica chemically modified by polar groups such as amino, cyano or diol groups. The main interaction of these sorbents is adsorption. Hopper[89] used diatomaceous earth to cleanup fruits and vegetables which were extracted with acetone to determine some organophosphorus pesticides. Lino *et al.*[90] used florisil SPE to cleanup human serum which was extracted with hexane or hexane : acetone (1:1) to determine some organochlorine pesticides.

1.2.5.7 Restricted access matrix sobents[51]

These sorbents called "restricted access materials (RAMs)" are combination of size exclusion and simultaneous enrichment of low molecular mass analytes at the inner pore surface. The low molecular mass analytes are retained by conventional retention mechanisms such as hydrophobic, ionic or affinity interactions.

Vielhauer *et al.*[91] determined 8-methoxypsoralen drug in plasma by using the novel internal surface reversed phase precolumn packing materials, alkyldiol silica (ADS). This sorbent has a hydrophilic and electroneutral outer

particle surface and hydrophobic internal pore surface. In the synthesis of ADS, silica is bonded with hydrophilic phase, i.e. glyceryl propyl-diol groups. Then the diol groups are reacted with fatty acid chlorides and finally treated with esterase to remove fatty acids exclusively from the outer surface. The molecular mass cut-off of this ADS in approximately 15 kD. This exclusion limit prevents macromolecules from interacting with the hydrophobic internal phase.

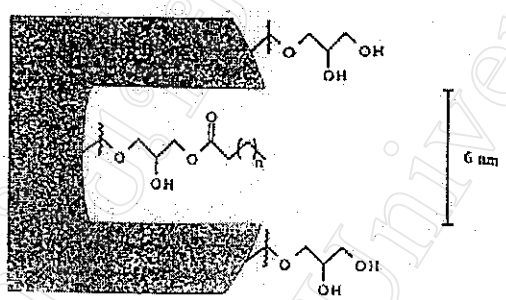


Figure 1.12 Schematic representation of bonded-phase topography of Alkyl-Diol silica particulate.[91]

1.2.5.8 Immunoaffinity extraction sorbents[51]

These sorbents involved antigen-antibody interactions, thus providing selective extraction methods based on molecular recognition. Antibodies are covalently bonded onto an appropriate sorbent to form a so-called "immunosorbent (IS)". These antibodies are highly selective towards the analyte used to initiate the immune response with a high affinity. Thus, the analyte was extracted from the complex matrices and the problems of matrices co-extraction is circumvented.

A typical SPE sequence of immonosorbent SPE is very similar to conventional C_{18} cartridge. It includes a conditioning step with water, the sample loading, a desorption step with a methanol-water mixture and reconditioning with PBS (phosphate–buffered saline) solution.

Farjam *et al.*[92] used a liquid chromatographic column-switching system containing a dialysis unit and an anti-aflatoxin immunoaffinity precolumn for the automated determination of aflatoxin M1 in milk. The water was used as the

acceptor phase for dialysis unit. Then the water containing aflatoxin was pumped into the immuno precolumn and desorbed with methanol-water (70:30 v/v) into HPLC-C_{18} system with the fluorescence detector.

1.2.5.9 Moleculary imprinted polymer (MIP) sorbents[51]

The MIP is a technique based on creating cavities in a highly cross-linked polymer matrix, that correspond to the size and shape of the print molecule.[93] The synthesis is made by assembly of monomers around a template molecule and a subsequent polymerization using a cross-linker providing a rigid material. Then, the template molecules are removed and the resulting polymers have cavities which are the "imprints". The recognition between polymers and the print molecules or related structure compounds is due to the formation of functional groups in a specific arrangement, the shape selectivity, and mixture of hydrogen bonding, hydrophobic and electronic interactions.

Bjarnason *et al.*[94] used simazine as the template molecule to produce MIP which selective to triazine herbicides. The MIP was coupled with C_{18}-SPE and used online with RP-HPLC for determination of some triazine herbicides in water, apple and urine samples.

