CHAPTER 1

INTRODUCTION

1.1 Pesticides[1]

Pesticides are chemicals designed to combat the attacks of various pests on agricultural and horticultural crops. They fall into three major classes: insecticides, fungicides, and herbicides (or weed killers). There are also rodenticides (for control of vertebrate pests), nematicides (to kill microscopic eelworms), molluscicides (to kill slugs and snails), and acaricides (to kill mites).

Pesticides may also be divided into two main types, namely contact or non-systemic pesticides, and systemic pesticides. Contact or surface pesticides do not appreciably penetrate plant tissues and are consequently not transported, or translocated, within the plant vascular system. The earlier insecticides, fungicides, and herbicides were of this type; their disadvantages are that they are susceptible to the effects of weathering (wind, rain, and sunlight) over long periods and new plant growth will be left unprotected and hence open to attack by insect and fungal pests.

In contrast, many of the more recent pesticides are systemic in character-these can effectively penetrate the plant cuticle and move through the plant vascular system. Examples are provided by the phenoxyacetic acid, selective herbicides; certain organophosphorus insecticides like schradan, and the more recently discovered systemic fungicides like benomyl.

Systemic fungicides are also sometimes termed plant chemotherapeutants and cannot only protect the plant from fungal attack, but also cure or

inhibit an established infection. They are little affected by weathering and will also confer immunity on all new plant growth.

1.1.1 Toxicological Properties[1]

In the early development of the pesticide, the toxicological properties have to be evaluated-this must cover not only determination of the acute toxicity, but also any possible longer-term effects on the environment. The acute toxicity is determined by testing the candidate chemical against various mammals, generally rats and mice. The toxicity is usually recorded as the LD_{50} value-this is the dose required to kill 50% of the population of test animals and is expressed as mg/kg of the body weight of the animal. There should be at least 10 animals in the experiment and administration of the chemical can be oral (in the animal's food), by intravenous injection, or to the skin (dermal). Dermal toxicities are often slightly less than the oral values while intravenous toxicities are higher than the oral figures. The average values of the oral toxicities (LD_{50} values) against rats are quoted to give some indication of the mammalian toxicities of the different types of pesticides. The smaller the LD_{50} value, the more toxic the chemical so that toxicities of chemicals can be graded by the LD_{50} values as follows:

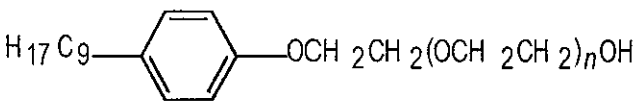
Table1.1 Toxicities of chemicals base on the LD_{50} values[1]

Level of Toxicities	LD_{50}(mg/kg)
1. Extremely toxic	1
2. Highly toxic	1-50
3. Moderately toxic	50-500
4. Slightly toxic	500-5000
5. Practically non-toxic	5000-15,000
6. Relatively harmless	>15,000

1.1.2 Formulations[1]

The formulator tries to bring the active ingredient into a convenient form for application, either directly as powders, granules, or self-dispensing packs, e.g. aerosols, or sometimes after mixing with water or other readily available liquid diluent. The product must be formulated so that it is as effective as possible, but it must also be stable and reasonably safe in storage and transport. The active ingredients of most pesticides are relatively insoluble in water, but are fairly soluble in organic solvents like petroleum and xylene. These are, however, insoluble in water and so if the pesticide is dissolved in a suitable organic solvent and the solution diluted with water the organic layer quickly separates out from the water in the spray vessel.

This problem can be overcome by addition of emulsifiers, which are surface-active agents (surfactants), to the solution of the pesticide enabling it to form a stable emulsion on mixture with water in the spray tank and the emulsion can then be sprayed onto the crop. Many water-insoluble pesticides are marketed in the form of thick self-emulsifiable concentrates consisting of a solution of the pesticide in an organic solvent, e.g. petroleum or other hydrocarbon oil containing oil-soluble surfactants. The formation of emulsifiable oils has been aided by the development of non-ionic emulsifying agents, e.g. polyglycol ethers, and polyethylene oxides such as (1), (where $n \approx 12$):



(1)

Such non-polar compounds have the advantage of being much more soluble in oils than ionic surfactants, and their effect is not greatly altered by the presence of saline impurities so that they do not form insoluble scum in hard water which was a major trouble when soaps were used. An effective self-emulsifying oil often requires the use of several different surfactants, for instance two or more non-ionic agents with widely differing numbers of ethylene oxide groups in their chains, or more generally a mixture of anionic and non-polar surfactants. Cationic surfactants are also used in certain cases, e.g. with some fungicides which are themselves cationic surfactants. In this case, use of anionic surfactants would lead to loss of surfactant properties and formation of and insoluble gum- for this reason pesticides should not be mixed with each other until it is clearly established that they are compatible.

If the active ingredient is soluble in water, the chemical can be dissolved in water to give an aqueous solution containing a suitable concentration of the active ingredient which can be sprayed directly onto the crop. The chemical may also be used as a wettable powder. The powder is prepared by grinding the material in a ball mill. However, few organic compounds produce a free-flowing powder without first mixing them with an inert inorganic mineral diluent, e.g. a talc or clay, and addition of dispersing and wetting agents is also needed to give a fine suspension when the powder is mixed with water in the spray tank, which must not settle out on the bottom of the tank for at least half an hour, otherwise frequent agitation will be needed to prevent formation of sticky powders that tend to cake on storage. In certain instances, especially with inorganic compounds like copper fungicides, the mineral diluent is not necessary since these chemicals have no tendency to stickiness. Clay mineral diluents contain strongly acidic centers which may

catalyze the decomposition of certain pesticides, whereas other chemicals may be sensitive to alkaline diluents. A knowledge of the chemical reactivity of the active ingredient, especially towards acidic or basic hydrolysis, is valuable information to aid selection of the mineral diluent to be used in the formulation of a wettable powder. The emulsion spray separates into two phases on impact; the water running off the plant surface while the pesticide solution remains on the surface. With non-systemic pesticides, it is essential to obtain a high coverage of the surface by the spray and this is often enhanced by the addition of various wetters and spreaders; further the persistence of the dried deposit is sometimes improved by the use of chemical additives termed "stickers". Currently approximately 75% of all pesticides are applied as sprays, chiefly as water emulsions made from emulsifiable concentrates.

