CHAPTER II

Literature Review

2.1 Principal types of Organophosphorus Insecticides

The organophosphorus pesticides (OPs) have several commonly used names such as,organic phosphate, phosphorus insecticides, nerve gas relatives, phosphates, phosphate insecticides, and phosphorus esters or phosphoric acid esters (George, 1978). They are all derived from phosphoric acid and are the most toxic of all pesticides to vertebrate animals. Because of their chemial structures and mode of action, they are related to the "nerve gases". Their insecticidal action was observed in Germany during World War II in the study of materials closely related to the nerve gases, sarin, soman, and tabun. Initially, the discovery was made in the searching of substitutes for nicotine, which was in critically short supply in Germany. The organophosphorus insecticides have two distinctive features. First, they are generally much more toxic to vertibrates than are the organochlorine insecticides, and, second, they are chemically unstable or nonpersistent. The latter quality brings them into the agricultural scene to substitute the persistent organochlorines, particularly DDT.

Normally, organophosphate is used as a general term of the insecticides containing phosphorus. But if we consider the atoms attached to the phosphorus as the combination of different alcohols and different phosphorus acids, then OPs are termed as esters. Esters of phosphorus have various combination of oxygen, carbon, sulfur,and nitrogen attached to the phosphorus atom and have different identities. Fig. 2.1 compares the chemical of the six subclasses of OPs structures including the seemingly odd chemical names given to these insecticides, such as phosphate and phosphonate. According to the different hydrocarbon functioning groups, the OPs can be characterised into three classes namely, aliphatic, phenyl, and heterocyclic derivatives.

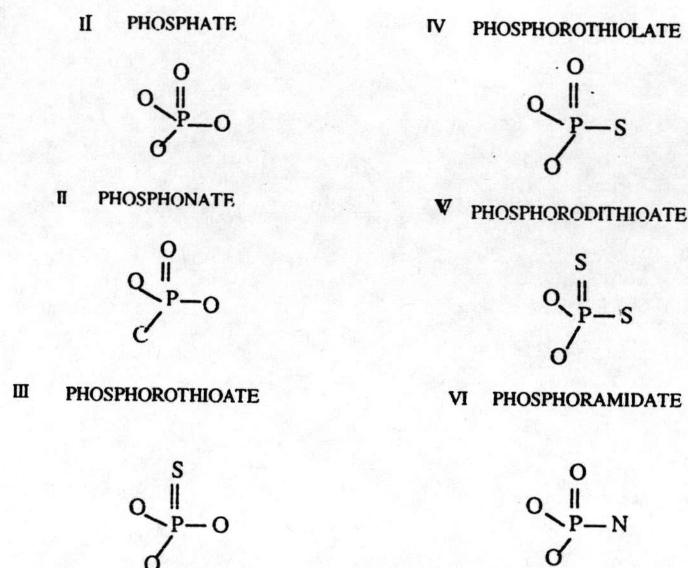


FIGURE 2.1 The subclasses chemical structures of OPs

2.1.1 Aliphatic Derivatives

The term aliphatic literally means "carbon chain," and the linear arrangement of carbon atoms differentiates them from ring or cyclic structures. All of the aliphatic OPs phosphoric acid derivatives bearing short carbon chains.

In 1946, the first organophosphorus TEPP (tetraethyl pyrophosphate) was introduced into agriculture as synthetic pest control agent. It is a useful pyrophosphate and is probably the most toxic. It was never available for home use and non-homogenous in water, it can hydrolyse quickly after spraying on crops and disappear within 10 to 12 hours. Fig 2.2 is the chemical structure of commonly use aliphatic OPs.

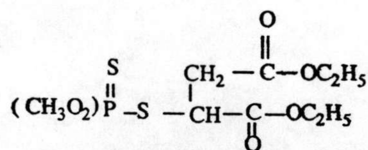
Malathion, however, is one of the most safe of the organophosphate and is commonly used in and around the home with little or no hazard either to humans or their pets. Malathion is normally detoxified by enzyme from the liver and so may not be considered extremely harmful to mammals. However, if other OPs inhibit the action of the liver enzyme system. The malathion's toxic effects will be increase (Niering,1968). **Trichlorfon** is a chlorinated OP, which has been useful for crop pest

control and fly control around barns and other farm buildings. **Monocrotophos** is a relatively new aliphatic OP containing nitrogen. It is a plant-systemic insecticide, but it has had limited use in agriculture because of its highly toxic to mamal and is not available to the homeowner.

Systemic insecticides are those that are taken into the roots of plants and translocated to the above-ground parts, where are toxic to any sucking insects feeding on the plant juices. Normally caterpillars and other plant tissue-feeding insects are not controlled, because they do not ingest enough of the systemic containing juice plants . However, there are several plant-systemic aliphatic OPs, such as dimethoate, dicrotophos, oxydemetomethyl, and disulfoton, which can be used safely by the homeowner (Fig 2.2). **Dichlorvos** (Fig 2.2 g) is a high vapor pressure aliphatic OP, giving strong fumigant quatities. It has been incorporated into vinyl plastic pet collars and pest strips, from which it is released slowly. It lasts several months and is useful for insect control in the home and other close areas. **Mevinphos** (Fig 2.2 h) is highly toxic OP used in commercial vegetable production.Because of its very short insecticidal life, it can be applied up to one day before harvest for insect control, yet it leaves no residues on the crop to be eaten by the consumer (Cheremisionoff,1991)

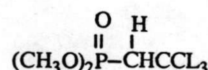
In summary, the aliphatic organophosphate insecticides are the simplest in structure of the organophosphate molecules. They have a wide range of toxicities, and several possess a relatively high water solubility, giving them plant-systemic qualities and several of which are useful around the home.

a. MALATHION



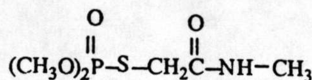
diethyl mercaptosuccinate,S-ester with O,O-dimethyl phosphorodithioate

b. TRICHLORFON



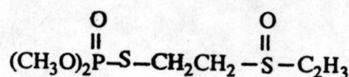
dimethyl(2,2,2-trichloro-1-hydroxyethyl) phosphonate