1.3 Gas Chromatography

In the late 1800s, Mikhail Tswett[95] used an adsorbent to separate and isolate various plant pigments. He later used the word "chromatography" to describe this process.[95] In the late 1930s and early 1940s, Martin and Synge[96] introduced liquid-liquid chromatography by supporting the stationary phase. They recommended that gas could be used as mobile phase instead of liquid phase. They concluded that the using of gas would be advantageous due to higher diffusion thus more efficient in separation[96]. In 1951, Martin and James returned to their previous concept, gas chromatography. They invented the simple gas chromatograph consisting of a straight packed column of three feet in length that was held vertically, thermostat in the vapour jacket and an automatic titration device as detector.

Today gas is used as the mobile phase and solid or liquid on the supporting material as the stationary phase in gas chromatography. The sample is injected into the hot injector then vaporized and brought through the column with the aid of carrier gas. At the end of the column, the detector is connected. The response signals from the detector are plotted with time or mobile phase volume thus chromatogram is obtained.

1.3.1 Some important terms in chromatography[97,98]

For purposes of clarity, some important terms in chromatography are presented here.

1.3.1.1 Distribution constant (K)

$$K = C_S/C_M \quad (1.1)$$

where C_S = concentration of analyte in the stationary phase

and C_M = concentration of analyte in the mobile phase

The larger the value of K, the more of analyte sorbs in the stationary phase and thus retain longer in the column.

1.3.1.2 Retention time and retention volume

V_R = retention volume, the volume of mobile phase required to carry the component molecules through the chromatographic system

V_O = void volume, the total volume of mobile phase within the length of the column

t_R = retention time , the time is taken by component molecules through the chromatographic system

t_M = hold-up time, the time is taken by the mobile phase or non sorbed species through the chromatographic system

$$V'_R = V_R - V_O \quad (1.2)$$

V'_R = adjusted retention volume

$$t'_R = t_R - t_M \quad (1.3)$$

t'_R = adjusted retention time

1.3.1.3 Capacity factor (k)

The capacity factor describes for the retention characteristics of the number of component molecules in the stationary phase to the number of component molecule in the mobile phase per unit volume.

$$k = (V_S C_S)/(V_M C_M) \quad (1.4)$$

where V_S = volume of the stationary phase

and V_M = volume of the mobile phase

From $K = C_S/C_M$ (1.1)

$$k = K V_S/V_M \quad (1.5)$$

$$k = (t_R - t_M) / t_M \quad (1.6)$$

1.3.1.4 Selectivity factor (α)

The selectivity factor for the adjacent peak of components A and B is a function of the type of mobile phase, stationary phase and column temperature. For the separation to occur, α is more than 1.0.

$$\alpha = k_B/k_A \quad , \quad k_B > k_A \quad (1.7)$$

$$= t'_{RB}/ t'_{RA} = V'_{RB}/V'_{RA} \text{ , } t'_{RB} > t'_{RA} \text{ and } V'_{RB} > V'_{RA} \quad (1.8)$$

1.3.1.5 Column efficiency

Two terms are used to indicate the column efficiency

- plate height (H)
- number of theoretical plates (N)

$$N = 16 (t_R /W_b)^2 \quad (1.9)$$

where W_b = peak width at baseline

Or

$$N = 5.54 (t_R/W_h)^2 \quad (1.10)$$

where W_h = peak width at half height

$$H = L/N \quad (1.11)$$

L = column length

From the equation above, the column efficiency increases as N greater or H smaller. Figure 1.13 illustrates a Gaussian peak and relevant chromatographic terms.