The quantities of pesticide formulation that have been imported into Thailand since 1984 are shown in Table 1.2.

In recent years synthetic insecticides have been widely used in agriculture and the most important groups of synthetic insecticides are the organochlorine, organophosphorus and carbamate compounds.

Table 1.2 Quantities of imported pesticide formulation in 1984[2]

Pesticide Formulation	Quantities of Imported Pesticide Formulation (Tons)		
	Insecticides	Fungicides	Herbicides
1. Powder for seed treatment (DS)	-	26.93	-
2. Emulsifiable Concentrates (EC)	2849.6	300.84	683.89
3. Electrochargeable liquid (ED)	9.76	-	-
4. Emulsion water in oil (EO)	8.2	-	-
5. Gas (GA)	303.36	-	-
6. Granule (GR)	105.6	5.0	51.0
7. Suspension concentrate (SC) or Flowable concentrate (FC or F)	34.91	26.87	-
8. Water soluble granule (SG)	25.0	-	-
9. Soluble concentrates (SL) or Water soluble concentrates (WSC)	724.47	-	-
10. Water soluble powder (SP)	216.84	2.0	19.0
11. Tablet (TB)	55.27	-	-
12. Technical material (TC)	2252.74	4.06	553.82
13. Wettable powder (WP)	1098.45	3555.73	4900.49
14. Crystal	273.5	-	-
15. Paste or Cream (PA)	-	1.27	-
16. Bait	1.8	-	-

1.2 Organophosphorus Insecticides[3]

The organophosphorus insecticides are all structurally related and undergo similar reactions. The chemical classification of the most widely used compounds of this type is given in Table1.3. All are inhibitors of the enzyme,cholinesterase. Their potency depends not only upon their intrinsic

enzyme affinity but also on anticholinesterase properties acquired through in vivo metabolism.

Table 1.3 Classification of Organophosphorus Insecticides[3]

Aliphatic Derivatives		Aromatic (Cyclic) Derivatives	
Butonate	Mevinphos	Azinphosmethyl	EPN
Demeton	Mipefox	Carbophenothion	Fenthion
Dichlorvos	Naled	Diazinon	Methyl parathion
Dimefox	Phorate	Dicapthon	Parathion
Dimethoate	Phosphamidon	Endothion	Ronnel
Dithiodemeton	Schradan		
Ethion	Sulfotepp		
Malathion	Tepp		
Methyl demeton	Trichlorofon		

There seems to be no limit to the number of toxic organophosphorus compounds that can be synthesized and that exhibit insecticidal activity. While many compounds of this type have been marketed, only about 20 of them comprise the bulk of the total tonnage. Parathion, methyl parathion, and malathion are perhaps the best known and most widely used of this class of insecticides. The relatively low cost of the first two, combined with their good performance against a broad spectrum of insects, probably accounts for their continuing popularity. The low order of toxicity of malathion to mammals has made it acceptable under many conditions where other, perhaps more insecticidally active, insecticides are restricted.

Some of the highly toxic insecticides of this type are used on many vegetable and fruit crops close to harvest because of their high degree of volatility; examples are tepp and mevinphos. Other insecticides of this type which are much less toxic are used primarily to control insects that attack livestock; examples are ronnel, dichlorvos, coumaphos, ciodrin, and trichlorfon. Still another group of these compounds is being developed for use in the soil to control soil-borne insects. Typical soil insecticides of this type are parathion, diazinon, and phorate.

These insecticides range from completely water-miscible compounds to essentially insoluble ones, as indicated in Table1.4.

Table 1.4 Water Solubilities of Some Organophosphorus Insecticides[3]

Organophosphorus Insecticides	Water Solubilities (ppm)	Organophosphorus Insecticides	Water Solubilities (ppm)
Carbophenothion	2	Phorate	85
Parathion	24	Malathion	145
Azinphosmethyl	33	Dichlorvos	1000
Diazinon	40	Dimethoate	7000
Methyl Parathion	50	Mevinphos	∞

Most highly water-soluble insecticides are systemic, that is, they are absorbed into the tissues of the growing crop, either through the leaves or through the roots. But some water-miscible compounds are so unstable that their toxicity is destroyed before systemic activity can be observed. Tepp, for example, has a half life in water of only 8 hours. While many other compounds in this class have only limited solubility in water, they are still effective systemics. Examples are demeton, methyl demeton, and phorate.

1.2.1 Mode of Action of Organophosphorus Insecticides[1]

The insecticidal organophosphorus compounds apparently inhibit the action of several enzymes, but the major action in vivo is against the enzyme acetylcholinesterase.