c. DIMETHOATE



O,O-dimethyl S-(N-methylcarmoylmethyl) phosphorodithioate

d. OXYDEMETONMETHYL



S-(2-(ethylsulfinyl)ethyl)O,O-dimethyl phosphorothioate

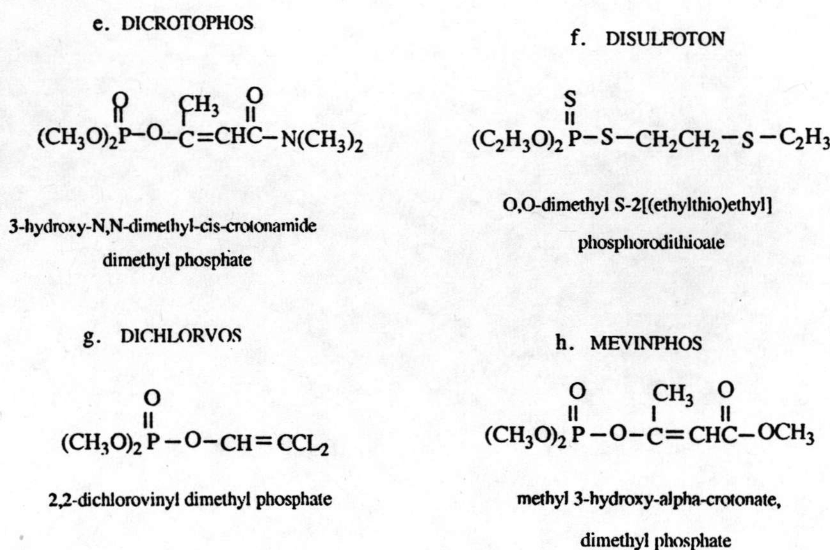


FIGURE 2.2 Chemical structures of Aliphatic Derivatives

2.1.2 Phenyl Derivatives

The phenyl OPs contain a benzene ring with one of the ring hydrogens displaced by attachment to the phosphorus moiety and others frequently displaced by Cl, NO_2, CH_3, CN, S etc. The phenyl OPs are generally more stable than the aliphatic OPs and consequently their residues are longer lasting.

Parathion is the most familiar of the phenyl OPs, developed in 1947, the second phosphate insecticide introduced into agriculture (Edwards,1987). As a result of its age and utility, parathion's total usage is greater than that of many of the less useful materials combined. **Ethyl parathion** was the first phenyl derivative commercially used and, because of its hazard, there is not available to the homeowner. **Methyl parathion** became available in 1949 and proved to be more useful than ethyl parathion because of its lower toxicity to humans and domestic animals and broader range of insect control. Its shorter residual life also makes it more desirable in certain instances. This material is also not used by the layperson.

Systemic insecticides are also found in the phenyl OPs. However, there are usually animal systemics used for the control of the cattle grub such as ronnel and crufomate. For examples, Gardona is a home-safe OP much like malathion in its overall usefulness against home and garden pests.

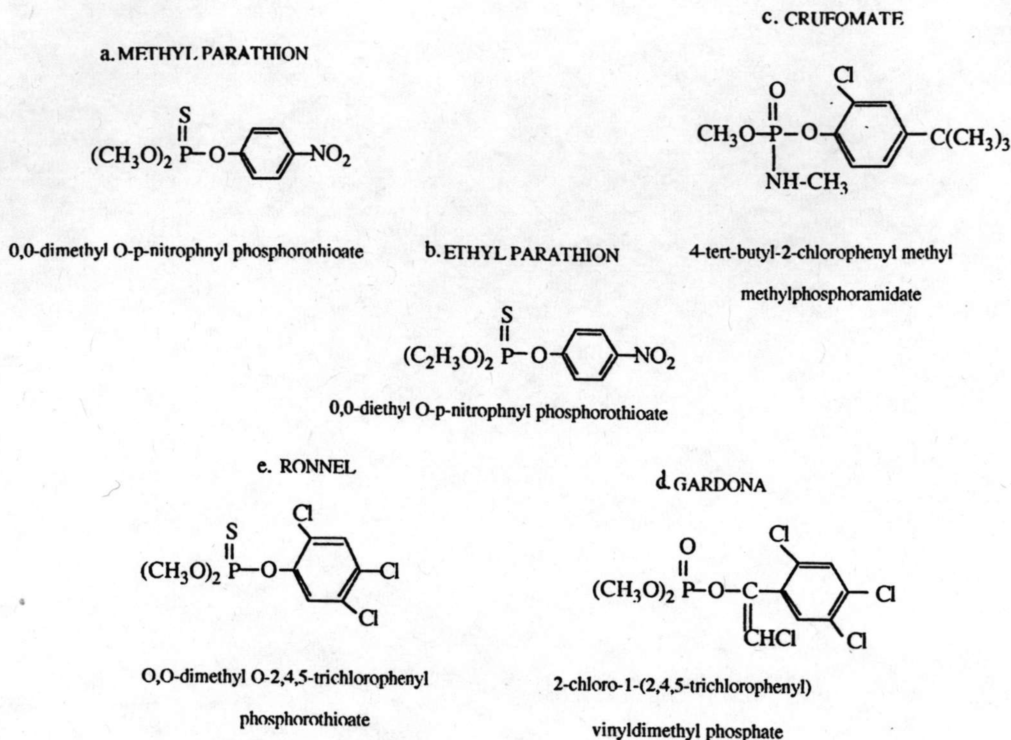


FIGURE 2.3 Chemical structure of Phenyl Derivatives

2.1.3 Heterocyclic Derivatives

The term heterocyclic means that the ring structures are composed of different atoms. In a heterocyclic compound, one or more of the carbon atoms are displaced by oxygen, nitrogen or sulfur, and the ring may have three, five, or six atoms. There are several widely use heterocyclic OPs such as Diazinon, Azinphosmethyl and Chlorpyrifos (Fig 2.4).

Diazinon is the first synthetic insecticides in this group, developed in 1952. Note that the six membered ring contains two nitrogen atoms, very likely the source of its proprietary name, since one of the constituents used in its manufacture is pyrimidine, a diazine. Diazinon is a relatively safe OPs that has an amazingly good track record around the home. It has been effective for practically every conceivable use; insects in home, lawn, garden, ornamentals around pets, and for fly control in stables and pet quarters. **Azinphosmethyl** is the second oldest member of this group and has been used in United States agriculture since 1954. It serves both as an

insecticide and acaricide in cotton production and is not available to the layperson. **Chlorpyrifos** has become the most frequently used insecticide by pest control operators in homes and restaurants for controlling cook roaches and other household insects.

In closing, the heterocyclic organophosphates are complex molecules and generally have longer-lasting residues than many of the aliphatic or phenyl derivatives. Their secondary breakdown products (metabolites) frequently make their residues difficult to detect in the laboratory. Consequently, their use by growers on food crops is somewhat less quantity than either of the other two groups of phosphorus-containing insecticides.