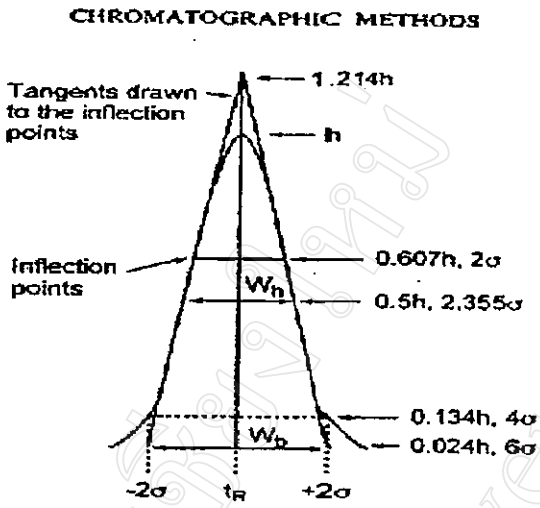


Figure 1.13 Peak heights and widths of a Gaussian.[98]

1.3.1.6 Resolution (R_S)

The resolution is measured to indicate the resolution power of the adjacent peaks, components A and B.

$$R_S = 2\Delta t_R/(W_{bB}+W_{bA}) \quad (1.12)$$

$$\Delta t_R = t_{RB} - t_{RA},\ t_{RB} > t_{RA} \quad (1.13)$$

W_{bB}, W_{bA} = peak width at baseline of components A and B

There are a number of relationships in chromatography. Some relationships are given in Table 1.10..

Table 1.10 Some relationships in chromatography[100]

Linear mobile phase velocity (u)	$u = L/ t_M$	
Volume of mobile phase (V_M)	$V_M = t_M F$ (F = mobile phase flow rate)	
Capacity factor (k)	$K = (t_R - t_M) / t_M$	$k = K V_S/V_M$
Partition coefficient (K)	$K = k V_M/ V_S$	$k = C_S/C_M$
Selectivity factor (α)	$\alpha = t'_{RB}/ t'_{RA}$	$\alpha = k_B/ k_A = K_B/K_A$
Resolution (R_S)	$R_S = 2\Delta t_R /(W_{bB}+W_{bA})$	$R_S = (\sqrt{N}/4)(\alpha-1/\alpha)[(k_B/(1+k_B)]$
Number of plates (N)	$N = 16(t_R /W_b)^2$	$N = 16R_S^2[(\alpha/(\alpha-1)]^2 [(1+k_B) / k_B]^2$
Plate height (H)	$H = L/N$	
Retention time (t_R)	$t_{R(B)} = 16R_s^2H/u\ [\alpha/(\alpha-1)]^2 [(1+k_B)^3 /k^2_B]$	

1.3.2 Chromatographic theory

All chromatographic separations are carried out using a mobile and stationary phase. The different interactions of analytes to mobile and stationary phase provide the different eluted rate and thus separation occurs.

For the component A in the chromatographic system, it is distributed between the mobile and stationary phase with an equilibrium.

$$[A]_S \leftrightarrow [A]_M$$

$[A]_S$ = concentration of A in the stationary phase

$[A]_M$ = concentration of A in the mobile phase

The components with higher affinity to the stationary phase tends to be more retained in the column and vice versa. There are two chromatographic theories widely used in explanation of chromatographic phenomena, plate theory and rate

theory. The plate theory[100] was originally adopted by Martin and Synge from the theory of distillation columns. The chromatographic columns were considered as a series of discrete narrow horizontal layers called "theoretical plates". In each plate, equilibration between analyte, stationary and mobile phase was assumed to occur. The migration of solutes were viewed as stepwise transfers from one plate to the next. In the finite column length, the smaller plate (the more number of plates) thus more efficiency in separation. The plate theory is based on equilibrium process but in the column it does not exist. Furthermore, the plate theory cannot explain the parameters which respond to zone broadening. Thus the new theory, rate theory, was proposed.

1.3.2.1 The rate theory[97]

The rate theory successfully describes the variables that influence the time at which elution band appears as well as the width of the eluted peak.