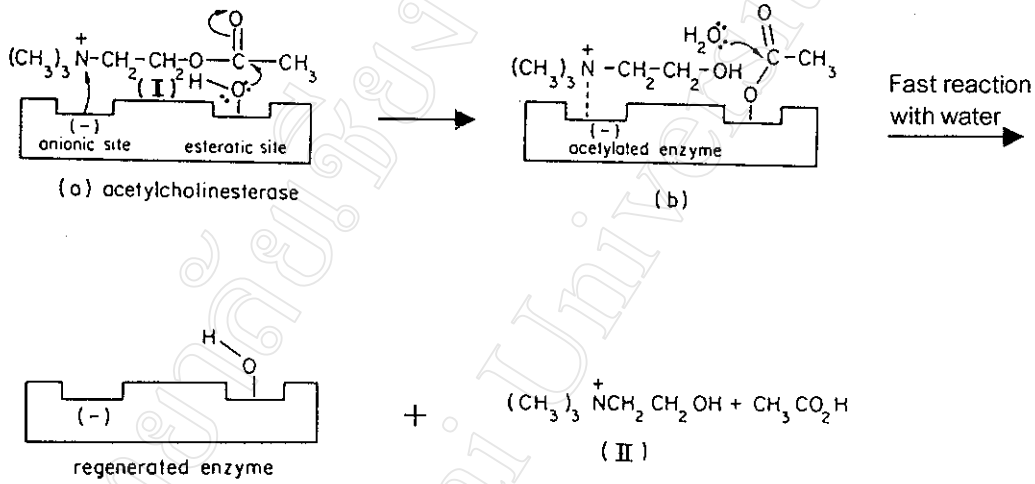


Figure 1.1 The normal enzymic hydrolysis of acetylcholine to choline; (a) depicts the formation of the initial enzyme-substrate complex by the orientation of the active centers of acetylcholinesterase to the substrate (acetylcholine). (b) Shows formation of the acetylated enzyme, which is subsequently rapidly hydrolyzed to choline (II) and acetic acid leaving the enzyme with both its active sites intact, so permitting it to repeat the enzymic hydrolytic process on further substrate molecules releasing several thousand choline molecules per second[1].

This controls the hydrolysis of the acetylcholine (I), generated at nerve junctions, into choline (II). In the absence of effective acetylcholinesterase, the liberated acetylcholine accumulates and prevents the smooth transmission of nervous impulses across the synaptic gap at nerve junctions. This causes loss of muscular coordination, convulsions, and ultimately death.

Acetylcholinesterase is an essential component of the nervous systems of both insects and mammals so the basic mechanism of toxic action of the organophosphorus compounds is considered to be essentially the same in insects and mammals. The active center of the enzyme acetylcholinesterase contains two main reactive sites: an 'anionic site' which is negatively charged and binds onto the cationic part of the substrate (acetylcholine, I), and the 'esteratic site' containing the primary alcoholic group of the amino acid serine which attacks the electrophilic carbonyl carbon atom of the substrate. The normal enzymic hydrolysis of acetylcholine (I) to choline (II) may therefore be illustrated as shown (Figure 1.1).

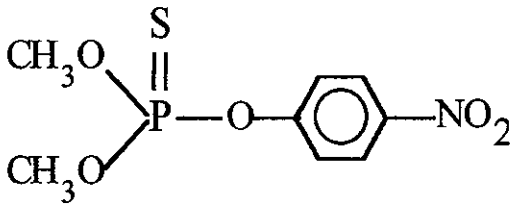
1.3 Methyl Parathion[4,5]

Methyl parathion is a man-made pesticide that may only be used lawfully on certain agricultural crops in open fields to control insects. It is most commonly used on cotton. Other major uses include field corn, peaches, wheat, barley, soybeans and rice fields.

1.3.1 Identity, Properties, and Uses

1.3.1.1 Chemical Name Methyl parathion is o,o-dimethyl o-(4-nitro phenyl)phosphorothioate.

1.3.1.2 Structure[6]



1.3.1.3 Synonyms The common name parathion-methyl (BSI, ISO) is in general use, except that methyl parathion and metaphos are used in the United States and USSR, respectively. Trade names have included: Bladan M, Dalf, Folidol-M, Metacide, Metron, Nitrox, Dithon 63, Ketokil 52, Seis-Tres 6-3, Metaspray 5E, Paraspray 6-3, Folez, Carlawin, Cabidon, Fonotox, Panadon.

1.3.1.4 Chemical and Physical Properties Methyl parathion has the empirical formula $C_8H_{10}NO_5PS$ and a molecular weight of 263.23. The pure material forms a white crystalline powder melting at 37-38 ^{o}C. The technical product is light to dark tan, crystallizes at about 29 ^{o}C, and is about 80% pure. The density of methyl parathion at 20 ^{o}C is reported variously from 1.20 to 1.36. The vapor pressure is 0.97×10^{-5} mm Hg at 20 ^{o}C. Methyl parathion is soluble in water at 25 ^{o}C to the extent of 55-60 ppm. It is slightly soluble in light petroleum and mineral oils and soluble in most other organic solvents. Methyl parathion is hydrolyzed by alkali at a higher rate than parathion and readily isomerizes on heating.

1.3.1.5 Formulations, Uses, and Production Formulations include emulsifiable concentrates, wettable powders, and dusts of various concentrations. Methyl parathion is a nonsystemic contact and stomach insecticide with some fumigant action. Its range of usefulness is similar to that of parathion, but its mammalian toxicity is lower.

1.3.2 Toxicity[4,5]

Short-term exposure to high levels of methyl parathion, an organophosphate, may affect the nervous system by inhibiting the activity of an enzyme called cholinesterase. At normal levels, cholinesterase breaks down a chemical called acetylcholine, which helps transmit signals in the

nervous system. When cholinesterase is inhibited, an excess of acetylcholine builds up and impairs the proper functioning of the nervous system.

Signs and symptoms of direct exposure to high levels of the more concentrated forms of methyl parathion may include headache, dizziness, loss of coordination, muscle twitching, tremor, nausea, vomiting, abdominal cramps, diarrhea and general weakness, blurred vision, excessive perspiration and salivation. These symptoms may result from a single exposure or from repeated exposures occurring over several days. Exposure may occur through inhaling the pesticide, absorbing it through the skin, or swallowing it. At higher levels of exposure, methyl parathion poisoning can lead to respiratory failure and death. Even diluted methyl parathion used indoors can lead to serious poisoning, especially in children and household pets.

A small percentage of those poisoned at the same high dose levels that cause short-term symptoms may experience long-term effects, including persistent neurological problems, such as visual disturbances (double or blurred vision), muscle weakness, mental confusion, short-term memory loss, depression, or difficulty concentrating.