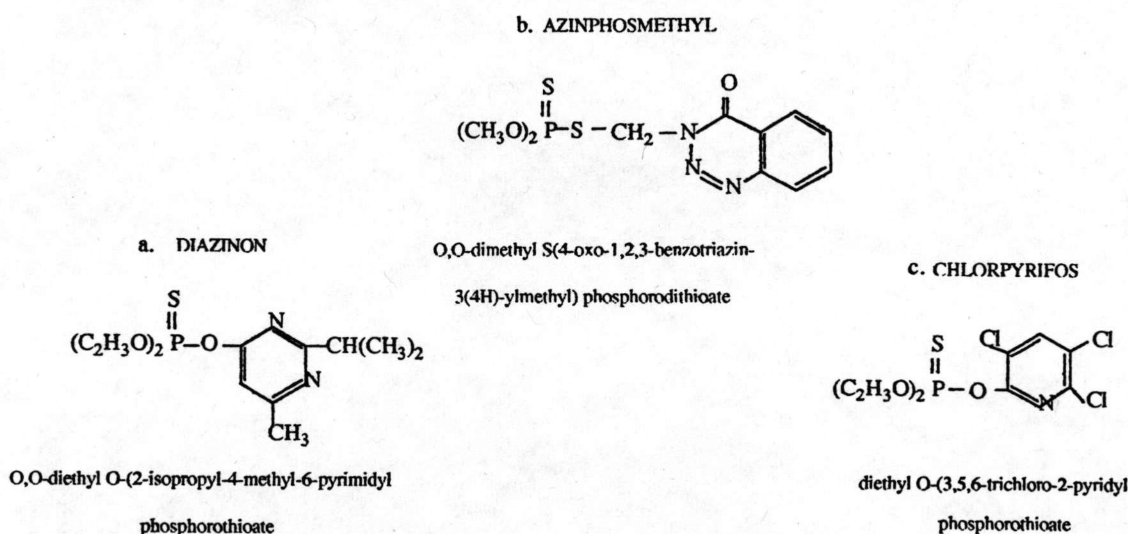
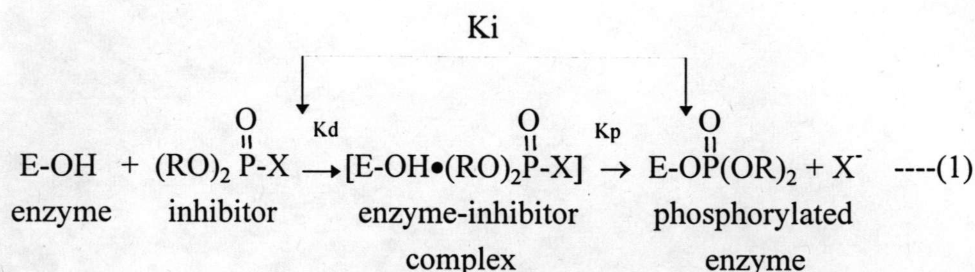


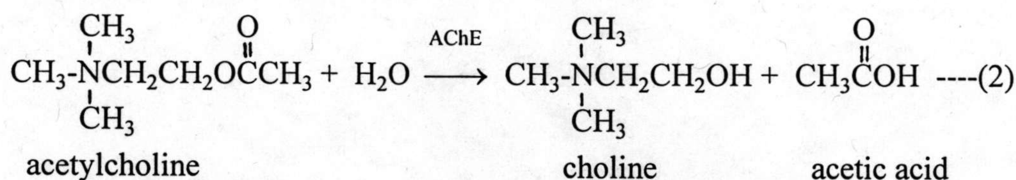
FIGURE 2.4 Chemical structures of Heterocyclic Derivatives

2.2 Mechanism of Action

The toxicity of organophosphorus esters to insects and mammals is generally associated with the inactivation of acetylcholinesterase (AChE), an enzyme that catalyzes the rapid hydrolysis of acetylcholine (ACh), the neurotransmitter (Aldridge and Davison, 1952).Inactivation is accomplished when the organophosphorus compound reacts with the enzyme, phosphorylating a serine hydroxyl moiety in the enzyme active site, as depicted in a simplistic manner by equation 1.



In eqation 1, hydroxy group(OH) in the enzyme (E-OH) represents the serine hydroxyl moiety, K_d is the dissociation constant, and K_p is the phosphorylation rate constant. Although the inhibition reaction is believed to take place in a two-step process-initial binding of the inhibitor to the enzyme to form an enzyme-inhibitor complex (K_d), and subsequent reaction to form the phosphorylated (inhibited) enzyme (K_p) the overall biomolecular inhibition constant K_i is usually used to provide an estimate of the speed of inhibition.The constant K_i is equal to K_p divided by K_d, and the anticholinesterase activity of an organophosphorus esters is proprotional to the value of K_i. Acetylcholinesterase is present in most animals, responsible for the rapid hydrolysis of acetylcholine into choline and acetic acid, (equation 2).



Acetylcholine is one of a number of physiologically important neurotransmitters that effect the transmission of nerve impulses across a synaptic junction, and in some case across a neuromuscular junction.

The role of AChE in regulating nervous transmission is attributable to the cation of ACh is represented in figure 2.5. Imagine a nerve impulse moving down a nerve axon, when the impulse reaches the nerve ending, either at a synapse or a neuromuscular junction, ACh (stored in vesicles) is released into the junction and quickly interacts with an ACh receptor site in the postsynaptic membrane (muscle or postsynaptic fiber), causing stimulation of the muscle or nerve fiber.Acetylcholines - terase regulates nervous transmission by reducing the concentration of ACh in the junction through enzymatic hydrolysis of ACh to choline and acetic acid. Choline is inactive as a neurotransmitting agent.

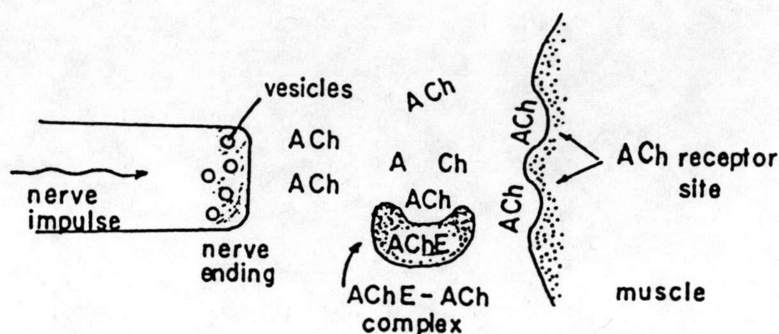


FIGURE 2.5 Acetylcholine-acetylcholinesterase interaction in a synapse or a neuromuscular junction. (Biggar, 1987)

In the reaction between AChE and the organophosphorus ester (equation 1), The moiety X is displaced from the phosphorus atom by the serine hydroxyl moiety of the enzyme, and is therefore refered to as a "leaving group" (it leaves the phosphorus atom in the inhibition reaction). The ability of X to leave the other moieties that are attached to the phosphorus atom. For a labile P-X bond, X must be an electronegative moiety or a group that contains electron-attracting substituents. Blood AChE levels are determined routinely as a way of estimating the degree of exposure to organophosphorus insecticides.

2.3 Detoxification

As esters, the organophosphorus insecticides are susceptible to hydrolytic degradation, resulting in detoxification products. Hydrolysis of the OPs compounds may be by enzymatic or chemical catalysis (Eto, 1974). Enzymatic hydrolysis is mediated by a variety of esterases that have generally been referred to as hydrolases or phosphoriester hydrolases (Dauterman, 1971). Hydrolases have been isolated from a variety of animals, including tissues from mammalian blood and liver, and from insects. Hydrolase catalyzed degradation of organophosphorus insecticides involves cleavage of a bond directly attached to the phosphorus center (a P-O bond in a phosphate ester or phosphorothionate ester). The examples of hydrolase-catalyzed

degradations resulting in P-OAr or P-OR bond cleavage are given in figure 2.6 with methyl parathion and its metabolic activation product metyl paraoxon. The O-demethylated products that occur by hydrolysis may also be formed by the action of glutathione(GSH) transferase, but in this case the CH_3-O bond is broken.