During the migration of analyte, many thousands of transferring between stationary and mobile phase occurs. The time interval in which components spend in either phase is highly irregular and depends upon their accidentally gaining the environmental energy. The particles which are incorporated in the mobile phase for a greater than average time will migrate more rapidly than the most particles and thus move towards the head of the peak. In contrast, the particles which are incorporated in the stationary phase for a greater than average time will migrate more slowly than the most particles and thus move towards the rear of the peak. From the random individual processes, the symmetric spread of velocity around the mean value occurs. The result is a Gaussian peak. The band broadening process is shown in Figure 1.14.

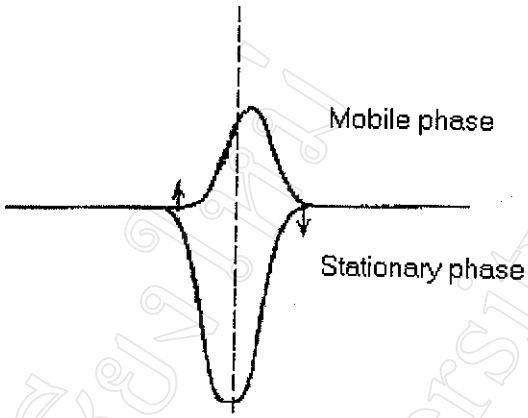


Figure 1.14 Band broadening process in the chromatographic column.[97]

The band broadening will increase as it moves down the column because more time is allowed for spreading to occur. Thus, the zone breadth is directly related to residence time in the column and inversely related to the velocity of mobile phase.

The important paper using the rate theory to explain band broadening in packed columns was published by van Deemter, Klinkenberg, and Zuiderweg[97] in 1956. The broadening can be explained in the term of plate height, H.

$$H = A + B/u + Cu \qquad (1.14)$$

H = plate height

u = linear velocity of mobile phase

A = eddy diffusion

B = longitudinal molecular diffusion

C = mass transfer in the stationary liquid phase

In the capillary columns,there is no packing inside and hence the term A does not exist. This has resulted into the new equation, Golay Equation,[97] as given below.

$$H = B/u + [C_S + C_M]\, u \qquad (1.15)$$

B/u = longitudinal diffusion

$C_S u$ = mass transfer to and from the stationary phase

$C_M u$= mass transfer to and from the mobile phase

From the equation above, plotting the graph between H and u gives the van Deemter plot, as shown in Figure 1.15.

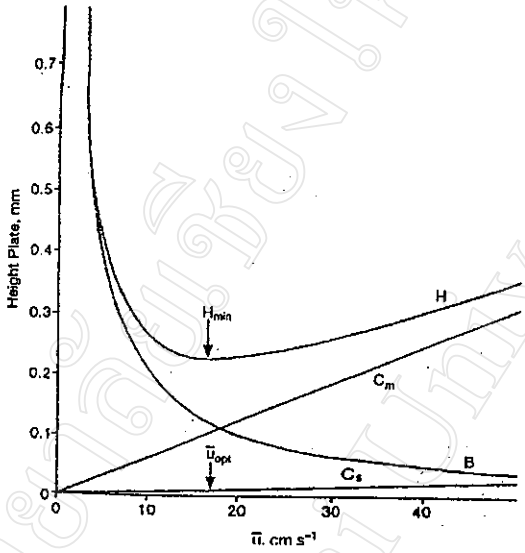


Figure 1.15 van Deemter plot.[97]

From the van Deemter plot, the optimum flow rate (u_{opt}) can be obtained. But in practice, a general chromatographic system is operated at the flow rate higher than this value.

1.3.3 Instruments for gas-liquid chromatography[100]

In the gas-liquid chromatography, the mobile phase is gas and stationary phase is the liquid on the inert support material. The analyte is injected to the hot injector port and then vaporized. The vaporized analyte is transferred through the column with the aid of carrier gas. The separation occurs according to ability of vaporization and affinity to the stationary phase of each compound. Then the eluted molecules are detected in the detector. The signals from the detector are plotted against time, giving what is called chromatogram.