1.3.3 Determination of Methyl Parathion

Methyl parathion is widely used as insecticide of the organophosphorus group. This insecticide is popular because of its broad range of activity and is preferred to organochlorine compounds because of its shorter persistence in the environment and in food commodities. Nevertheless, it is found in agricultural produce, and its characterization is necessary to ascertain contamination levels.

The wide use at high toxicity levels has necessitated the development of a new sensitive and selective method for the determination of methyl parathion.

Most methods involve separatory techniques prior to quantitation such as GC[7-10], TLC[11-14], GLC[15-25], and HPLC[26-31] with detection limits in the ng/ml levels. However, due to the thermal lability of methyl parathion, the results obtained using GC are less feasible. Other techniques such as polaro graphy[6,32] gave the best analytical sensitivity.

The detection technique most frequently used has been Spectrophoto metry. Direct spectrophotometric analysis is always preferred[33-36], but derivatization reactions using different chromogenic reagents are usually employed. A brief review of methyl parathion determination is shown in Table 1.5.

Moreover, there are many techniques that have been utilized for the determination of organophosphorus pesticides such as flow injection analysis[40,41], spectrophotometry[42-49], chemiluminescence[50], fluorimetry[51]. However, these methods are nonspecific for methyl parathion.

Table 1.5 A brief review of methyl parathion determination

Method or reagent	References	Remarks
Thin layer chromatography	11,12,13,14	Limited accuracy.
Gas liquid chromatography	15,16,17,18,19 20,21,22,23,24 25	Sensitive but cumbersome, expensive and time consuming.
High performance liquid chromatography	26,27,28,29,30 31	Sensitive but cumbersome, sophisticated instrumentation.
Polarography	6,32	Chromatographic separation would be required prior to analysis in real samples.
Enzyme inhibition	37,38,39	Time consuming, poor enzyme's activity stability.
Guaiacol	33	Complicated procedure.
Alkaline Hydroxylamine	34,35	Reagent unstable.
4-(p-nitrobenzyl)pyridine	36	Slow procedure.

1.4 Flow Injection Analysis (FIA)

1.4.1 Historical Perspectives[52,53]

The inception of FIA in turn is the result of along search for better laboratory techniques in solution manipulation, which could match the efficiency of the computer age. The important stages of development in this

quest for efficiency and automation in the chemical laboratory are shown in Figure 1.2 and 1.3, which also show the relation between the various techniques for automated solution analysis and the scheme for their classification.

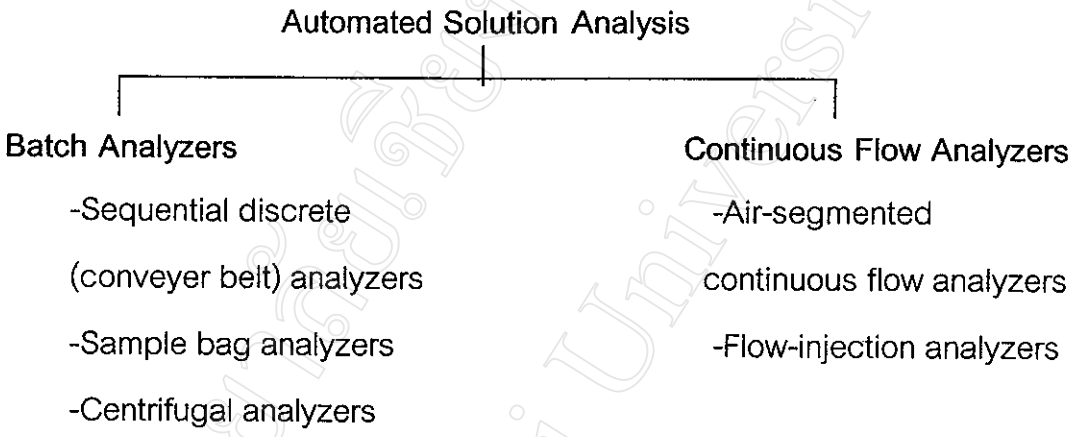


Figure 1.2 Stage of development and classification of automated solution analysis[52].

The first attempts for automation of solution handling which made up the bulk of labor in an analytical laboratory was simply mechanizing and simulating the traditional manual batch operations under a conveyer-belt concept. This was an approach, which never proved to be cost effective and efficient enough to gain widespread acceptance. Batch analyzers, which were more successful, include the Dupont "sample bag" analyzers and the parallel centrifugal analyzers. They are, however, expensive devices, and their use is rather limited, moreover, they are not designed to perform separations.

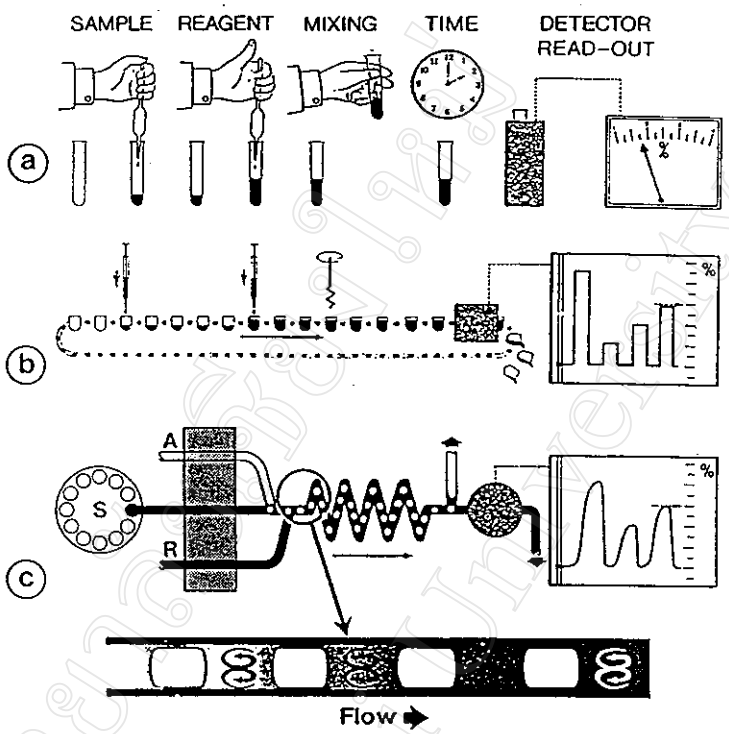


Figure 1.3 The parallel between manual and automated operations that have to be performed in the course of a typical colorimetric assay: (a) Manual handling; (b) a discrete belt-type analyzer; (c) a continuous-flow air-segmented analyzer, with a detail of the air and liquid segments, showing a mixing pattern that leads to homogenization of individual liquid segments[53]