An organophosphorus insecticides may also be degraded by hydrolysis of the compound at a place other than the phosphorus center. Examples of the carboxyl - esterase catalyzed hydrolysis of malathion to the nontoxic monoacid or diacid hydrolysis products (Ryan and Fukuto, 1984). This reaction takes place rapidly in mammals, and the low mammalian.The detoxification fate of malathion is fast by carboxylesterase. Which is faster than the activation to malaoxon. Insects generally have low carboxylesterases and therefore are susceptible to intoxication by malathion.

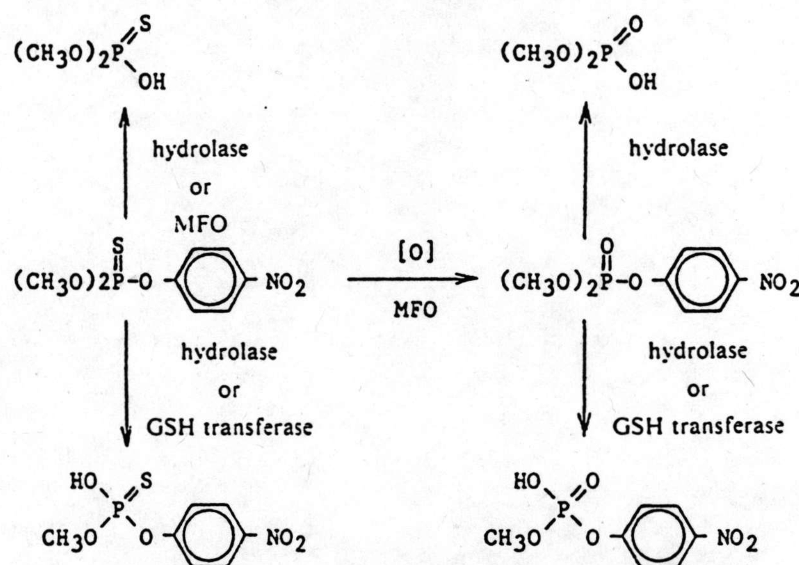


FIGURE 2.6 Hydrolase catalyzed degradation of methyl parathion and and methyl paraoxon. (Biggar and Seiber, 1987)

2.4 Toxicity

The acute toxicity of the organophosphorus insecticides to mammals ranges from highly toxic to very safe. Rat oral LD_{50} values for typical materials in the toxic category (less than 100 mg per kg) and in the safe category are given in table.2.1 (Worthing and Walker, 1983). The data reveal a broad range in the acute toxicity of these materials to the rat. In many case, the high toxicity or safety of one of these insecticides has been explained by elucidation of its metabolic and biochemical behavior in mammals. For instance, the low mammalian toxicity of malathion is explained in terms of its metabolic behavior.

TABLE 2.1 Rat oral toxicity of organophosphorus insecticides. (Worthing and Walker, 1983)

Toxic compounds			"safe" compound		
Common name	Trade name	LD_{50} (mg/kg)	Common name	Trade name	LD_{50} (mg/kg)
TEPP	-	1-2	Diazinon	-	108-250
Phorate	Thimet	1-5	Chlorpyrifos	Dursban	163
Disulfoton	Disyston	2-12	Fenthion	-	255-298
Demeton	Systox	2-12	Dimethoate	-	250-500
Mevinphos	Phosdrin	4-7	Naled	Dibrom	430
Parathion	-	7	Trichlorfon	Dipterex	450-469
Methamidophos	Monitor	12-30	Fenitrothion	Sumithion	250-670
EPN	-	7-65	Surecide	Surecide	1,000
Fonophos	Dyfonate	8-16	Acephate	Orthene	866-945
Azinphosmethyl	Guthion	13-16	Malathion	-	885-2800
Monocrotophos	Azodrin	21	Phenthoate	Cidial	200-4700
Dicrotophos	Bidrin	22	Abate	Abate	1000-3000
Methyl parathion	-	25-50	Stirifos	Rabon	4000-5000
Dichlovos	DDVP	80			

2.5 Introduction to High Performance Liquid Chromatography (HPLC)

High performance liquid chromatography (HPLC) is a technique that has arisen from the application of liquid chromatography (LC) of theories and instrumentation which were originally developed for gas chromatography (GC).

Classical liquid chromatography has been around for quite a long time. In the original method an adsorbent, for instance alumina or silica, is packed into a column and is eluted with a suitable liquid. A mixture to be separated is introduced at the top of the column and is washed through the column by the eluting liquid. If a component of the mixture (a solute) is adsorbed weakly onto the surface of the solid stationary phase it will travel down the column faster than another solute that is more strongly adsorbed. Thus separatation of the solute is possible if there are differences in their adsorption by the solid. This method is called adsorption chromatography or liquid solid chromatography (LSC).

Theoretically, the efficiency of the chromatography can be improved if the particle size of the stationary phase materials used in LC can be reduced. High performance liquid chromatography has been developed steadily during the late 1960s since the high efficiency packing materials were produced. In addition, the improvement of the instrumentation allowed the full potential of these material to be realised. As HPLC has been developed, the particle size of the stationary phase used has become progressively smaller. The stationary phase used today are called **microparticulate** column packings and are commonly uniform, porous silica particles, with spherical or irregular shape, and nominal diameters of 10, 5 or 3 μm. The different separation mechanism mentioned earlier can be operated by bonding different chemical groups to the surface of the silica particle to produce the bonded phase. There are various chromatography suppliers list of packing materials, but about 75% of the works in HPLC at the moment have been operated by using a bonded phase in which C_{18} alkyl groups attached to the surface of the silica particles. These types are called **ODS (octadecylsilane) bonded phase**. The sorption mechanisms of bonded phase are still unclear so there are much the oretical and experimental works going on at the moment attempting to clarify such mechanisms (Lindsay, 1987).

In the packed column, the small size of these particles leads to a considerable resistance to solvent flow, so that the mobile phase has to be pumped through the column under high pressure. Hence, it typically requires 10-25 cm length and 4.6 mm internal diameter columns. Although these columns are expensive, they are re-usable, so that the cost can be spread over a large number of samples. The column and all the associated pumbing must be able to with stand the optimum pressures and must also be chemically resistant to the mobile phase solvents. Columns are usually made of stainless steal, althrough glass or plastics are favoured by some manufactures. At the moment there is considerable interest in the properties of columns that have a diameter of 2 mm or less (known as small bore or microbore columns) and it is possible that these type of columns may become widely used in the future (Lindsay, 1987).

In analytical HPLC, the mobile phase is pumped through the column at a flow rate of 1-5 cm^3 min^{-1} . If the composition of the mobile phase is constant, the method is called **isocratic elution**. Alternatively, the composition of the mobile phase can be made to change in a predetermined way during the separation, which is a technique call **gradient elution**. Gradient elution is used in situations similar to those requiring temperature programming in GC, and is necessary when the range of retention times of solute on the column is so large that they cannot be resolved and eluted in a reasonable time using a single solvent or mix solvents. In the case of adsorption chromatography which polar solutes are strongly adsorbed, the more polar solvents are required. If the sample contains a wide range of polarities, the separation could be done by changing the polarity of the solvent mixture during the separation. In other case it may be necessary to use gradient elution where other properties of the solvent (e,i., pH or ionic strength) are changed.