The basic components of the gas chromatography are shown in Figure 1.16.

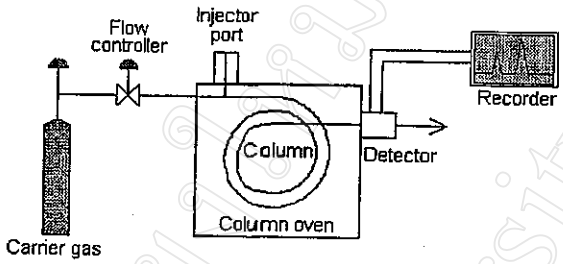


Figure 1.16 Gas chromatograph components.[101]

1.3.3.1 Carrier gas supply

Several gases, including helium, hydrogen and nitrogen can be used as the carrier gas in a gas chromatographic system. The carrier gas must be chemically inert. The suitable carrier gas is dictated by the type of detector used. The carrier gas supply is associated with pressure regulators, gauges and flow meters.

1.3.3.2 Sample injection system

The most common method of sample injection is the use of a microsyringe.

1.3.3.3 Column and column oven

The column oven is used to control the column temperature to a few tenths of a degree for precise work with the aid of a thermostat. The temperature used in separation depends on the analyte's boiling point and the degree of separation required. In the broad boiling point range sample, the temperature program is required to provide reasonable separation and analytical time.

The packed and capillary columns are normally used in GC. The capillary column is preferred for today with its high resolution property. The capillary column is normally produced from fused silica with the length 10-100 m and 0.1-0.53 mm. internal diameter. The capillary column consists of three types namely, wall-coated open tubular (WCOT), support-coated open tubular(SCOT) and porous-layer

open tubular(PLOT) column. The WCOT is widely used. In WCOT, the liquid stationary phase is coated in the inner wall of column with 0.1-5 μm film thickness. But recently, the bonded phase of which the liquid stationary phase is chemically bonded with the silanol groups in the inner wall of the column is widely used. This provides more chemically inert and higher thermal stability. Many types of liquid stationary phase are commercial available. In this work, three types of the liquid stationary phase are used, i.e., DB-1, HP-608 and DB-1701.The chemical structure of DB-1 and DB-1701 liquid stationary phase are shown in Figure 1.17 and 1.18, respectively.

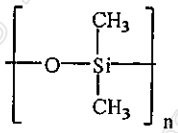


Figure 1.17 Structure of DB-1 liquid stationary phase, 100% dimethylpolysiloxane.[102]

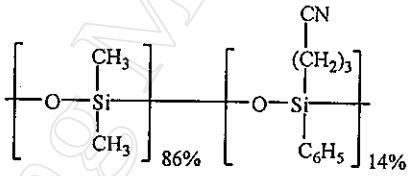


Figure 1.18 Structure of DB-1701 liquid stationary phase, (14% cyanopropylphenyl)-dimethylpolysiloxane.[102]

As for the column HP-608, the structure is not provided by the manufacturer but it is often regarded as a stationary phase with intermediate polarity.

1.3.3.4 Detector

Some characteristics of an ideal gas chromatographic detector are

- Adequate sensitivity
- Good stability and reproducibility

- Large linear dynamic range
- Response is not dependent on flow rate
- Easy to use
- Short response time
- Similarity in response toward all analytes or alternatively selective response toward one or more classes of analytes
- Nondestructive of sample

In reality, no detector exhibits all of these characteristics. Many types of detector are used in the gas chromatographic system such as flame ionization detector (FID), thermal conductivity detector(TCD), electron capture detector(ECD), nitrogen-phosphorus detector(NPD), flame photometric detector(FPD), atomic emission detector(AED), photoionization detector(PID), mass spectrometer(MS) and infrared detector(IR).