An important breakthrough in laboratory automation was the introduction of continuous flow analysis by Skeggs in 1957. With this system analytical chemistry was for the first time performed in conduits instead of discrete vessels, which greatly improved the efficiency of serial assays. After being marketed by Technicon under the trade name AutoAnalyzer, the system became the most widely accepted equipment for automated solution analysis before the advent of FIA. Yet the conventional concept of performing chemistry under equilibrium conditions was strictly adhered to in this system, as for discontinuous batch procedures. To achieve this, air bubbles were

introduced into the flow to ensure homogeneous mixing, which constituted the most important feature of Skeggs's system, i.e., air-segmented continuous flow. This approach, although effective in serving its purpose of steady state reading under flow conditions, later confined the further development of continuous flow analysis. The requirement of achieving equilibrated conditions seriously restricted the efficiency of the technique, so that sampling frequencies were typically only in the range of 20-40 h^{-1}. A new stage of important development was initiated in the mid-seventies by substituting the segmented flow principle by the non-segmented principle of FIA, and abandoning the principle of steady state readout.

1.4.2 Basic Principles of FIA[52-54]

Ruzicka and Hansen defined FIA as "A method based on injection of a liquid sample into a moving unsegmented continuous stream of a suitable liquid. The injected sample forms a zone, which is then transported toward a detector that continuously records the absorbance, electrode potential, or any other physical parameter, as it continuously changes as a result of the passage of sample material through the flow cell, and the technique is base on a combination of sample injection, controlled dispersion, and exact timing"- later often referred to as the three basic principles or cornerstones of FIA. The basic components of an FIA system described by this definition may be illustrated by a very simple schematic diagram (Figure 1.4 a).

This diagram consists of a pump, which is used to propel the carrier stream through a narrow tube; an injection port, by means of which a well-defined volume of a sample solution is injected into the carrier stream in a reproducible manner; and a microreactor in which the sample zone disperses

and reacts with the components of the carrier stream, forming a species that is sensed by flow through detector and recorded.

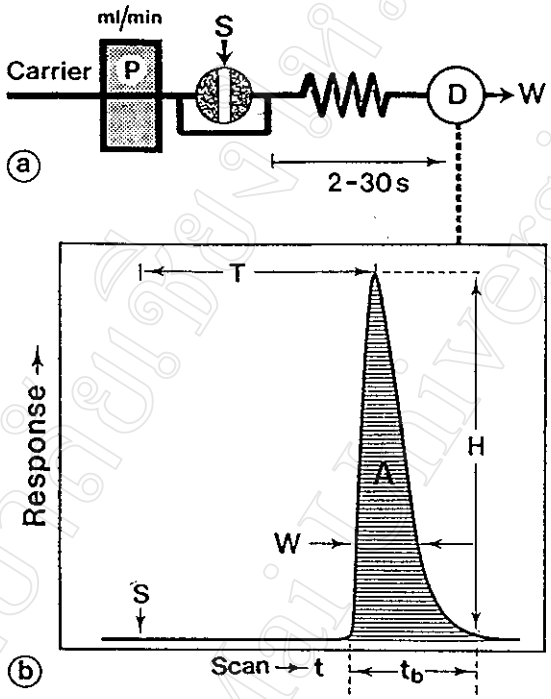


Figure 1.4 The basic components of an FIA system; (a) The simplest single-line FIA manifold utilizing a carrier stream of reagent; S is the injection port, D is the flow cell, and W is the waste. (b) The analog output has the form of peak, the recording starting at S (time of injection to). H is the peak height, W is the peak width at a selected level, and A is the peak area, T is the residence time corresponding to the peak height measurement, and t_b is the peak width at the baseline[53]

A typical recorder out put has the form of a peak (Figure 1.4 b), the height *H*, width *W*, or area *A* of which is related to the concentration of the analyte. The time span between the sample injection *S* and the peak maximum, which yields the analytical readout as peak height *H*, is the residence time *T* during which the chemical reaction takes place. A well-designed FIA system has an extremely rapid response, because *T* is in the range of 5-20 s. Therefore, a sampling cycle is less than 30 s (roughly $T+ t_b$),

and thus, typically, two samples can be analyzed per minute. The injected sample volumes may be between 1 and 200 μL (typically 25 μL), which in turn requires no more than 0.5 ml of reagent per sampling cycle. This makes FIA a simple, automated microchemical technique, capable of having a high sampling rate and a minimum sample and reagent consumption.

The FIA system itself provides the reproducible physical conditions, in contrast to batch methods or air-segmented continuous flow analyzers. This is true since the FIA system contains no components that create random turbulence. Only reproducible convection will be observed. The next question is whether a steady state signal is a requirement for precise quantitation in an FIA system. Since identical physical and chemical conditions can consistently be obtained, as long as the system configuration is not changed, steady state is not a necessary requirement. Figure 1.5 is a printout of multiple injections of a dye and is a typical example of the physical reproducibility of FIA.