After passing through the column, the separated solutes are detected by an in-line detector. The output of the detector is an electical signal, the variation of which is displayed on a potentiometric recorder, a computing integrator. Most of the popular detectors in HPLC are selective devices, which means that they may not respond to all of the solutes that are present in a mixture. Since there is no univeral detector for HPLC that can compare with the sensitivity and performance of the flame ionization detector used in gas chromatography, some solutes are not easy to detect in HPLC.

In that case, they have to be converted into a detectable form after emerging from the column. This approach is called a **post-column derivatisation** (Lindsay, 1987).

As in other forms of chromatography, the time taken for the solute to pass through the chromatographic system (the retention time) is a characteristic of the solute for particular set of conditions. However, to use retention data on its own for the identification of unknown solutes would be rather like trying to identify an unknown organic compound simply by measuring its melting or boiling point. Many different solutes will have identical retention times for particular set of condition. Chromatography is an excellent method for the separation mixtures, but it does not provide the detail necessary for the clear identification of the separated compounds, although, some detectors can record and store the UV spectra of solutes as they emerge from the column. A much more powerful method is the direct combination of liquid chromatography and mass spectrometry. In both case, modern then using data processing methods match the obtained spectra with the standard spectra, obtained from libraries of reference spectra.

GC and HPLC are different in term of detection phase. In GC, the mixture samples are examined in the vapour phase. The analysed substances are changed into some stable vapour forms so these are depressed on the vapourization property of the samples. In the case of non-volatile samples,they are commonly converted to volatile derivatives forms. Only about 20% of chemical compounds are suitable for GC without any modification; the remainder are thermally unstable or involatile. In addition, the substances with highly polar or ionisable functional groups often show poor chromatographic behaviour by GC, being very prone to tailing. Thus HPLC is the better technique for macromolecules, inorganic or other ionic species, labile natural products, pharmaceutical compound, biochemicals and pesticides. (Fig.2.7) is shown the relation of molecular weight vs. polarity of compounds using HPLC and GC. There are less than 10% of total organic contents found by GC and GC/MS.

In GC there is only one phase (the stationary liquid or solid phase) that is available for interaction with the sample molecules. Because the mobile phase is a gas, allsample vapours are soluble in it in all proportions. In HPLC both the stationary phase and the mobile phase can interact selectively with the sample. Interactions such as complexation or hydrogen bonding that are absent in the GC mobile phase may

occur in the HPLC mobile phase. The variety of these selective interactions can also be increased by suitable chemical modification of the silica surface, HPLC is a more versatile technique than GC, and can often achieve more difficult separations.

HPLC instrument requires a high pressure pump and a supply of mobile phase, column packed with a high efficiency stationary phase, an injection unit for samples on to the column, an inline detector and some method of displaying the detector signal. Fig. 2.7 is a block diagram showing the way in which these different components are arranged to form a high performance liquid chromatograph.

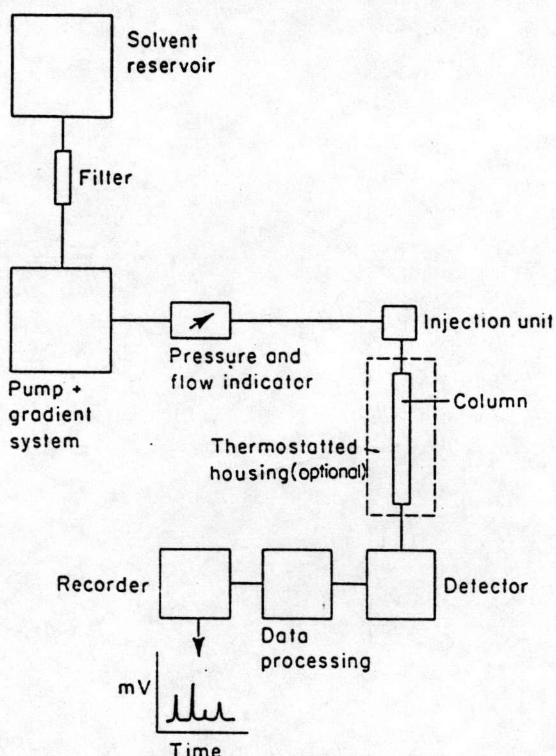


Figure 2.7 Block diagram of a High Performance Liquid Chromatograph systems

2.5.1 UV Absorbance Detectors

When the mobile phase from the column passed through a small flow cell held in the radiation beam of a UV/visible photometer or spectrophotometer, the absorbed energy has been detected. These detectors are selective in the sense that they will detect only those solutes that absorb UV (or visible) radiation. Such solutes include alkenes, aromatics and compounds having multiple bonds between C and O, N or S. The mobile phase that use, on the other hand, should absorb little or no

radiation. Absorption of radiation by solutes as a function of concentration, c, is described by the Beer-Lambert law:

$$A = \varepsilon . bc$$

Where A = absorbance, b = path length of the cell in cm.,c = is the sample concentration in mole/l and ε = molar absorptivity of the sample, which is a constant for a given solute and wavelength.

The Beer-Lambert law applies only monochromatic radiation. However, the detector system does not provide truly monochromatic radiation, If test the law for a solute at a wavelength in the spectrum where the absorbance is changing rapidly with wavelength, then the different wavelengths comprising the band may be absorbed by quite different amounts and the law may not be obeyed.

Both fixed and variable wavelength UV/Visible detectors are available. The variable types use a deuterium and/or a tungsten filament lamp as the radiation source and can operate between about 190-700 nm. They will have a number of absorbance ranges (range are given in "aufs" which means **a**bsorbance **u**nits corresponding to **f**ull **s**cale deflection on the recorder). Fixed wavelength detectors can operate at 254 nm, 280 nm or at other wavelengths. Table. 2.2 shows some of the specification of two modern variable wavelength detectors (Lindsay, 1987).

	Radiation source	wavelength range,nm	abrorbance ranges,aufs	noise,au
Phillips 4025	Deuterium lamp	190-380	0.005-1.28 (9 ranges)	10^{-4} at 230 nm
	Tungsten lamp	190-600		
Uvikon 735	Deuterium lamp	195-350	0.0025-2.56 (11 ranges)	5×10^{-4} at 250 nm

TABLE 2.2 Specification of two variable wavelength detectors
nm = nanometer, au = absorbtion unit,
aufs = absorption unit of full scale

หอสมุดกลาง สถาบันวิทยบริการ
จุฬาลงกรณ์มหาวิทยาลัย

In UV absorbance detection, it is often useful to be able to detect different peaks in the chromatogram at different wavelengths. This may occurrs because the certain solutes have only a small absorptivity at the wavelength selected.