In this work, ECD and mass spectrometer were used.

- Electron capture detector[98] (ECD)

The ECD is widely used in trace environmental pollutants analysis especially in high electron affinity compounds such as organohalogens. The ECD cell consists of two electrodes, namely source electrode and collector electrode. The source electrode produces β-particles with high energy. Then this particle interacts with the carrier gas or auxillary gas N_2 or Ar to produce large amounts of thermal electrons which are collected by the collector electrode. The interaction between the thermal electrons and collector electrode produces the standing current or baseline signal. The molecules, AB, which high electron affinity capture the thermal electrons as they pass through the detector thus reduce the detector current and give the negative signals. These signals are subsequently electronically processed to form the chromatogram. Nickel (^{63}Ni) is commonly used as the β-radiation source. Figure 1.19 summarizes the electron capture process and Figure 1.20 illustrates an electron capture detector.

$$N_2 + \beta^- \rightleftharpoons N_2^+ + e^-(\text{thermal})$$

$$2Ar + \beta^- \rightleftharpoons Ar + Ar^+ + e^-(\text{thermal})$$

$$AB + e^-(\text{thermal}) \longrightarrow AB^- \text{ and } A^{\bullet} + B^-$$

Figure 1.19 Electron capture process.[98]

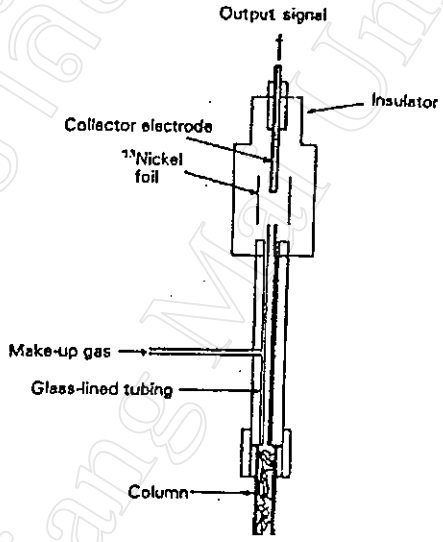


Figure 1.20 Electron capture detector.[98]

• Mass spectrometer

The simple fundamental theory of mass spectrometer is producing the analyte ions either positive or negative ions then separating them according to m/z (mass-to-charge ratio).

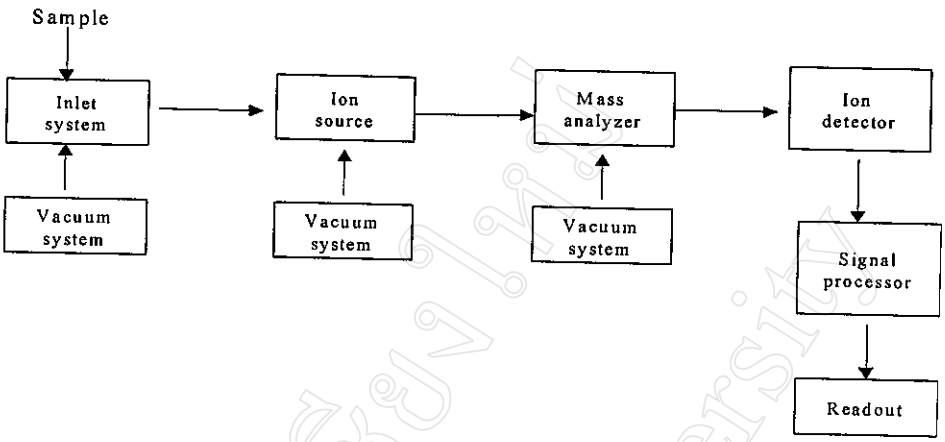


Figure 1.21 Components of a mass spectrometer.[100]