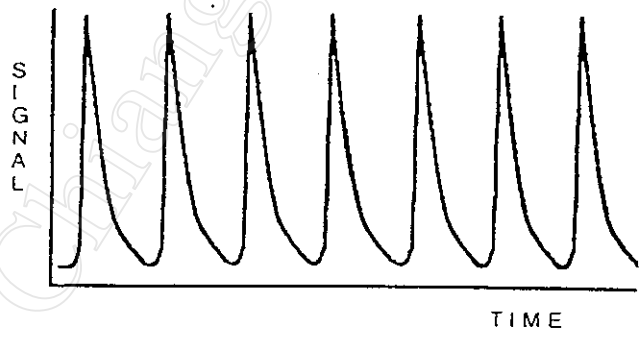


Figure 1.5 Typical detector output of an FIA system into which a dye solution is repeatedly injected[54].

1.4.3 Dispersion of Sample Zone[52,53,55]

When the sample is first injected, it forms a well-defined sample plug in the stream. As the sample is swept downstream through the analytical

conduits of narrow bore tubing, the plug disperses into and, thus, mixes with the carrier stream under laminar flow conditions to form a gradient.

A simple dispersion experiment is used to describe the dispersion by means of the dispersion coefficient (Figure 1.6). A sample solution, contained within the valve cavity prior to injection, is homogeneous and has the original concentration C^0 that, if it could be scanned by a detector, would yield a square signal the height of which would be proportional to the sample concentration (Figure 1.6, left).

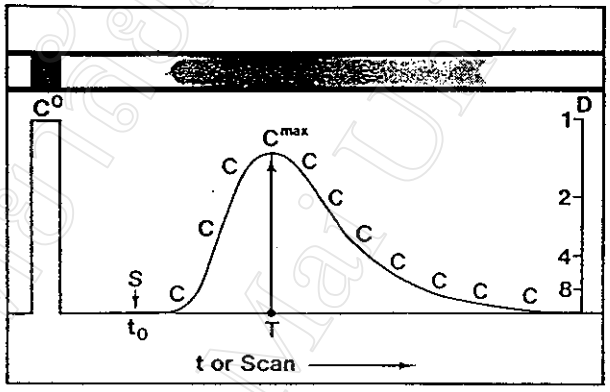


Figure 1.6 Dispersed sample zone in flow system; an originally homogeneous sample zone (top left) disperses during its movement through a tubular reactor (top center), thus changing from an original square profile (bottom left) of original concentration C^0 to a continuous concentration gradient with maximum concentration C^{max} at the apex of the peak[53]

When the sample zone is injected, it follows the movement of the carrier stream, forming a dispersed zone whose form depends on the geometry of the channel and the flow velocity. Therefore, the response curve has the shape of a peak reflecting a continuum of concentrations (Figure 1.6, right), forming a concentration gradient, within which on single element of fluid has the same concentration of sample material as a neighboring one. It is useful, however, to view this continuum of concentrations as being composed of individual

elements of fluid, each of them having a certain concentration of material *C*, since each of these elements is a potential source of a readout.

The dispersion coefficient D, is used for evaluation of dispersion, and is the ratio of the concentration of the constituent of interest in a fluid element of the injected zone before and after the dispersion has taken place, expressed by[52]:

$$D = \frac{C^o}{C} \qquad (1.1)$$

Where C^o is the original concentration of the constituent of interest in the solution to be injected, and *C* the concentration of that fluid element of the dispersed solution zone which is under consideration. When the fluid element with the highest concentration is concerned (i.e. readout at FI peak maximum), equation 1.1 is expressed as[52]:

$$D = \frac{C^o}{C^{max}} \qquad (1.2)$$

Where C^{max} is the concentration of the constituent at peak maximum of the dispersed zone. When fluid elements on the rising or falling slopes of the concentration gradient of the dispersed zone are concerned, the equation is expressed as[52]:

$$D = \frac{C^o}{C^{grad}} \qquad (1.3)$$

Where C^{grad} is the concentration of the constituent in that fluid element on the gradient of the FI peak which is under consideration.

D is therefore dimensionless, and is a value greater than unity, the value reflecting the dilution factor of the fluid element being studied. FI systems with dispersion coefficients below 2 are limited dispersion, which can be attained to feed such detectors as electrodes and atomic absorption spectrometers to effect high analytical rates. Medium dispersion, those between 2 and 10, can be applied to attain a wide variety of reaction configurations to develop some detectable entity such as color, fluorescence or an electroreactive product. While large dispersion, those above 10, can be utilized to give a substantial degree of mixing between sample and carrier stream to form a well-developed concentration gradient, as is necessary when performing continuous flow titrations or for investigating the chemistry at the sample-stream interface.

The magnitude of this dispersion is dependent on the operating parameters applied to the system, including sample volume, tubing bore size, tubing length, flow rate, sample volume and, possibly, coil diameter. Varying the values of these parameters confers a significant degree of control over the dispersion characteristics and facilitates optimization of a flow injection system for many diverse applications.

1.5 General Instrumentation of FIA

The flow infection system should be constructed in such a manner that[56]:

1.The carrier stream flows through a narrow tube of uniform diameter, including the injection and detector sections.

2.The sample solution is injected as an instant pulse of exact volume and short duration, in such a way that the movement of the carrier stream remains undisturbed.

3.Side streams are added to the main stream in an easily reproducible manner.

4.The flow of all streams is pulse-free, and their movement can be started and stopped instantaneously.

5.The detector instantly and selectively responds to the analyte concentration with maximum signal yield.

Obviously, these criteria are difficult to meet in full, especially if the individual components (i.e., the pumps, infection ports, reaction coils, detectors, and readout devices) should be simple in construction, inexpensive, and reliable in use. Therefore, a compromise must be made between the ideal, availability, and cost.

1.5.1 Liquid Delivery Units (Pumps) [52,54,56]

The liquid delivery unit is a critical component in an FIA system. There are three types of liquid delivery devices that are utilized; pressurized bottle (including constant head), peristaltic pump, and syringe pump. By far the peristaltic pump has gained the largest popularity and acceptance. The peristaltic pump consists of a motor-driven wheel with peripherally placed rollers and a compression cam (or band) which is squeezed against the rollers. One or several pump tubes are affixed so that they rest on a minimum of two of the rollers at all times, see Figure 1.7.