2.5.2 HPLC

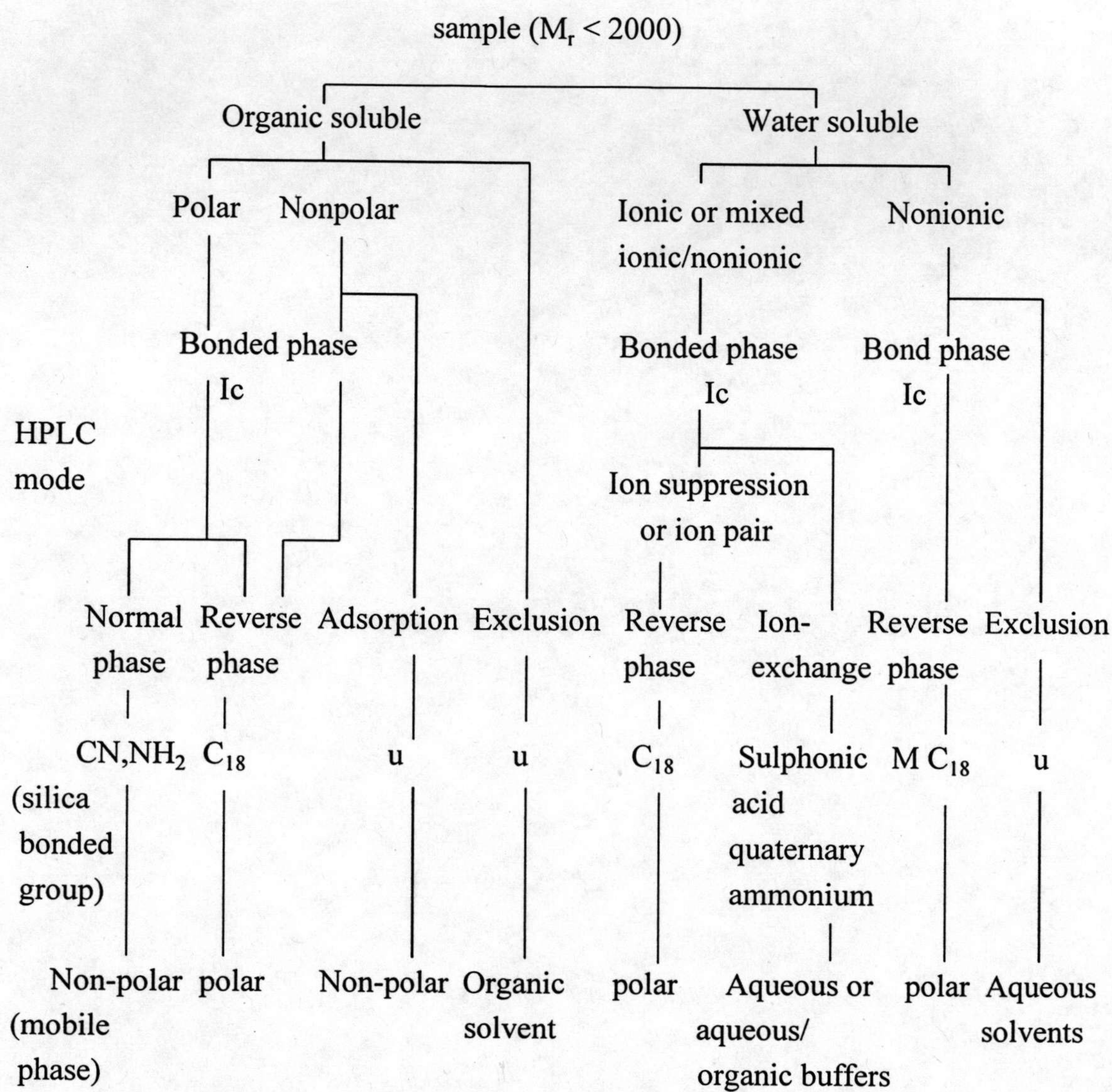
Silica have been used in a number of different ways in HPLC column as adsorbents, supports for stationary liquids in partition chromatography and as bonded phase. Bonded phase chromatography is experimentally much easier than adsorption or liquid-liquid partition. It is more versatile, quicker, and has better reproducibility than the older modes. In a bonded phase, the highly polar surface of the silica is altered by the chemical attachment of different functional groups. These attached groups can be non polar (i,e., C_8, phenyl, C_{18}), polar (-NH_2, -CN) or ionisable (sulphonic acid, quaternary ammonium). The range of function groups that can be bonded to silica is very wide, and for specialised applications some fairly toxic bonded phase are available.

The term **normal phase** and **reverse phase** are used to decribe adsorption and many bonded phase separations. Normal phase means that the polarity of the stationary phase is higher than of the mobile phase, for example, when silica is used in adsorption chromatography. Reverse phase means that the polarity of the stationary phase is less than that of mobile phase, which is the case with hydrocarbon-type bonded phases. Polar bonded phases can be used in either normal or reverse phase modes. With both tecniques, solutes are eluted in order of polarity (increasing or decreasing), and it can change the retention times of solutes by changing the polarity of the stationary phase or mobile phase. These facts are summarised in Table 2.3 compares the different characteristics of these two chromatographic types.

TABLE 2.3 Summary of characteristics of normal and reverse phase chromatography

	Normal phase Chromatography	Reverse phase Chromatography
Stationary phase polarity	High	Low
Mobile phase polarity	Low-medium	Medium-high
Typical mobile phase	heptane/$CHCl_3$	CH_3OH/H_2O
Order of elution	Least polar first	Most polar first
To increase retention of solutes	Decrease mobile phase polarity	Increase mobile phase polarity

Table 2.3 shows how the HPLC mode of analysis is chosen on the basis of the solubility of the sample and the sort of functional groups that it contains. For some modes stationary phase are available that are based on materials other than silica. From Fig.2.8 there is always a choice of mode and also that almost any separation can be achieved by reverse phase chromatography using a bonded silica stationary phase. This is the mode that would tend to look at first; it is often faster, cheaper and experimentally easier than the alternatives. C_{18} bonded phases were used in over half of them. Since these data were collected the use of C_{18} columns has increased; they are probably now used in around 75% of applications (Lindsay, 1987).



u = unmodified

Ic = ion chromatography

M_r = molecular wieght

FIGURE 2.8 Choice of an HPLC mode

2.6 Solid-phase Extraction (SPE)

This method of extracting organic pollutants from water or, whenever possible, from solid matrices, has been introduced rather recently as an alternative to liquid-liquid extraction and soxhlet extraction. Two main advantages characterise this technique, namely, a lower solvent volume and faster operation. As a consequence of the first, a lower volume of glassware is necessary, while the second makes it possible to carry out a larger number of extraction processes within a given time.

SPE is a technique based on the principle of liquid-solid chromatography just as sampling and thermal desorpion of air pollutants are based on the principles of gas-solid chromatography. In solid-phase extraction a very short column is filled with an adsorbent in a fine mesh range(150-400) and with a thickness of about 0.5 cm. In general , the column is made of glass or plastic syringe tubing. It is schematically show in fig 2.9.