According to Figure 1.21, the sample is introduced into the mass spectrometer via the inlet systems. There are many types of the inlet system such as GC, HPLC, CE, FIA, DIP (Direct insertion probe) etc. Then the sample molecules are passed into the ion source in which ionization occurs. There are several methods to ionize the sample molecules such as electron impact (EI), chemical ionization (CI), fast atom bombardment (FAB), electrospray ionization (ESI) etc. The ionization method is selected on the basis of type of analyte and the analysis objective. After ionization, the ions are passed into the mass analyzer which separates the ions according to mass to charge ratio(m/z). There are many types of mass analyzer commercially available such as magnetic sector, quadrupole, ion trap, time of flight (TOF) and ion cyclotron resonance (ICR). Then the separated ions are detected by the detector, normally electron multiplier. The produced signals are stored and manipulated in the computer.The GC/MS gives the two important data, retention and mass spectrum data . These data are used in the qualitative purpose.

In this work, GC/MS with the ion-trap mass spectrometer was used to identify the interference compounds in Mae-ping water sample. In the ion-trap, the sample molecules were ionized in EI mode to produce the positive ions. These ions were stored at the storage voltage inside the trap. Then the radio frequency voltage was ramped to eject the unstable m/z ions to the detector thus scanning was obtained. Figure 1.22 shows a diagram of a GC/MS with ion trap mass spectrometer.

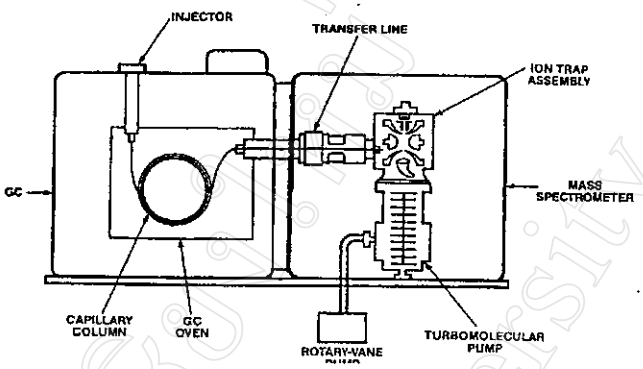


Figure 1.22 The ITS40, GC/MS with ion trap mass spectrometer.[103]

1.3.4 Qualitative analysis

The retention data of the reference standards and sample, retention time, relative retention time or index, is normally used for the qualitative purpose. But the retention data alone can only indicate the probability which the two substances, reference standard and solute in the sample, are the same. Because many compounds can give the same retention data in each GC column, using the stationary phase with different polarity will give more accurate results. Nevertheless, the positive identification should be confirmed by using the spectroscopic methods such as MS or IR in conjunction with the gas chromatographic method.

1.3.5 Quantitative analysis

Quantitative analysis in GC is normally based on comparison of either peak height or peak area of analytes and reference standards. In the peak height analysis, the high precision will be obtained if the peak width variations are controlled such as column temperature, eluent flow rate, rate of sample injection and overloading of the column. This measurement is more accurately determined than peak area analysis in the case of narrow peaks.[100] In the peak area analysis, it is more satisfactory than peak height analysis due to independence of broadening effects. The calculation of analyte concentration is normally based on internal standard or external standard method.

In the internal standard method, a known quantity of internal standard is added to each standard and sample. The ratio of analyte to the internal standard peak areas serves as the analytical parameter with the suitable internal standard, the precision of better than 1% can be obtained.

In the external standard method, the series of standards are injected to the gas chromatograph. The chromatographic response, i.e. peak area or peak height, is plotted versus the concentration. Then the concentration of analytes in the sample is calculated from the constructed calibration graph.

1.4 Aims of the Research

The aims of this research work can be summarized as follows.

1.4.1 To study and obtain the optimum GC conditions for PCBs analysis in milk and water

1.4.2 To investigate and optimize the extraction conditions for C_{18} SPE in analysis of milk and water