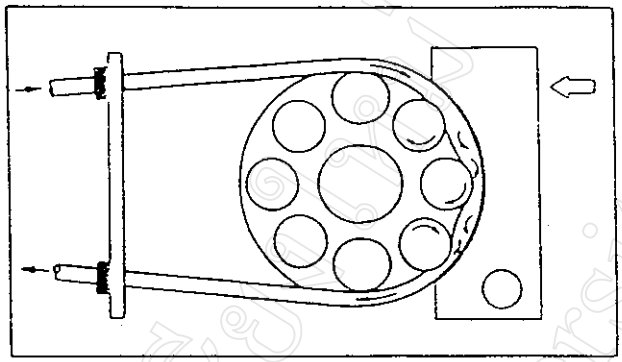


Figure 1.7 Relationship between the rollers of a peristaltic pump and the pump tubes[54].

A peristaltic pump is the most suitable means of propelling the carrier stream (s) in a FIA apparatus, because it may accommodate several channels whereby, according to individual tube diameters, equal or different pumping rates may be obtained. As each tube might either be used for aspirating or delivering solution, a propelling as well as a withdrawing motion may be executed by the same pump. A well-constructed pump stops and starts instantly, thus allowing precise control of the movement of all streams for stopped-flow or intermittent pumping functions.

The main disadvantages of such devices seem to be their flow pulsation, a lack in long term flow-rate stability, and low resistance (of the pump tubes) to organic solvents and high concentrations of strong acids. However, when used properly, most of the drawbacks of the peristaltic pump can often, though not always, be overcome.

There is no universal method to reduce the pulsation in a system containing a peristaltic pump. Lubrication and a correct adjustment of the tube tension are the basic means to prevent pulsation.

1.5.2 Injection Valves[54]

The following aspects should be considered when choosing an injector for FIA:

1. **Volumes of liquid**

The injection volume must be changeable at least in the range 30-200 μL. Once chosen, the volume should be perfectly constant. The sample volume needed to fill the injector is inevitably larger than the injection volume but it should not be significantly larger.

2. **Filling of sample**

The most convenient way to fill the sample into the injector is by aspiration since a minimum of manual intervention is required. Filling from a syringe can be acceptable if very few samples are to be run, but it is totally unacceptable for large numbers of samples.

3. **Mode of activation**

Automatic activation predominates and is definitely preferable. Manual activation often involves turning a movable part of the valve, which is a difficult operation to carry out in a reproducible way. The activation period should be short. The carrier stream into which the sample is to be inserted is, for many injectors, interrupted during the activation period. This means that the proportions between the carrier and the reagent are changed temporarily. In some injector designs this effect has been decreased through implementation

of a by-pass tube so that the carrier can continue to flow during the activation period.

From a functional point of view, there are two types of injectors, rotary valve injectors and syringe injectors (Figure 1.8 and 1.9).

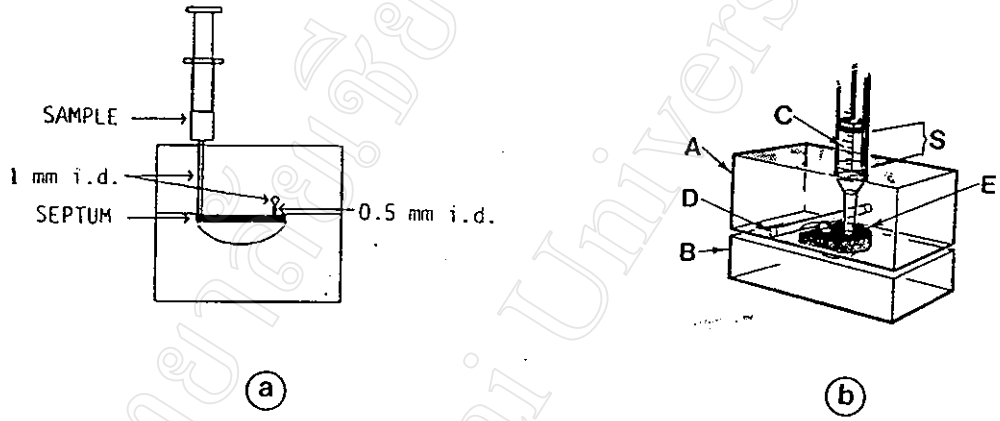


Figure 1.8 Syringe injector; (a) Schematic drawing of syringe injector. (b) Syringe injector for manual sample introduction, consisting of two parts (A and B) between which is placed a rubber septum E. Sample solution S is metered by syringe C, which is then placed into a tightly fitting sleeve in A. When the plunger is depressed, the pressure of the sample liquid will make the septum yield so that the sample solution is forced into tube D, transporting the carrier solution[54,56].

The syringe injection mode approximates the pulse injection, which theoretically has some advantages as to the geometrical form of the injected sample zone, whereas the rotary valve injection mode approximates the plug injection and is a more practical way of introducing well-reproducible sample volumes into a flowing carrier stream without disturbing its motion. This is why the valve injection has gained wide acceptance for FIA applications. The injection valve, schematically shown in Figure 1.9.