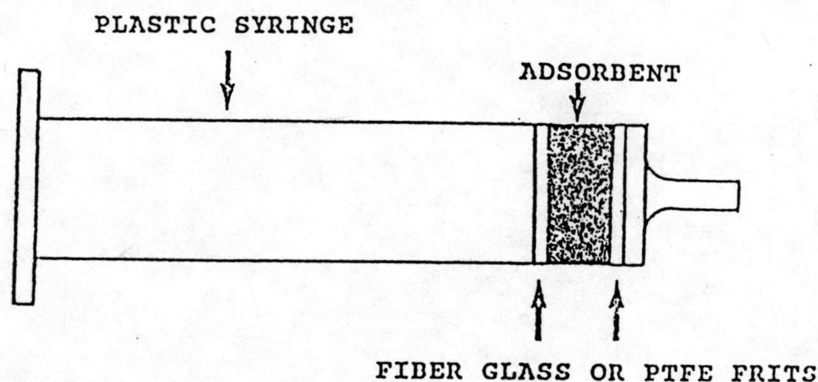


FIGURE 2.9 Typical cartridge for SPE

There are several ways in which SPE can be performed. First, it can be used for **trace enrichment,** in which a large volume of dilute sample is passed over the stationary phase. Phase are selected because they exhibit a very high affinity for the compound of interest and little or no affinity for the matrix. The enriched sample then can be quantitatively recorded by displacement with a small volume of strong eluent. In a similar, SPE can be used for **sample isolation**, in which the compound of interest can be selectively sorbed onto the solid phase while matrix interferences are allowed

to pass through the SPE column. In the reverse manner, **matrix isolation** can be used to bind interferences to the solid phase, allowing the sample of interest to pass through and be collected. Contaminants remain on the cartridge, which are discarded. Finally, SPE can be used for sample storage and transport. In this approch, samples are collected in the field, transported to the laboratory, displaced, and analyzed. This approach is especially useful for labile or volatile compounds, as well as in trace analysis, for which the transport of large amounts of sample becomes impractical. Compounds are often stabilized if they have been sorbed in a solid state (Majors, 1986).

2.6.1 Mechanisms of solid-phase extraction

The two major mechanisms of analyte retention on solid support are desorption and partition (Font, 1993). Extraction of trace amounts of organic compounds from water with solid sorbent is a method in which adsorption on a solid substance is used in order to isolate compounds dissolved in water. Sorbent extraction can also be based on the distribution of dissolved compound between the solid sorbent and water.

Solid-phase extraction generally consists of four basic steps (Majors, 1986).

1. Condition the column for aqueous samples. This is generally done by wetting the sorbent with a volume of methanol, which is then replaced by a volume of water. The role of this step is to secure perfect and maximum mutual contract of the liquid and solid phase. Prewetting of chemically bonded silicas causes opening of the hydrocarbon chains of the stationary phases, thus increasing its surface area. Organic solvents commonly used as prewetting media are methanol, acetonitrile, and acetone.
2. Apply the sample to the column. This results in compounds (including the compound of interest) being retained on the sorbent while others pass through unretained.
3. Rinse the column to remove interfering compounds.
4. Elute the compound of interest in a small volume of an appropriate solvent.

2.6.2 General properties of bonded silicas

Bonded silicas are formed by the reaction of organosilanes with activated silica. The product is a sorbent with the functional group of the organosilane attached to the silica substrate through a silyl ether linkage. Fig.2.10 shows the reaction, in which HCl is eliminated between a surface silanol group and silylating agent.

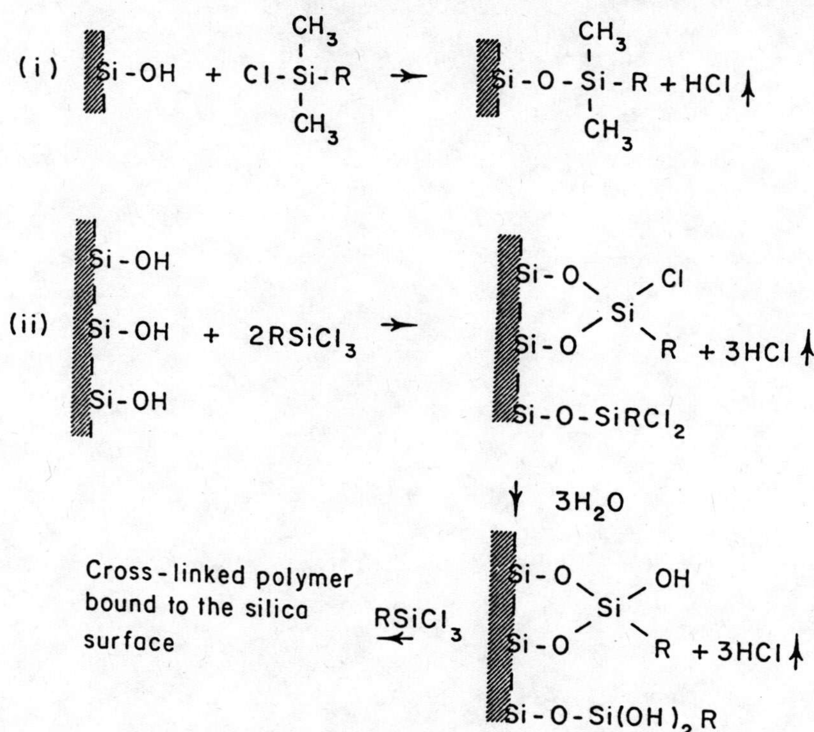


FIGURE 2.10 Preparation of bonded phase. Reaction of silica with substituted chlorosilanes to form (i) monomeric (ii) polymeric bonded phases

It is not possible to bond all of the surface silanol groups. Unreacted silanols are capable of adsorbing polar molecules, and will thus affect the chromatographic properties of the bonded phase. Usually, the unreacted silanols produce undersirable effects, such as tailing and excessive retention in reverse phase separations, although there have been cases reported where the unreacted silanols improve such separations. The concentration of unreacted silanols in non-polar bonded phases is normally reduced by the process of " end-capping", in which most of the remaining silanols are reacted with a small silylating agent, such as trimethylchlorosilane. fig.2.11 shows

the surface structure of an ODS bonded phase, containing bonded C18 alkyl groups, end-capped silanol groups and a small number of free silanols.

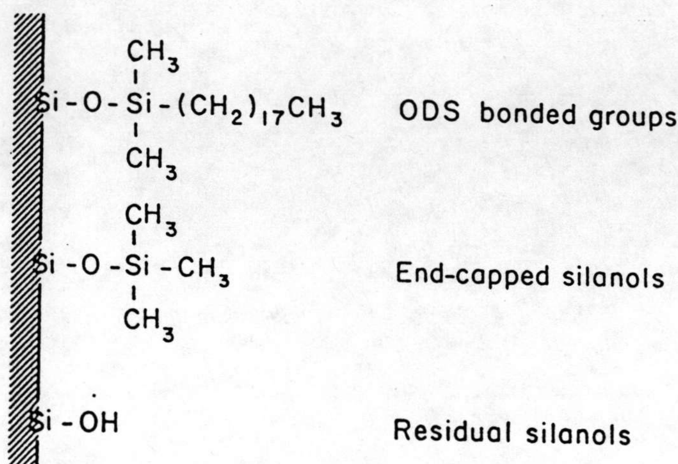


FIGURE 2.11 Structure of an ODS (C_{18}) surface

2.6.2 Sorbents used in solid-phase extraction

2.6.2.1 Carbon Sorbents

Carbon was the first medium used for the accumulation of organic compounds from water. The advantage of activated carbon was high sorption capacity, and high thermal stability; however, the use of heterogeneous nature of activated carbons caused problems such as irreversible sorption, affinity for some groups of compounds only, or catalytic activities of the carbon surface (liska, 1989).