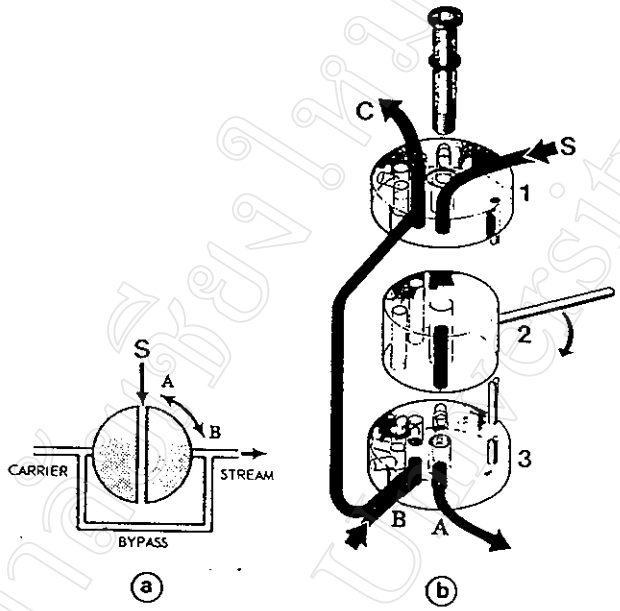


Figure 1.9 Rotary injection valve; (a) The principle of a simple of a simple rotary valve, the bore of which determines the injected volume. The valve is furnished with a bypass of higher hydrodynamic flow resistance than the volumetric bore so that the carrier stream can flow continuously through the manifold lines when the valve is turned from the inject (B) to the sampling (A) position. (b) Multi-injection valve consisting of a rotor (2) sandwiched between two stators (1 and 3), the whole system being clamped together by a bolt when assembled. The rotor (20 mm high) has three volumetric bores of which one is shown filled by sample solution S, the excess of which is drained through the bottom stator at (A). In the rotor position shown, the carrier stream C bypasses the rotor through a shunt, entering the valve through the bottom stator at (B). Thus, after turning the rotor (as indicated by the arrow), the precisely measured sample zone is swept by the carrier stream into the system, because the bypass conduit has a higher hydrodynamic flow resistance[57].

1.5.3 Mixing Reactors[52-54]

The main function of mixing reactors is to promote the reproducible radial mixing of two or more components merged through a T-piece. The reactor is usually made of PTFE tubing with the same range of dimensions as

for the transport conduits. The tubes are coiled Knotted, or knitted (Figure 1.10) to produce a secondary flow in the radial plane by varying the flow direction. This enhances radial mixing, and decreases the axial dispersion of an injected sample plug.

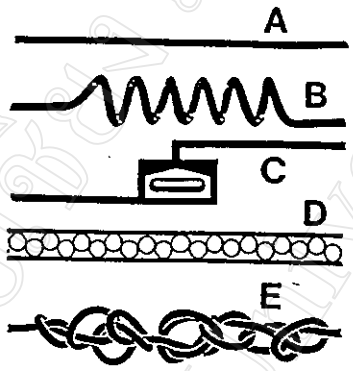


Figure 1.10 The microreactor geometries most frequently used in FIA: A, straight open tube; B, coiled tube; C, mixing chamber; D, single-bead string reactor (s.b.s.r.); and E, knitted reactor[53].

Although coiled reactors of about 10-mm coil diameter are most often used, the most dramatic effects are obtained by using knitted or knotted reactors. These reactors were first introduced by Engelhardt and Neue for applications in HPLC, and referred to in some references as three-dimensionally disoriented reactors (or 3-D. reactors). While in coiled reactors the flow directions are changed mainly on a two-dimensional basis, and hence its name. Owing to its strong capability in limiting dispersion, it has been recommended not only for mixing but also for transport conduits and sample loops.

The single bead string reactor (s.b.s.r.) was described as early as 1981 by Reijn et al. Dry glass beads with a mean diameter of 0.4 mm were carefully

packed in a polyethylene tube, i.d. 0.6 mm, using a pipette and a funnel. The single bead string reactors showed less dispersion and enhanced micro-mixing. Several other studies have confirmed these findings.

1.5.4 Detectors[52]

In principle, any detection system, which could be adapted for flow-through detection, may be used as detectors for FIA. However, some detectors are inherently more suitable than others in the interfacing, and therefore are used more frequently in FI systems. These include the spectrophotometer (visible and UV), atomic absorption and ICP spectrometer, chemiluminescence and various electrochemical detectors.

In this research, only spectrophotometer has been used as a detector in FI system, and it will be discussed here in more detail.

1.5.4.1Spectrophotometers[52]

Visible and UV spectrophotometers are by far the most frequently used type of detectors in FI systems. Provided the light source intensity is strong enough, a conventional batch spectrophotometer can easily be converted into a flow-through spectrophotometer by substituting the conventional cuvette with a flow-through cell.

Flow-cells

A most often used flow-cell configuration, which may be furnished with either glass or quartz windows, is shown in Figure1.11. Flow cells of 18 μL capacity in the light path (1.5 mm diameter, 10 mm long) are commonly used.

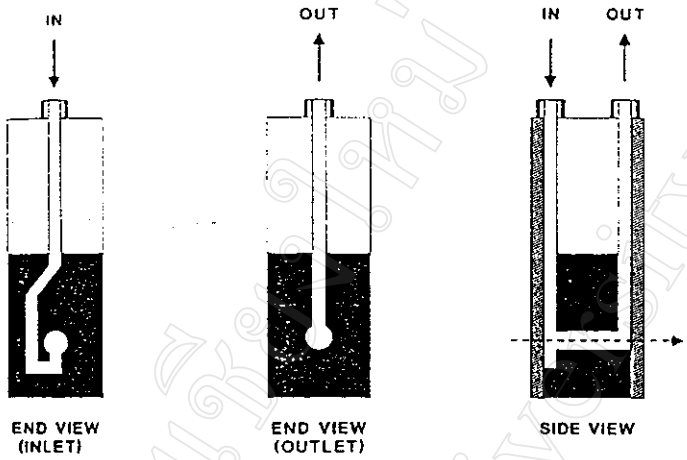


Figure 1.11 A spectrophotometric flow-cell. Arrow shows light path[52].

The inlet and outlet conduits of the cell are so arranged that the stream is fed in from the bottom of the cell and leaves from the top to facilitate the release of accidentally introduced air bubbles. Care should be taken not to reverse the flow directions through the cell, in order to avoid the trapping of air bubbles in it.

1.6 Research Aims

The aims of this research can be summarized as follows:

1) To design and construct a flow injection system for rapid and simple determination of methyl parathion.

2) To investigate the optimum conditions for the determination of methyl parathion by flow injection spectrophotometric method.

3) To apply the proposed method for determining methyl parathion in environmental samples.