2.6.2.2 Polymer Sorbents

Polymer have been reported to use as alternative sorbents for trace enrichment, instead of carbon, since the late 1960s. Their homogeneous structure, results in a greater reproducibility of the trace enrichment experiments. The most often used types of sorbents are styrene-divinylbenzene copolymers. This group comprises, e.g., Amberlites XAD-1, XAD-2, XAD-4, Chromosorb 102, PRP-1, or Ostion SP-1. Columns packed with these materials have significant utility for certain type of separations, but they are available in a limited range of functionality. In addition, only limited separation experience with these materials has been reported.

Of particular concern are studies by Vign and co-workers that report severe nonlinear adsorption isotherms on commercial polystyrene-divinylbenzene column packing for low molecular weight solutes. These data suggest that isocratic retention times vary significantly with changes in sample loading, an effect that is not characteristic of silica-based, bonded-phase columns (Glajch, 1990).

2.6.2.3 Bonded Silicas

These materials are the most popular and widely applied sorbents. The first attempts to use them as preconcentration media date to the middle of the 1970s. The growth in acceptance of bonded silica extraction is due to its superior selectivity resulting in very good recoveries with high purity of the compound of interest. Because of the wide variety of bonded silica sorbents available, a large selection of sorbent/solvent combination is possible. Solid-phase extraction provides a rapid and effective way to clean up and concentrate a variety of compounds for analysis. But developing a reliable extraction method depends upon understanding the properties of bonded silica materials (Liska, 1989).

The sample preparation step of analytical method is the most important process. There are several extraction and preconcentration method which can be used in the determination of the organophosphorus insecticides, such as partitioning (Font, 1993),liquid-liquid extraction (Driss, 1993) and solid-phase extraction (Brooks,1990). Classical analytical procedures based on solvent partitioning in seperation funnels are long time consuming and requires large volumes of costly and toxic solvent .

The determination of pesticides in water sample using solid-phase extraction using a variety of sorbents such as C_{18} (Foster and Synovec, 1996), C_8 (Mill, 1993), activation carbon (Font, 1993), silica gel (Markell, 1985), amberlite XAD (Dietrich, 1988), synthetic polymer (Naidong, 1991) and empore membrane extraction discs. The solid-phase extraction (SPE) system using bonded silica sorbents applied to the extraction of pesticides from water offers the advantages of a shorter analysis time, lower cost and the consumption of very low volumes of organic solvent (Vinuesa, 1989).

Solid-phase extraction has gained in popularity over the last ten years and some procedures have been validated by social institution, e.g., the U.S. Environmental Protection Agency (EPA) (Bidlingmeyer, 1984). This technique has already been applied to a variety of pesticides such as organochlorine, organophosphorus and carbamate pesticides.

Studies of environmental sample by SPE technique are widely reported.

Larson and Houglum (1991) describes a reverse phase liquid chromatographic method for assay of samples that combine dicamba, 2, 4-D and MCPP in herbicide formulation. Samples are diluted with isopropanol-water (2:1) and chromatographed on a C-18 column with a gradient mobile phase. Dicamba, 2, 4-D and MCPP elute at 6, 13 and 16.5 minute, respectively. The within day efficient of variation was 0.1 to 3.2% between day variation was 0.6 to 5.5%. The method seperates the 3 herbicides from major potential impurities.

Hanks *et al.* (1992) determined methamidophos and five different formulated products. Samples were dissolved in water, seperated by LC and detected at 210 nm. Quantitation was done by peak area measurements. Relative standard deviations for repeatability and reproducibility ranged from 0.35 to 0.80 and from 1.14 to 1.60, respectively.

Brooks *et al.* (1990) developed method for analysis chlorpyrifos, isofenphos, carbaryl, iprodione and triadimefon in groundwater by solid-phase extraction. This method involves the extraction of pesticides onto C_{18} column and elution with methylene chloride and change to hexane. The extracts are analyzed by gas chromatography using nitrogen-phosphorus detection. Recoveries average higher than 90% with a detection limit of 1 ppb for carbaryl, iprodione and triadimefon and 0.1 ppb for chlorpyrifos and isofenphos.

Molto *et al* (1991) used octadecyl (C_{18}) banded porous silica to remove triazine and organophosphorus pesticides from natural water. The adsorbed compounds were removed with ethyl acetate, evaporated to 200 μL and determined by gas chromatography. The average recoveries were higher than 85% except for dimethoate and trichlorfon.

Mcdonnell *et al* (1993) developed sample preparation for natural waters using the empore disk. The Empore disk is a new solid-phase sample preparation technology. In order to increase the volume of water. The eluate was concentrated and determined by gas chromatography by using electron capture detection. The use of the empore disks offered some advantages.

Marvin *et al* (1990) developed method to determined trace concentrations of propoxur, carbaryl, propham, captan, chloropropham, banban and liquid chromatography. The analytes are eluated from the cartridge with acetonitrile. The analytes are seperated on 25-cm C_{18} analytical column and determined by UV absorption at 220 nm. The recoveries for the eight pesticides ranged from 84% to 93%. The detection limit are in the range of 0.02-0.92 μg/L for the eight pesticides.

Driss *et al.* (1993) used on-line liquid chromatographic preconcentration techniques for the determination of carbaryl and seven organophosphorus pesticides in drinking water. Between 100 and 300 mL of water sample is passed through 1.5 cm. precolumn, packed with C-18 bonded silica or styrene-divinylbenzene copolymer (PRP-1) sorbent at flow rate 3 mL/min. The revoveries for most of the examined pesticides were 90%, except for carbaryl (54%). The detection limits are in the range 0.03-0.2 μg/L.

Bornes *et al.* (1995) develope and validated for the determination of the insecticide diflubenzuron in mushrooms using high-performance liquid chromatography atmosphere pressure chemical ionization mass spectrometry (HPLC-APCI-MS). This insecticide was extracted into dichloromethan-cyclohexane and cleaned-up by size-exclusion chromatography. The limit of detection was 0.02 ng/μL. Revovery of diflubenzuron from spiked mushrooms was 85.5% with a relative standard deviation of 14.5%.

Buchheit and Witzenbacher.(1996) described the results of a comparison of extraction efficiency between a new polymeric sorbent for solid-phase extraction (SPE) and RP-18. The pesticides were separated using HPLC and detected by diode array detection.

Okumura and Yoshikawa (1995) determined traces of fourteen organophosphorus pesticides in environmental samples such as river water, sediment and fish by capillary GC-MS with selected -ion monitoring. The pesticides could be determined with range 0.02-0.75 ng/mL in water with relative standard deviations of 1.0-31.4%. The detection limits of the pesticides were 0.013-0.120 ng/mL in water.

Bardalaye and Wheeler (1986) described a method for the determination of trace quantities of triazine herbicides, terbutryn, prometryn and ametryn in water. The procedure involved preconcentration of water samples by sorption on C_{18} cartridges and desorption with 2-propanol. The determinative step was achieved by capillary gas chromatography on supelcowax-10 fused silica column using a nitrogen-phosphorus detector. The limit of detection was 0.1-10 μg/L.